ICEERS

INTERNATIONAL CENTER FOR ETHNOBOTANICAL EDUCATION, RESEARCH & SERVICE

Ayahuasca

Scientific Literature Overview

conducted by José Carlos Bouso for
The International Center for Ethnobotanical Education, Research & Service

2012

International Center for Ethnobotanical
Education, Research & Service
Nieuwe Zandstraat 4
4661AP Halsteren
Netherlands
tel.: +31624841232
email: info@iceers.org
www.iceers.org

Literature Research conducted by
José Carlos Bouso
jcbouso@gmail.com

Tabel of Content

Clinical Trials &
Short Term Effects

Literature research conducted by José Carlos Bouso for
The International Center for Ethnobotanical Education, Research & Service

ELSEVIER

Journal of Ethnopharmacology 65 (1999) 243–256

Journal of ETHNO-PHARMACOLOGY

Pharmacokinetics of *Hoasca* alkaloids in healthy humans

J.C. Callaway [a,*], D.J. McKenna [b], C.S. Grob [c], G.S. Brito [d], L.P. Raymon [e], R.E. Poland [c], E.N. Andrade [f], E.O. Andrade [f], D.C. Mash [g]

[a] *University of Kuopio, Department of Pharmaceutical Chemistry, POB 1627, Kuopio FIN-70211, Finland*
[b] *Botanical Dimensions, POB 807, Occidental, CA 95465, USA*
[c] *Department of Psychiatry, Bldg. D-6, Harbor/UCLA Medical Center, 1000 West Carson Street, Torrance, CA 90509, USA*
[d] *Centro De Estudos Medico, Da União Do Vegetal, Caixa Postal 71505, 05020-990 São Paulo, Brazil*
[e] *Department of Pathology, Forensic Toxicology Laboratory, 12500 SW, 152nd Street, Building B, Miami, FL 33177, USA*
[f] *University of Amazanas, Medical School, Department of Internal Medicine, Manaus, Amazônia, Brazil*
[g] *University of Miami School of Medicine, Department of Neurology, 1501 NW 9th Avenue, Miami, FL 33136, USA*

Received 21 April 1998; received in revised form 1 September 1998; accepted 15 September 1998

Abstract

N,*N*-Dimethyltryptamine (DMT), harmine, harmaline and tetrahydroharmine (THH) are the characteristic alkaloids found in Amazonian sacraments known as *hoasca*, *ayahuasca*, and *yajè*. Such beverages are characterized by the presence of these three harmala alkaloids, where harmine and harmaline reversibly inhibit monoamine oxidase A (MAO-A) while tetrahydroharmine weakly inhibits the uptake of serotonin. Together, both actions increase central and peripheral serotonergic activity while facilitating the psychoactivity of DMT. Though the use of such 'teas' has be known to western science for over 100 years, little is known of their pharmacokinetics. In this study, *hoasca* was prepared and administered in a ceremonial context. All four alkaloids were measured in the tea and in the plasma of 15 volunteers, subsequent to the ingestion of 2 ml *hoasca*/kg body weight, using gas (GC) and high pressure liquid chromatographic (HPLC) methods. Pharmacokinetic parameters were calculated and peak times of psychoactivity coincided with high alkaloid concentrations, particularly DMT which had an average T_{max} of 107.5 ± 32.5 min. While DMT parameters correlated with those of harmine, THH showed a pharmacokinetic profile relatively independent of harmine's.

Keywords: *Ayahuasca*; Serotonin; Psychoactivity; Religion; Addiction

1. Introduction

Hoasca is a Brazilian word for a decoction of the woody liana *Banisteriopsis caapi*, which is

* Corresponding author. Tel.: +1 358 17163601; fax: +1 358 17162456; e-mail callaway@uku.fi

0378-8741/99/$ - see front matter
PII: S0378-8741(98)00168-8

pounded and then boiled with the leaves of *Psychotria viridis*. This beverage, also known as *ayahuasca*, *caapi*, *daime*, *yagé*, *natem*, and by many other local names, is found throughout the extensive river regions of Brazil, Bolivia, Ecuador and Peru. Such beverages have been used throughout the Amazon and Orinoco River Basins for both medicinal and ceremonial purposes since antiquity, and remain as sacraments to indigenous religions of these forested regions (Spruce, 1908; Schultes and Hofmann, 1992). While other plants may be added to the brew, and many variations of this beverage have been described (Schultes, 1957; Pinkley, 1969; McKenna and Towers, 1984; McKenna et al., 1984a,b; Luna and Amaringo, 1991; Schultes and Raffauf, 1992; Ott, 1994), the salient common denominator is the presence of harmala alkaloids (Rivier and Lindgren, 1972); particularly harmine, harmaline and tetrahydroharmine (THH). Aside from a brief mention in a congress abstract (Rivier and Holmstedt, 1982), pharmacological studies of this beverage in humans have not been reported.

The harmala alkaloids, obtained from *B. caapi*, were initially identified as the primary components of this beverage (Hochstein and Paradies, 1957), and were subsequently considered to be responsible for the visionary effects (Naranjo, 1967). In this regard, however, these alkaloids function primarily as specific and reversible inhibitors of type-A monoamine oxidase (MAO–A), particularly the more potent harmine and harmaline (Udenfriend et al., 1958; Buckholtz and Boggan, 1977). While not a strong inhibitor of MAO, THH possibly contributes neuroactivity by weakly inhibiting the uptake of serotonin (5-hydroxytryptamine, 5-HT) at presynaptic sites, like other 1-methyl-tetrahydro-β-carbolines (Airaksinen et al., 1980). Subsequently, concentrations of 5-HT increase in the body when both its metabolism by MAO–A and presynaptic uptake are simultaneously blocked by these harmala alkaloids.

The inhibition of MAO also allows for the oral activity of *N*,*N*-dimethyltryptamine (DMT), a potent psychedelic agent often found in these beverages and is obtained from the leaves of *P. viridis*. DMT binds to serotonergic sites in the brain and typically facilitates novel perceptions of reality with complex mental imagery (Holmstedt and Lindgren, 1967; McKenna and Towers, 1984; McKenna et al., 1984a,b, 1990; Deliganis et al., 1991; Ott, 1994; Strassman et al., 1994). The psychoactivity of DMT was first described in the medical literature over 40 years ago (Szára, 1956), at an effective dose of about 1 mg/kg intermuscularly. Ordinarily, DMT is rapidly oxidized by functional MAO to an inactive metabolise (Barker et al., 1980; Suzuki et al., 1981). For this reason it is orally inactive. Harmine and harmaline allow the oral activity of DMT by temporarily inhibiting the activity of MAO, and the resulting visionary effects are a hallmark of this unique plant combination. Fig. 1 shows the molecular structures of DMT and harmala alkaloids, illustrating their chemical similarities to 5-HT.

Aside from conferring oral activity on DMT, MAO inhibition may also contribute to actions of other psychoactive alkaloids that are sometimes found in these beverages; e.g. nicotine from *Nicotiana* species, cocaine from *Erythroxylum coca*, caffeine from *Ilex guayusa*, atropine, scopolamine and other tropane alkaloids from members of the *Solanaceae* family etc. (Schultes, 1957; Pinkley 1969; Ott, 1994). The inherently complex pharmacology of such combinations are essentially unknown to modern medicine, although their utility is indicated by a legacy of human use throughout a large geographical region (Schultes and Hofmann, 1992).

Stemming from indigenous usage, syncretic churches have developed over the last 75 years within urban populations of northern South America, particularly in Brazil. These sects evolved by blending the psychoactive effects of these beverages with Judeo–Christian, African or other Old World religious doctrines. Of these modern religions, the Santo Daime (perhaps the oldest Christian church using this beverage), the União do Vegetal (UDV, the largest unified congregation), and the Barquinha (an Afro–Brazilian church) presently have some of the largest followings. In 1987, the use of such beverages within a religious context was officially recognized and protected by law in Brazil, after lengthy investigations into its alleged threats to public health and national security (Ott, 1994).

Excluding users from the indigenous population, the present number of regular (e.g. monthly) users within the urban populations of South America could be over 15000 individuals (Luna, 1997). In most indigenous groups only a small percentage of the total population use the tea on a regular basis, although most individuals have had it at some time during their lives. In the syncretic churches, however, it is routinely consumed by all adult members on a weekly or bimonthly basis within a ceremonial context. Some physical tolerance may develop through regular use (Callaway et al., 1994), as a reaction to the subsequent and periodic surge in neurotransmitter levels that follow the ingestion of *hoasca* (especially 5-HT), although it is not physically addictive nor has psychological dependence been demonstrated for these beverages. In fact, dedicated members of these modern religions typically lose their interest in the habitual use of alcohol, tobacco, cocaine and other addictive substances (Grob et al., 1996).

During the Summer of 1993, at the invitation of the UDV's Center for Medical Studies, a clinical study was initiated in order to investigate the psychopharmacologic properties of *hoasca*. This invitation was accepted by an international team of medical researchers who were interested in examining the contextual use of this beverage, in addition to examining its potential applications in modern medicine. Our previous articles for this investigation described the analytical methodologies that were used to assay the *hoasca* alkaloids, conduct psychologic inventories of the volunteers before, during and after *hoasca* ingestion, and identify changes in 5-HT uptake site densities on thrombocytes after long term (>10 years) periodic (biweekly) use of *hoasca* (Callaway et al., 1996; Grob et al., 1996; Callaway et al., 1994, respectively). Herein are reported the pharmacokinetic results following the ingestion of *hoasca* in healthy volunteers, and their correlation with some pharmacodynamic effects.

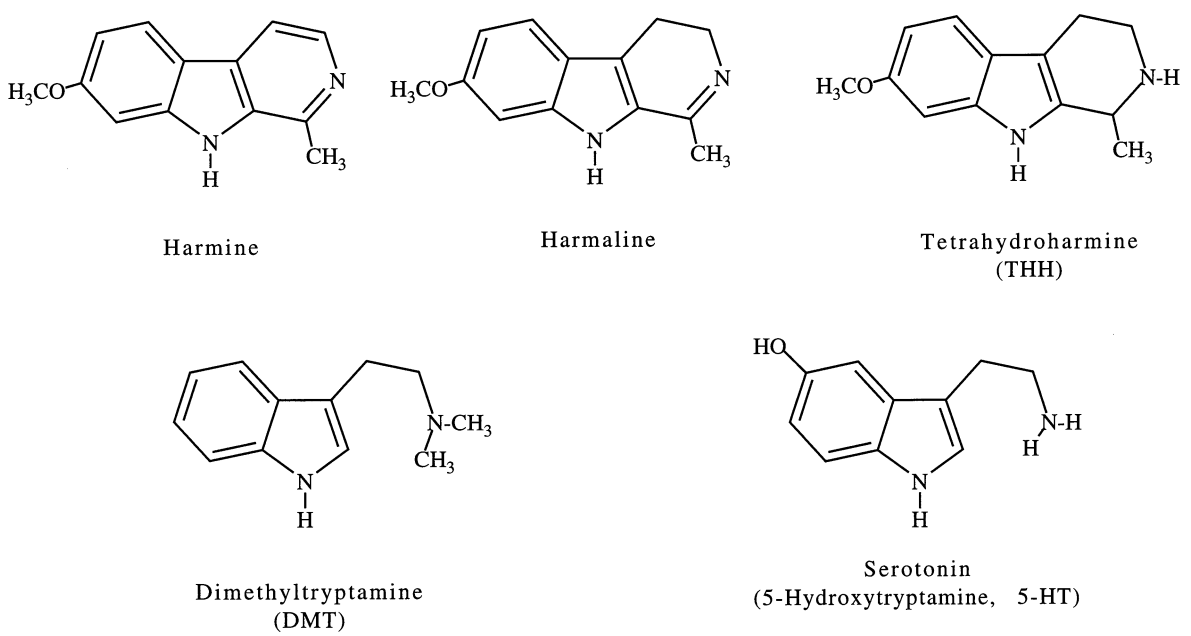


Fig. 1. Molecular structures of *N*,*N*-dimethyltryptamine (DMT) and three harmala alkaloids found in *hoasca*, along with the neurotransmitter serotonin (5-hydroxytryptamine, *S*-HT).

2. Methodology

2.1. The study site

The *Nucleo Caupurí*, a UDV temple on the outskirts of Manaus, Amazônia, was the designated site for this study. Approximately 40 years in existence, this temple is the second oldest *nucleo* of the UDV and has the highest proportion of long-term members.

2.2. The hoasca

Sufficient amounts of *B. caapi* Spruce. ex Grisebach (Malpighiaceae), known as *mariri* to the UDV, and *Psychotria viridis* Ruíz et Pavón (Rubiaceae), known as *chacrona* to the UDV, were gathered on site at the *Nucleo Caupurí* to prepare *hoasca* for the purpose of this study. Plant collection began shortly after dawn. Voucher specimens were collected and authenticated by Dr. D.J. McKenna, and subsequently deposited in the herbarium at the Puutarha Botanical Garden at the University of Kuopio, in Kuopio, Finland (*B. caapi* # 98-147, *P. viridis* # 98148). In preparing the *hoasca*, the woody *B. caapi* was carefully washed in water and pounded with wooden mallets, and the leaves of *P. viridis* were simply rinsed with water.

The plant materials were carefully combined, boiled and concentrated over several hours to produce approximately 120 l of the tea before midnight of the same day. This process was supervised entirely by *mestres* of the UDV, and executed by members of the UDV, according to their religious practice. The tea was tested for quality and potency, in a ceremonial context, prior to the pharmacokinetic study. Alkaloid content of the tea was later quantified by high-pressure liquid chromatography (HPLC), using fluorescence detection (Callaway et al., 1996).

Each of the 15 volunteers (74.2 ± 11.3 kg; average $\pm$ SD) ingested 2 ml/kg of *hoasca* after a baseline blood sample had been collected from a cubital vein, through an indwelling catheter. The actual dose was always rounded up to the nearest power of 10 (e.g. an individual weighing 66 kg received 140 ml of the brew, rather than 132 ml). In every case, the *hoasca* was administered by a *mestre*, in keeping with their tradition, and the entire amount was rapidly consumed (within less than 10 s).

2.3. The volunteers

Fifteen male members of the UDV, between 26 and 48 years of age (35.9 ± 6.9 years; avg. $\pm$ SD), were randomly selected from a larger group of 24 volunteers who had used *hoasca* as part of their regular religious practice for at least 10 years, and who had also passed a physical examination administered by a medical doctor. The medical evaluation included an extensive blood-chemistry panel (SMAC-24), basal cardiac measures, ECG and other standard measures of health. Psychological evaluations were made throughout the study, and these results have been published elsewhere (Grob et al., 1996). In addition to the experimental volunteers, an age matched group of 15 males who had never consumed *hoasca* were subjected to the same medical evaluation, which included blood samples, as previously described (Callaway et al., 1994; Grob et al., 1996).

UDV members typically ingest *hoasca* once every other week, though seldom more often than once a week. All volunteers used some caffeinated beverage on a daily basis. None had used alcohol, tobacco or other drugs for many years, although eleven (73%) were once dependent on tobacco, alcohol and/or other drugs prior to their regular sacramental use of *hoasca* (Grob et al., 1996). All participants gave informed consent to the study. All of the volunteers abstained from their use of *hoasca* for at least 1 week prior to the study. All studies began at 09:00 h, and volunteers were instructed to fast on the morning of their study day.

2.4. Subjective effects

The hallucinogenic rating scale (HRS) is an instrument that was developed to measure the psychotropic effects of injected DMT (Strassman et al. 1994). The HRS was administered to each volunteer before, during and after the acute pharmacokinetic study in an attempt to obtain some

numerical measure of *hoasca's* psychotropic effect, and these results have already been published (Grob et al., 1996).

2.5. Plasma collection

Whole blood was allowed to flow freely through a heparinized line and into an opened test tube that contained aqueous EDTA as an anticoagulant (13 mg EDTA per 10 ml purple top tube, Termo Oy, Espoo, Finland). Samples were capped and briefly mixed by repeated inversions, then centrifuged at $200 \times g$ for 10 min at ambient Amazonian temperatures (33–38°C). The plasma was rapidly transferred to clean glass tubes and frozen on dry ice and subsequently stored at −80°C. All samples remained frozen until analysis. A maximum of three volunteers were studied in a single day, where each administered dose was staggered by 15 min. Blood samples were collected prior to ingestion, then at the following time points (in min): 0, 20, 40, 60, 90, 120, 180, 240, 360, 480 and finally a 24 h sample on the next day. A sample was discarded if collection began over 1 min past the designated time.

2.6. Plasma alkaloid analyses

Harmine, harmaline and THH were measured from plasma using HPLC with fluorescence detection, while plasma DMT was quantified by gas chromatography using nitrogen-phosphorus detection (GC-NPD), as previously described (Callaway et al., 1996).

2.7. Pharmacokinetic calculations

The software package PCNONLIN (Version 4.0, Scientific Consulting) was used to determine concentration–time curves (AUC) for the alkaloids. This program afforded a nonparametric analysis of the pharmacokinetic data, using Levenberg's modification of the Gauss–Newton method for estimating parameters from non-linear regression analyses.

2.8. Neuroendocrine assays

Growth hormone (GH) and prolactin were measured in plasma against standards obtained from the National Pituitary Agency, as previously described (Odell et al., 1967; Poland and Rubin, 1981). GH was iodinated by the glucose-oxidase method and its suitability for radio-immuno assay (RIA) was determined by the talc-resin-TCA method. All samples were analyzed in duplicate. Assay sensitivity was 0.2 ng/ml for both GH and prolactin. Plasma cortisol was determined by RIA, using ^{125}I-cortisol and anti-cortisol antisera (Radio Assay Systems Laboratories), and analyzed as previously described (Poland and Rubin, 1982). Control samples were analyzed in duplicate at the beginning, middle and end of each assay, giving maximum intra- and inter-assay coefficients of variation of approximately 9.0 and 15%, respectively.

2.9. Autonomic measurements

Heart rate, blood pressure, respiration, oral temperature and pupillary diameter were measured after each blood draw until 240 min, each time by the same clinicians, using simple standard techniques.

3. Results

3.1. The hoasca

Emperical testing of the freshly prepared tea, by experienced members of the UDV, provided verification that the beverage was typical and suitable for the pharmacokinetic study. Subsequently, it was decided that a standard dose of 2 ml/kg body weight would be used throughout the study. In retrospect, this particular amount was considered to be somewhat mild in effect, according to the volunteers. Analytical analyses revealed the alkaloid content of the *hoasca* to be: harmine 1.70 mg/ml, harmaline 0.20 mg/ml, THH 1.07 mg/ml and DMT 0.24 mg/ml.

Table 1
Averages and ranges of body weight, amount of *hoasca* ingested (2 ml/kg) and amounts of alkaloids consumed by 14 volunteers

	Body weight (kg)	Tea (ml)	DMT (mg)	THH (mg)	Harmaline (mg)	Harmine (mg)
Average	74.2 ± 11.3	148.4 ± 22.6	35.5 ± 5.3	158.8 ± 24.2	29.7 ± 4.5	252.3 ± 38.4
Range	58–90	120–180	28.8–43.2	128.4–192.6	24.0-36.0	204.0–306.0

3.2. The volunteers

From the preclinical investigations, no significant differences were found between the experimental and control groups (note: the latter group of *hoasca*-naive individuals were not included in the pharmacokinetic phase of this study). The ranges and averages of body weights for the 14 experimental volunteers included in these analyses, and averaged amounts of tea, and measured alkaloids consumed, are presented in Table 1. One of the 15 volunteers vomited during the pharmacokinetic study, approximately 45 min after ingestion, and experimental data from this individual were not included in the final analyses of this report.

3.3. The subjective effects

The duration of psychoactivity from the tea was coincidental with alkaloid plasma levels. In particular, peak plasma levels of DMT were associated with intricate and colored eyes-closed visual imagery, complex thought processes and a general state of heightened awareness. Overall perceptual, cognitive, and affective processes were significantly modified while maintaining the presence of a clear sensorium. All 15 volunteers experienced these subjective effects at this dosage (2 ml/kg).

3.4. Pharmacokinetics

Although peak plasma levels for DMT were determined in all volunteers, only 12 had sufficient concentrations for all pharmacokinetic calculations. The following data are reported as the mean $\pm$ SD, in plasma. The C_{max} for DMT ($n = 12$) was 15.8 ± 4.4 ng/ml, with a T_{max} of 107.5 ± 32.5 min. Pharmacokinetic values for harmine were determined for 14 volunteers, giving a C_{max} of 114.8 ± 61.7 ng/ml and a T_{max} of 102.0 ± 58.3 min. Levels of harmaline were already low in the beverage, thus C_{max} (6.3 ± 3.1 ng/ml) and T_{max} (145.0 ± 66.9 min) were determined for only five volunteers. Pharmacokinetic values for THH were determined for all 14 volunteers, giving a C_{max} of 91.0 ± 22.0 ng/ml and a T_{max} of 174.0 ± 39.6 min. These and other pharmacokinetic parameters of the four alkaloids are summarized in Table 2. Plasma alkaloid concentrations for harmine, THH and DMT were averaged and plotted against time for the 14 volunteers, and these data are presented in Fig. 2. None of these alkaloids were detected in blank samples collected before the ingestion of *hoasca*, nor at the zero time point. Only THH was detected, at low levels, in three of the volunteers at the 24 h time point.

3.5. Neuroendocrine effects

All measures of neuroendocrine response showed sharp increases over basal levels for each volunteer (Fig. 3). The following are reported as the mean $\pm$ SEM. After 20 min, levels of plasma growth hormone began to increase to a maximum at 90 min (9.44 ± 2.41 ng/ml), then returned to basal levels (0.54 ± 0.48 ng/ml) by 360 min (Fig. 3A). Plasma prolactin levels began to increase after 40 min, to a maximum at 120 min (33.90 ± 8.86 ng/ml), then also returned to basal levels (7.60 ± 1.26 ng/ml) by 360 min (Fig. 3B). Plasma cortisol values increased to a maximum at 60 min (132.60 ± 10.72 ng/ml), dipped below basal levels (66.80 ± 10.12 ng/ml) after 360 min, and showed a significant increase ($P < 0.05$) to 73.90 ± 11.40 ng/ml over basal values in the 24 h samples (Fig. 3C).

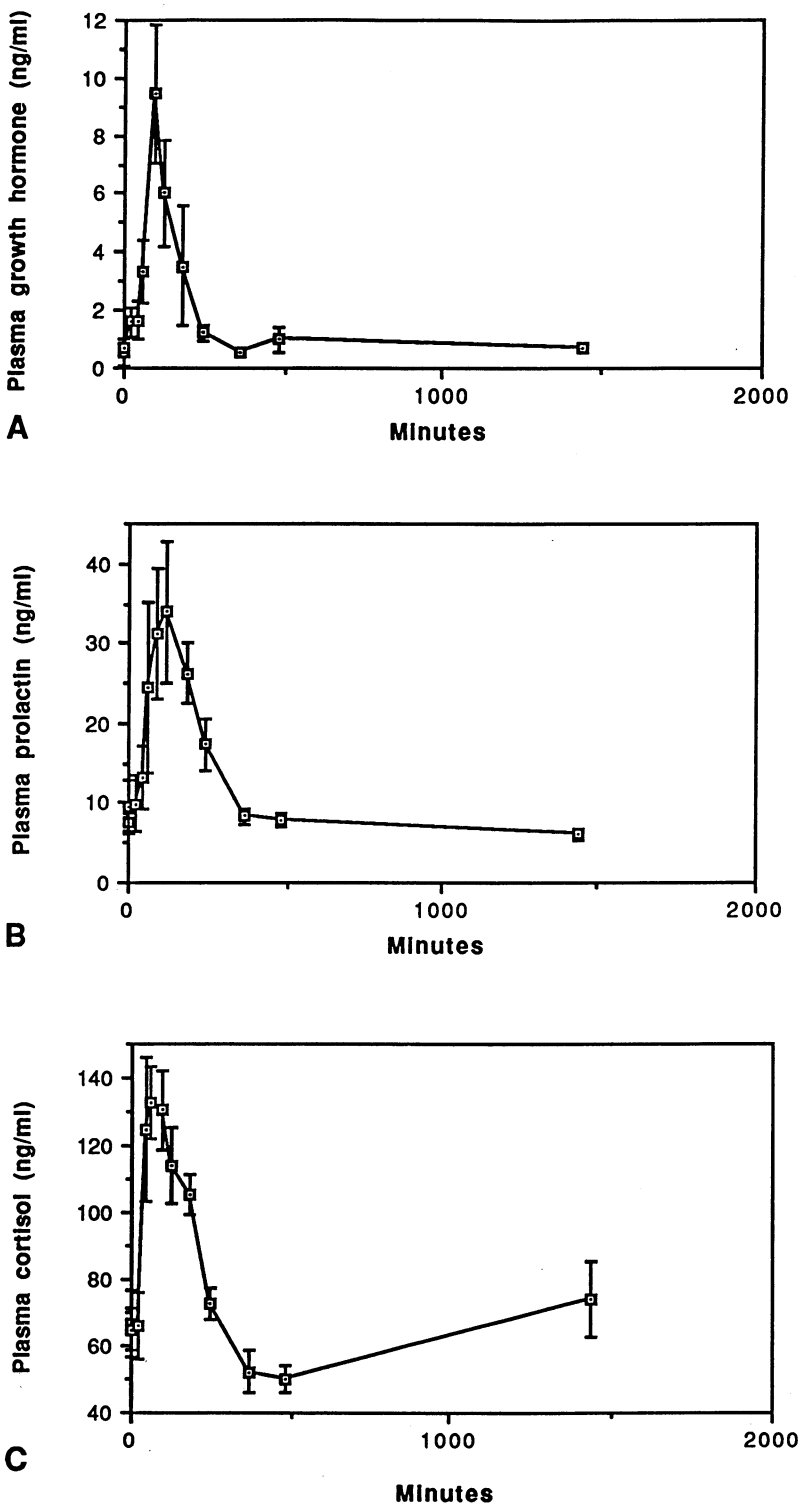


Fig. 3. Averaged values and standard errors for the following neuroendocrine values, in ng/ml plasma, are shown for the 14 volunteers after *hoasca* (2 ml/kg); growth hormone 3A, prolactin 3B, and cortisol 3C.

3.6. Autonomic effects

All measures showed increases over basal levels for each volunteer (Fig. 4). The following results are reported as the mean ± SEM. Pupillary diameter increased over basal values (3.7 ± 0.2 mm) after 40 min, to a maximum of 4.9 ± 0.2 mm at 180 min, and pupils remained dilated after the last measurement at 240 min (Fig. 4A). Pupil size typically returned to normal after approximately 6 h at this dosage (2 ml/kg). Respiration rate increased slightly over basal values (18.4 ± 0.7 breaths/min), to a maximum of 21.5 ± 1.0 at 90 min, and fluctuated throughout the study, showing an overall increase after 240 min (Fig. 4B). Oral temperature increased slightly over basal measures (37.0 ± 0.1°C), reaching a maximum of 37.3 ± 0.1°C by 240 min (Fig. 4C). It should be noted here that ambient room temperature also increased (33–38°C) throughout each day as the study sessions progressed from morning to afternoon.

3.7. Cardiovascular effects

All measures showed increases over basal levels for each volunteer. The averaged values and SEM of all 14 volunteers are illustrated in Fig. 5. The following results are reported as the mean ± SEM. Heart rate initially increased over basal values (71.9 ± 2.9 bpm) to a maximum of 79.3 ± 0.3 bpm by 20 min, decreased to a minimum of 64.5 ± 2.2 bpm by 120 min, then increased towards basal levels by 240 min. Both systolic and diastolic pressures increased to maxima after 40 min (137.3 ± 3.2 and 92.0 ± 3.0 mmHg, respectively) over basal values (126.3 ± 3.9 and 82.7 ± 2.9 mmHg, respectively), gradually returning to basal levels by 180 min. At 240 min, the systolic pressure was 123.9 ± 3.2 mmHg and the diastolic pressure was 81.1 ± 2.8 mmHg.

Table 2
Pharmacokinetic parameters (avg. ± SD) of *hoasca* alkaloids from 14 volunteers

	C_{max} (ng/ml)	T_{max} (min)	$T_{1/2}$ (min)	k_{obs} (min^{-1})	C1/F (ml/min/kg)	V_{ss}/F (l/kg)	AUC_{inf} (mg min/ml)	MRT (min)
Harmine ($n = 14$)								
Avg.	114.8	102.0	115.6	0.016	271.7	49.6	22.88	180.2
± S.D.	61.7	58.3	60.1	0.027	180.3	40.4	11.69	55.7
Harmaline								
Avg.	6.3	145.0	—	—	—	—	—	—
± S.D	3.1	66.9						
THH ($n = 14$)								
Avg.	91.0	174.0	531.9	0.003	63.3	43.5	47.78	548.9
± SD	22.0	39.6	290.8	0.002	21.9	8.0	25.88	404.2
DMT ($n = 12$)								
Avg.	15.8	107.5	259.4	0.008	221.8	54.8	5.60	357.7
± SD	4.4	32.5	207.2	0.016	129.9	14.8	4.53	271.5

Due to low concentrations, parameters for DMT could only be determined in 12 volunteers, while only C_{max} end T_{max} could be calculated for harmaline in five volunteers.
Avg, average.

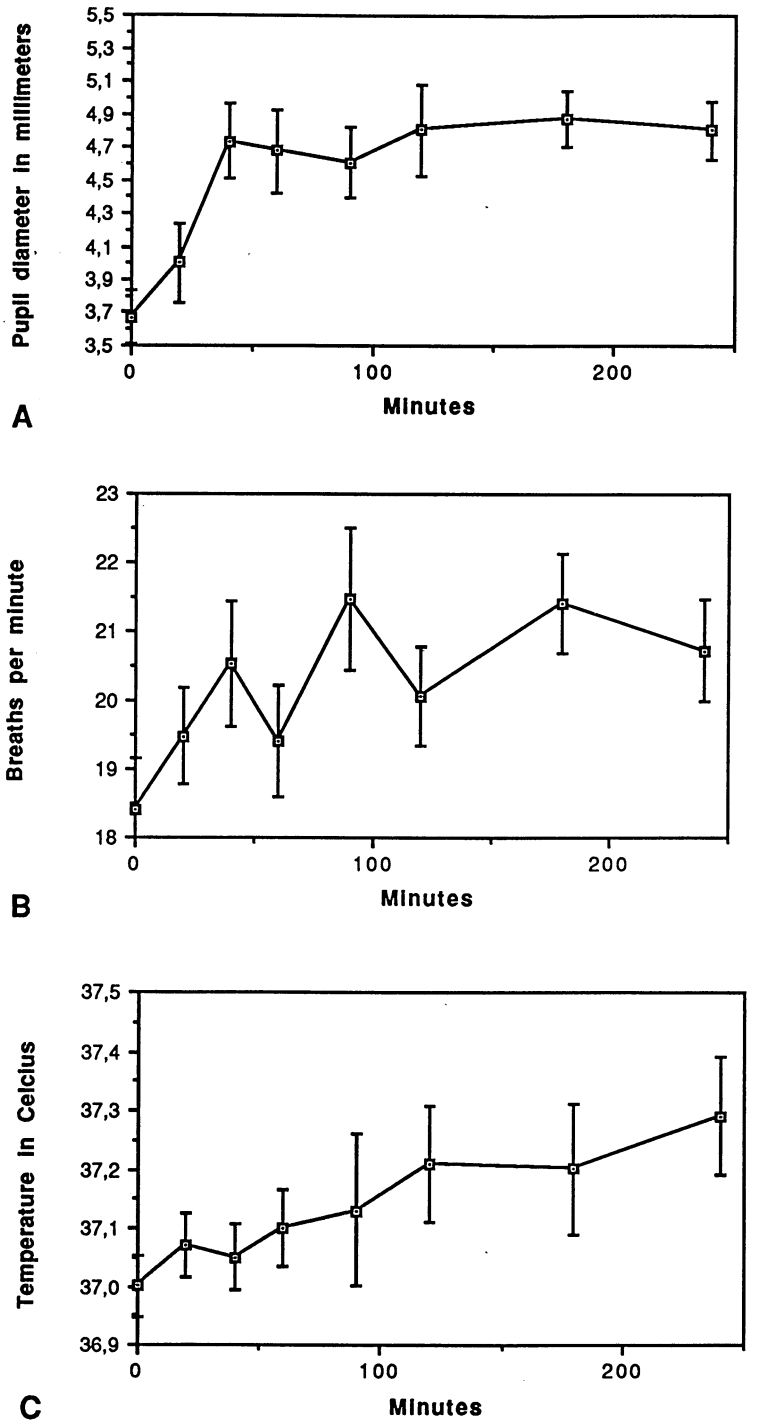


Fig. 4. Averaged values and standard errors for the following autonomic responses, after *hoasca* (2 ml/kg), are shown for the 14 volunteers; pupillary diameter 4A, respiration rate 4B, and oral temperature 4C.

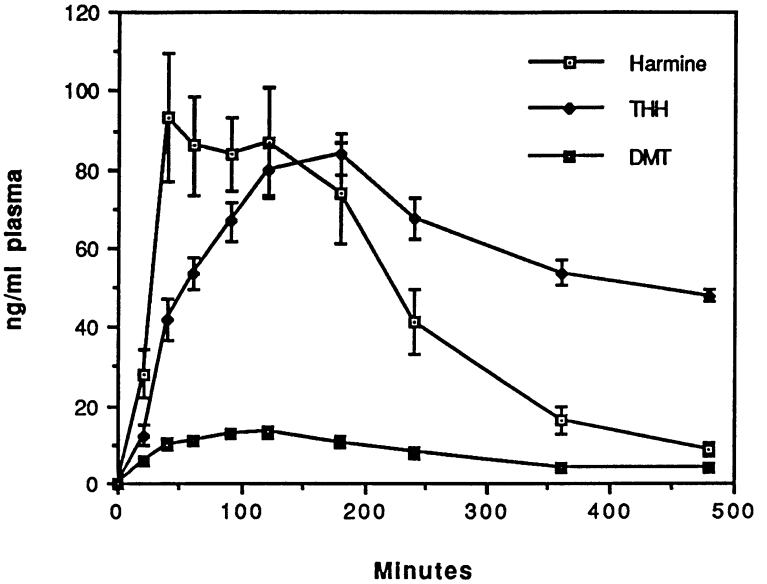


Fig. 2. Averaged values and standard errors of harmine, THH and DMT concentrations, in ng/ml plasma, are shown for the 14 volunteers after *hoasca* (2 ml/kg).

4. Discussion

The purpose of this study was to examine the pharmacokinetic effects of *hoasca* in healthy humans. The alkaloid content of the *hoasca* used in this study are in agreement with published reports of tea dosages from other sources (McKenna et al., 1984a; Liwszyc et al., 1992; Casale and Koles, 1995; Don et al., 1998), and the beverage itself was considered typical of *hoasca* by experienced volunteers. While the dosage of *hoasca* in this study was considered mild, by comparison, the plasma alkaloid levels were sufficient for analytical detection, and larger amounts of the tea could have increased the risk of nausea and vomiting. The *hoasca*-naive agematched control group was not included in the acute pharmacokinetic study. The control group was only used in this study to provide a standard baseline measure of health, and a measure of platelet uptake site density in a *hoasca*-naive population of the region (Callaway et al., 1994).

Changes in DMT pharmacokinetic profiles were reflected in autonomic and neuroendocrine responses, and subjective effects, as previously reported (Strassman and Qualls, 1994; Strassman et al., 1994). The intensity and duration of subjective effects between *hoasca* versus intravenous DMT, however, differed considerably. In the present study, the most intense visionary effects were reported to occur between 60 and 120 min after ingesting the tea, which corresponds with the average T_{max} for DMT (Table 2). Moreover, the results from our psychological inventory with *hoasca* use (Grob et al., 1996) indicate qualitative differences between comparable levels of injected DMT, where the onset of maximal effect tended to be more rapid, singular in effect, and of shorter duration (Strassman et al., 1994). The quantitative difference is obviously due to the inherent differences in routes of administration; i.e. intravenous versus orally activated DMT. The qualitative differences can be explained by the suggestion that the visionary effects of DMT manifest through interactions at central serotonin receptor sites (Deliganis et al., 1991), where subjective effects are modified by increased levels of 5-HT, which provides competition for DMT at these sites.

The purgative effects of *hoasca* are considered to be tonic, rather than toxic, according to those who use this beverage with regularity. Variable

degrees of nausea, vomiting, and occasionally simultaneous diarrhea, are not uncommon. These effects vary according to the individual, dosage, and alkaloid composition of the tea. They are probably symptomatic of the increasing levels of unmetabolized 5-HT throughout the acute phase of this experience, which is a consequence of MAO-A inhibition by both harmine and harmaline (Buckholtz and Boggan, 1977). Vomiting, for example, results from increased vagal stimulation by central 5-HT, and increased peripheral 5-HT can stimulate intestinal motility to the point of diarrhea. A fine transient tremor and nystagmus were also observed in some cases. This may be due to receptor mediated interactions of harmala alkaloids on tryptamine binding receptors (Rommelspacher and Bruning, 1984; Airaksinen et al., 1987).

Increased heart rate and blood pressure may be due to unmetabolized catecholamines after MAO inhibition, where increasing levels of central 5-HT later attenuate this effect by decreasing cardiac response through vagal stimulation (Udenfriend et al., 1958). Similar modifications in cardiac performance have already been reported in humans and other animals for both harmine and harmaline (Goldberg and Sjoerdsma, 1959; Sjoerdsma et al., 1959; Pletscher et al., 1960). While increases in cardiac responses were remarkable, they were not hypertensive. Four individuals presented heart rates less than 60 bpm at 120 min after *hoasca* ingestion (59, 59, 58 and 52 bpm), where the two lowest measures had basal heart rates below the group average (65 and 62 bpm, respectively).

With the regular use of *hoasca*, subsequent periodic increases in levels of 5-HT may signal a compensatory upregulation of 5-HT uptake sites on blood platelets (Callaway et al., 1994). Since none of the volunteers showed signs of active or current depression (Grob et al., 1996), which might be expected from a net lack of synaptic 5-HT activity through its increased uptake, it is conceivable that such an upregulation could actually stimulate 5-HT production to fill these receptor sites during the times between *hoasca* sessions.

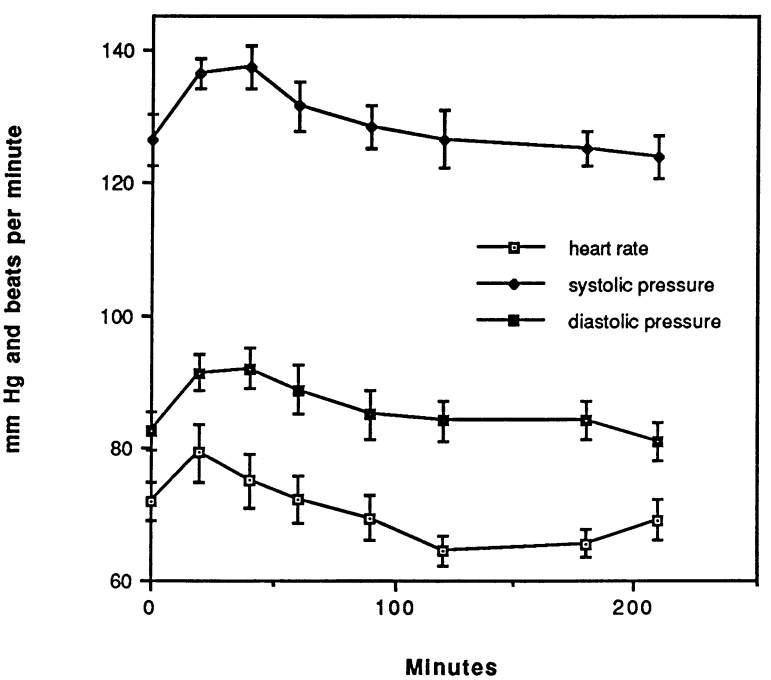


Fig. 5. Averaged values and standard errors of heart rate, systolic and diastolic pressures, after *hoasca* (2 ml/kg), are shown for the 14 volunteers. Heart rate, in beats per min (bpm) and pressure in mmHg share the same numeric scale on the vertical axis.

The mechanism of action for *hoasca* and analogous beverages apparently begins with the inhibition of MAO by harmine and, to a lesser extent, harmaline. This action allows for the oral activity of DMT, a mechanism suggested over 30 years ago (Holmstedt and Lindgren, 1967). Where the T_{max} of injected DMT (0.4 mg/kg) was only about 2 min with a resulting C_{max} of 15.8 ng/ml (Strassman and Qualls, 1994), gastrointestinal absorption and subsequent MAO inhibition lengthened T_{max} to 108 ± 32.5 min and increased C_{max} to 90.0 ng/ml at a comparable oral dose of DMT from 2 ml/kg *hoasca* (i.e. 0.48 mg DMT/kg). Ordinarily, DMT is not orally active, even at 25 times the oral dosage used in the present study (Ott, 1994).

The oral activity of DMT in *hoasca* is apparently facilitated by the presence of harmala alkaloids. This has been tested in man (Ott, 1994) by achieving *hoasca*-like psychoactivity through the simultaneous oral ingestion of pure harmine (1.5 mg/kg) with DMT (0.44 mg/kg). The same has been demonstrated for 5-methoxy-DMT (Callaway, 1993), which is also orally inactive. An earlier study using iproniazid, a non-specific MAO inhibitor, had already demonstrated that DMT is primarily metabolized by MAO (Barker et al., 1980). As the harmala alkaloids are known to preferentially inhibit MAO-A (Buckholtz and Boggan, 1977), it follows that DMT would be the preferred substrate for this particular isozyme (i.e. MAO-A). However, one report has suggested that DMT is preferentially metabolized by MAO-B (Suzuki et al., 1981), while 5-methoxy-DMT is preferentially metabolized by MAO-A (Squires, 1975). A metabolic study on DMT showed this alkaloid to be rapidly metabolized in the blood to dimethylkynuramine by an unknown enzymatic reaction (Hryhorczuk et al., 1986). Moreover, it is also conceivable that increasing levels of 5-HT could compete with DMT for any of these reactions, and effectively slow its eventual metabolism in that way.

The EC_{50} for the inhibition of MAO-A has been reported to be 8×10^{8} M for harmine, 6×10^{-8} M for harmaline and 1.4×10^{-5} M for THH, and at higher concentrations both harmine and harmaline begin to inhibit MAO-B (Pletscher et al., 1960; Buckholtz and Boggan, 1977). In the present study, plasma concentrations of harmine alone were several orders of magnitude greater than its reported EC_{50}. By considering the high concentrations of harmala alkaloids in *hoasca* that are typically ingested, it could be argued that this amount is aufficient to inhibit both isozymes of MAO.

Due to its weak affinity, and in the presence of high harmine concentrations, THH may not play a significant role in the inhibition of MAO. Instead, THH may contribute psychoactivity indirectly by inhibiting the uptake of 5-HT in platelets and presynaptic neurons (Airaksinen et al., 1980), further increasing extracellular 5-HT levels over those seen from MAO inhibition alone, as significant amounts of this alkaloid are known to occur in *B. caapi* (Rivier and Lindgren 1972; Callaway et al., 1996). The pharmacokinetic profile of THH (Fig. 2) and related parameters (Table 2) also suggests some independence from interactions between harmine and MAO. It is possible that the activity of THH may even be potentiated by MAO inhibition.

All changes in neuroendocrine responses correlated with subjective effects. Growth hormone and prolactin are under the influence of the serotonergic system, and serve as indicators of increased serotonergic action (Van de Karr, 1991). The neuroendocrine challenge by *hoasca* provides information on the functionality of the serotonergic system. Increased cortisol and prolactin levels were comparable to previously reported values after injected DMT, although the action of MAO inhibition seems to have prolonged the time response by a factor of 4–5 in the present study. Increased levels of growth hormone followed, as well, which was also seen after injected DMT (Strassman and Qualls, 1994). The increased levels of prolactin and growth hormone that were observed in the present study probably reflect increased activation of 5-HT receptors, again through increased levels of 5-HT (Scheinin et al., 1990). Increased cortisol levels also follow this sudden surge in neurochemical activity.

Increased pupillary diameter, oral temperature and cardiac effects were also reported earlier for 0.4 and 0.2 mg/kg i.v. doses of DMT (Strassman

and Qualls, 1994), but these effects were of shorter duration than the increases seen in the present study.

5. Conclusions

A long and continuous history of regular use indicates the utility of *hoasca*. Signs of physical or psychological deterioration were not observed as a consequence of its use. Instead, the regular use of *hoasca* in a ceremonial context seems to increase one's ability to psychologically adapt to the larger process of life (Grob et al., 1996).

By investigating human reactions to psychotropic agents, we begin to bridge the gap between neurochemistry and cognition. The clinical and pharmacokinetic data obtained from this prospective study provide some direction for further investigations into the complex psychopharmacology of *hoasca*, and related substances, in healthy human volunteers. Although preliminary in nature, the results from this study suggest that such neurochemical agents are powerful tools that can enable a more comprehensive study of the mind.

Acknowledgements

This study was made possible by ground support from members of the União do Vegetal (UDV) and the Instituto Nacional de Pesquisas da Amazônia (INPA). Funding was provided by The Heffter Institute, Botanical Dimensions, the Aurora Institute, the Multidisciplinary Association for Psychedelic Studies (MAPS) and The Brain Research Fund, University of Miami School of Medicine.

References

Airaksinen, M.M., Svensk, H., Tuomisto, J., Komulainen, H., 1980. Tetrahydro-β-carbolines and corresponding tryptamines: in vivo inhibition of serotonin and dopamine uptake by human blood platelets. Acta Pharmacologia et Toxicologia 46, 308–313.

Airaksinen, M.M., Lecklin, A., Saano, V., Tuomisto, L., Gynther, J., 1987. Tremorigenic effects and inhibition of tryptamines and serotonin receptor binding by β-carbolines. Pharmacology and Toxicology 60, 5–8.

Barker, S.A, Monti, J.A., Christian, S.T., 1980. Metabolism of the hallucinogen *N*,*N*-dimethyltryptamine in rat brain homogenates. Biochemical Pharmacology 29, 1049–1057.

Buckholtz, N.S., Boggan, W.O., 1977. Monoamine oxidase inhibition in brain and liver by β-carbolines: structure-activity relationships and substrate specificity. Biochemical Pharmacology 26, 1991–1996.

Callaway, J.C., 1993. Tryptamines, β-carbolines and you. MAPS Newsletter of the Multidisciplinary Association of Psychedelic Studies 4 (2), 30–32.

Callaway, J.C., Airaksinen, M.M., McKenna, D.J., Brito, G.S., Grob, C.S., 1994. Platelet serotonin uptake sites increased in drinkers of *ayahuasca*. Psychopharmacology 116, 385–387.

Callaway, J.C., Raymon, L.P., Hearn, W.L., McKenna, D.J., Grob, C.S., Brito, G.S., 1996. Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with *Ayahuasca*. Journal of Analytical Toxicology 20, 492–497.

Casale, J.F., Koles, J.E., 1995. Analysis of *ayahuasca* ('Santo Daime [*sic*]'). Microgram 28 (9), 296–299.

Deliganis, A.V., Pierce, P.A., Peroutka, S.J., 1991. Differential interactions of dimethyltryptamine (DMT) with 5-HT1a and 5-HT2 receptors. Biochemical Pharmacology 41 (11), 1739–1744.

Don, N.S., McDonough, B.E., Moura, G., Warren, C.A., Kawanishi, K., Tomita, H., Tachibana, Y., Bohlke, M., Farnsworth, N.R., 1998. Effects of *ayahuasca* on the human EEG. Phytomedicine 5 (2), 87–96.

Goldberg, L.I., Sjoerdsma, A., 1959. Effects of several monoamine oxidase inhibitors on the cardiovascular actions of naturally occurring amines in the dog. Journal of Pharmacology and Experimental Therapeutics 127, 212–218.

Grob, C.S., McKenna, D.J., Callaway, J.C., Brito, G.S., Neves, E.S., Oberlander, G., Saide, O.L., Labigalini, E., Tacla, C., Miranda, C.T., Strassman, R.J., Boone, K.B., 1996. Human psychopharmacology of Hoasca, a plant hallucinogen used in ritual context in Brasil. Journal of Nervous and Mental Disorder 184 (2), 8694.

Hochstein, F.A., Paradies, A.M., 1957. Alkaloids of *Banisteria caapi* and *Prestonia amazonicum* [*sic*]. Journal of the American Chemical Society 79, 5735–5736.

Holmstedt, B.R., Lindgren, J.-E., 1967. Chemical constituents and pharmacology of South American snuffs. In: Efron, D.H., Holmstedt, B., Kline, N.S. (Eds.), Ethnopharmacologic Search for Psychoactive Drugs (No. 1645). US Public Health Service, Washington, DC, pp. 339–373.

Hryhorczuk, L.M., Rainey, J.M., Froham, C.E., Novak, E.A., 1986. A new metabolic pathway for *N*,*N*-dimethyltryptamine. Biological Psychiatry 21, 84–93.

Liwszyc, G.E., Vuori, E., Rasanen, I., Issakainen, J., 1992. Daime—a ritual herbal potion. Journal of Ethnopharmacology 36 (1), 91–92.

Luna, L.E., Amaringo, P., 1991. Ayahuasca Visions: The Religious Iconography of a Peruvian Shaman. North Atlantic Books, Berkeley.

Luna, L.E., 1997. Personal communication.

McKenna, D.J., Towers, G.H.N., 1984. Biochemistry and pharmacology of tryptamines and β-carbolines: A mini review. Journal of Psychoactive Drugs 16 (4), 347–358.

McKenna, D.J., Towers, G.H.N., Abbot, F., 1984a. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of *ayahuasca*. Journal of Ethnopharmacology 10 (2), 195–223.

McKenna, D.J., Towers, G.H.N., Abbot, F., 1984b. Monoamine oxidase inhibitors in South American hallucinogenic plants. Part 2: constituents of orally-active myristicaceous hallucinogens. Journal of Ethnopharmacology 12 (2), 179–211.

McKenna, D.J., Repke, D.B., Lo, L., Peroutka, S.J., 1990. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. Neuropharmacology 29, 193–198.

Naranjo, C., 1967. Psychotropic properties of the harmala alkaloids. In: Efron, D.H., Holmstedt, B., Kline, N.S. (Eds.), Ethnopharmacologic Search for Psychoactive Drugs (No. 1645). US Public Heatlh Service, Washington, DC, pp. 385–391.

Odell, W.D., Rayford, P.L., Ross, G.T., 1967. Simplified, partially automated method for radioimmunoassay of human thyroid-stimulating, growth, luteninizing, and follicle stimulating hormones. Journal of Laboratory and Clinical Medicine 70, 937–980.

Ott, J., 1994. *Ayahuasca* Analogues: Pangæn Entheogens. Natural Products Co., Kennewick, WA.

Pinkley, H.V., 1969. Plant mixtures of Ayahuasca, the South American hallucinogenic drink. Lloydia 32, 305–314.

Pletscher, V.A., Gey, K.F., Zeller, P., 1960. Monoamineoxydase-hemmer: chemie, biochemie, pharmakologie, klinik. Progress in Drug Research 2, 417–590.

Poland, R.E., Rubin, R.T., 1981. Radioimmunoassay of haloperidol in human serum: correlation of serum haloperidol with serum prolactin. Life Sciences 29, 1837–1845.

Poland, R.E., Rubin, R.T., 1982. Saliva cortisol levels following dexamethasone administration in endogenously depressed patients. Life Sciences 30, 177–181.

Rivier, L., Lindgren, J.E., 1972. Ayahuasca, the South American hallucinogenic drink, an ethnobotanical and chemical investigation. Economic Botany 26, 101–109.

Rivier, L., Holmstedt, B., 1982. Ethnopharmacology of ayahuasca: recent observations and new perspectives. In: Laite, J. (Ed.), 44th International Congress of Americanists, Abstracts of Papers. Manchester University, Manchester, p. 290.

Rommelspacher, H., Bruning, G., 1984. Formation and function of tetrahydro-β-carbolines with special reference to their action on [^{3}H]tryptamine binding sites. In: Schloßberger, H.G., Kochen, H., Linzen, A., Steinhart, R. (Eds.), Tryptophan. DeGryter, Berlin.

Scheinin, M., Koulu, M., Karhuvaara, S., Zimmer, R.H., 1990. Evidence that the reversible MAO-A inhibitor moclobemide increases prolactin secretion by a serotonergic mechanism in healthy volunteers. Life Science 47, 1491–1499.

Schultes, R.E., 1957. The identity of malpighiaceous narcotics of South America. Botanical Museum Leaflets. Harvard University 18, 1–56.

Schultes, R.E., Hofmann, A., 1992. Plants of the Gods: Their Sacred, Healing and Hallucinogenic Powers. Healing Arts Press, Rochester, VT.

Schultes, R.E., Raffauf, R.F., 1992. Vine of the Soul: Medicine Men, Their Plants and Rituals in the Columbian Amazon. Synergistic Press, Oracle, AZ.

Sjoerdsma, A., Gillespie, L., Udenfriend, S., 1959. A method for measurement of monoamine oxidase inhibition in man: Application to studies in hypertension. Annals of the New York Academy of Sciences 80, 969–980.

Spruce, R., 1908. Notes of a Botanist on the Amazon and Andes (two volumes). Wallace, A.R., (Ed.). Macmillian, London. Reprinted by Johnson Reprint, New York, 1970.

Squires, R.F., 1975. Evidence that 5-methoxy-*N*,*N*-dimethyltryptamine is a specific substrate for MAO-A in the rat: implications for the indoleamine dependent behavioural syndrome. J Neurochem. 24, 47–50.

Strassman, R.J., Qualls, C.R., 1994. Dose-response study of *N*,*N*-dimethyltryptamine in humans I. neuroendocrine, autonomic, and cardiovascular effects. Archives of General Psychiatry 51, 85–97.

Strassman, R.J., Qualls, C.R., Uhlenhuth, E.H., Kellner, R., 1994. Dose-response study of *N*,*N*-dimethyltryptamine in humans II. Subjective effects and preliminary results of a new rating scale. Archives of General Psychiatry 51, 98–108.

Suzuki, O., Katsumata, Y., Oya, M., 1981. Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. Biochemical Pharmacology 30 (1), 1353–1358.

Szára, S., 1956. Dimethyltryptamin [*sic*]: its metabolism in man; the relation of its psychotic effect on serotonin metabolism. Experentia 12, 441–442.

Udenfriend, S., Witkop, B., Redfield, B., Weissbach, H., 1958. Studies with the reversible inhibitors of monamine oxidase: harmaline and related compounds. Biochemical Pharmacology 1, 160–165.

Van de Karr, L.D., 1991. Neuroendocrine pharmacology and serotonergic neurons. Annual Review of Pharmacology and Toxicology 31, 289–320.

Psychopharmacology (2001) 154:85–95
DOI 10.1007/s002130000606

ORIGINAL INVESTIGATION

**Jordi Riba · Antoni Rodríguez-Fornells
Gloria Urbano · Adelaida Morte · Rosa Antonijoan
Maria Montero · James C. Callaway
Manel J. Barbanoj**

Subjective effects and tolerability of the South American psychoactive beverage *Ayahuasca* in healthy volunteers

Received: 4 July 2000 / Accepted: 3 October 2000 / Published online: 14 December 2000

Abstract *Rationale: Ayahuasca* is a South American psychoactive beverage that contains the naturally occurring psychedelic agent *N*,*N*-dimethyltryptamine (DMT). This "tea" has been used for centuries in religious and medicinal contexts in the rain forest areas of South America and is presently gaining the attention of psychedelic users in North America and Europe. *Objectives:* In the present study, the psychological effects and tolerability of *ayahuasca* were assessed. *Methods:* Three increasing doses of encapsulated freeze-dried *ayahuasca* (0.5, 0.75, and 1.0 mg DMT/kg body weight) were administered to six healthy male volunteers with prior experience in the use of this tea, in a single-blind crossover placebo-controlled clinical trial. *Results: Ayahuasca* produced significant dose-dependent increases in five of the six subscales of the Hallucinogen Rating Scale, in the LSD, MBG, and A scales of the Addiction Research Center Inventory, and in the "liking", "good effects" and "high" visual analogue scales. Psychological effects were first noted after 30–60 min, peaked between 60–120 min, and were resolved by 240 min. The tea was well tolerated from a cardiovascular point of view, with a trend toward increase for systolic blood pressure. Modified physical sensations and nausea were the most frequently reported somatic-dysphoric effects. The overall experience was regarded as pleasant and satisfactory by five of the six volunteers, while one volunteer experienced an intensely dysphoric reaction with transient disorientation and anxiety at the medium dose and voluntarily withdrew from the study. *Conclusions: Ayahuasca* can be described as inducing changes in the perceptual, affective, cognitive, and somatic spheres, with a combination of stimulatory and visual psychoactive effects of longer duration and milder intensity than those previously reported for intravenously administered DMT.

Keywords *Ayahuasca* · DMT · Subjective effect · Tolerability · Human

J. Riba · G. Urbano · A. Morte · R. Antonijoan · M.J. Barbanoj (✉)
Àrea d'Investigació Farmacològica, Institut de Recerca,
Hospital de la Santa Creu i Sant Pau (HSCSP),
St. Antoni Maria Claret, 167, 08025 Barcelona, Spain
e-mail: mbarbano@santpau.es
Tel.: +34-93-2919019, Fax: +34-93-2919286

J. Riba · G. Urbano · A. Morte · R. Antonijoan · M.J. Barbanoj
Departament de Farmacologia i Terapèutica,
Universitat Autònoma de Barcelona, Barcelona, Spain

A. Rodríguez-Fornells
Medizinische Hochschule Hannover, Hannover, Germany

M. Montero
Servei de Psiquiatria, Hospital de la Santa Creu i Sant Pau (HSCSP),
Barcelona, Spain

J.C. Callaway
Department of Pharmaceutical Chemistry, University of Kuopio,
Kuopio, Finland

Introduction

Ayahuasca, a potent psychotropic drink that has been used for centuries for magico-religious purposes and folk medicine in the Amazon and Orinoco river basins (Dobkin de Ríos 1972; Schultes and Hofmann 1982), is becoming increasingly popular in Europe and North America as a sacramental drug (Metzner 1999). In recent years, the use of *ayahuasca* has spread outside South America, and several groups using this tea have become established in Spain and other European countries (Marshall 1997; López 1999), where the tea is reportedly used to facilitate self-knowledge and introspection. A relevant facet in expanding *ayahuasca* use can be attributed to the growing interest of the many individuals who are interested in shamanic practices, in addition to the activities of a number of Brazilian syncretic religions, particularly the *Santo Daime* and the *União do Vegetal*, that have combined Old World religious beliefs with the indigenous use of *ayahuasca*. Because this tea contains measurable amounts of *N*,*N*-dimethyltryptamine (DMT), the *ayahuasca* churches are actively working to obtain legal exemption for *ayahuasca* use within a religious context outside Brazil, the only country where it currently enjoys legal protection, analogous to the status held

by the Native American Church for the use of *peyote* (*Lophophora williamsii*, a mescaline-containing cactus) in the United States. Even though the number of users is still relatively small outside of Brazil, *ayahuasca* use has raised concerns for public health (Callaway and Grob 1998), and extensive clinical data on its somatic, psychological, and neurophysiological effects are indicated.

Ayahuasca, also known as *Daime* or *Hoasca* in Brazil, *Yajé* in Colombia, or *Natem* in Ecuador, is generally obtained by infusing the shredded stalk of the malpighiaceous vine *Banisteriopsis caapi* with the leaves of *Psychotria viridis* (Rubiaceae) or *Diplopterys cabrerana* (Malpighiaceae). *B. caapi* contributes a mixture of β-carboline alkaloids to the tea, particularly harmine, tetrahydroharmine (THH), and trace amounts of harmaline (Rivier and Lindgren 1972). *P. viridis* and *D. cabrerana* are rich in the psychedelic indole DMT (River and Lindgren 1972; Schultes and Hofmann 1980; Callaway et al. 1996).

DMT, the main psychotropic agent of *ayahuasca*, is capable of eliciting an intensely emotional dream-like experience characterized by vivid visual imagery, perceptual and cognitive changes, and profound modifications in the sense of self and reality, when administered parenterally (Strassman et al. 1994). On the molecular level, DMT has affinity for 5-HT_2 and 5-HT_{1A} binding sites, similarly to LSD (Pierce and Peroutka 1989; Deliganis et al. 1991), and is structurally similar to serotonin. Interestingly, DMT is known for its lack of psychoactivity when orally ingested, even in quantities in the order of grams (Ott 1999), due to metabolism by monoamine oxidase (MAO; Suzuki et al. 1981). The β-carbolines present in *ayahuasca*, particularly harmine and harmaline, have been found to inhibit MAO (McKenna et al. 1984), an effect that apparently allows the viable access of DMT to the systemic circulation and the central nervous system. In addition to the action of DMT on serotonin receptors, it has also been suggested that *ayahuasca*'s psychoactive effects may also be partly due to a general increase of catecholamines and serotonin (Callaway et al. 1999). This increase would be due to both the inhibited metabolic breakdown of serotonin in addition to its uptake inhibition by THH and also competition with DMT for receptor sites (Callaway et al. 1999). Thus, *ayahuasca* constitutes a very complex psychoactive preparation, acting at least through three different pharmacologic mechanisms.

In the present paper we report a single-blind placebo-controlled clinical trial conducted with *ayahuasca*, in which the subjective effects and tolerability of three different doses of *ayahuasca* were evaluated in healthy volunteers. This study is part of a wider research project designed to further characterize the pharmacologic effects of this tea.

Materials and methods

Volunteers

For ethical reasons, participation in this initial study was limited to six healthy male volunteers having previous experience with *ayahuasca*. Volunteers were contacted by word of mouth in the Barcelona area of Spain, and all had previous exposure to the "tea", but had no formal connections to any *ayahuasca* church. The volunteers were given a structured psychiatric interview (DSM-III-R) and completed the trait-anxiety scale from the state-trait anxiety inventory (Spielberger et al. 1970). Exclusion criteria included a present or past history of axis-I disorders and alcohol or other substance dependence, and high scores on trait anxiety. Following the psychiatric interview, participants underwent a complete physical examination that included a medical history, laboratory tests, ECG, and urinalysis. Mean age was 32.2 years (range: 26–44), mean weight 71.5 kg (range: 66–85), and mean height 174.3 cm (range 167–186). All volunteers had previous experience with cannabis, cocaine, psychedelics, and other illicit substances. Regarding their prior experience specifically with *ayahuasca*, volunteers 1 and 2 had previously consumed it on 10 occasions, volunteer 3 on about 60 occasions, volunteer 4 on 2 occasions, volunteer 5 on 6 occasions, and volunteer 6 on 30 occasions. The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans, and was approved by the hospital's ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of *ayahuasca*, the general psychological effects of psychedelics, and their possible adverse effects, as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drug

A 9.6 litre batch of *ayahuasca* (*Daime*) was obtained from CEFLURIS, a Brazilian-based religious organization related to the *Santo Daime* church. The tea had the appearance of a brown-reddish suspension with a characteristic bitter-sour taste and smell, and a markedly acidic pH (3.63). In order to mask the drug in the single-blind design and establish accurate dosings, the tea underwent a freeze-drying process that yielded 611 g of a yellowish powder, which was subsequently homogenized and analyzed for alkaloid contents by an HPLC method previously described in the literature (Callaway et al. 1996). One gram of freeze-dried material contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg THH. Thus, the alkaloid concentrations in the original tea were as follows: DMT 0.53 mg/ml, harmine 0.90 mg/ml, harmaline 0.06 mg/ml, and THH 0.72 mg/ml. The DMT concentration found in the tea was similar to that reported previously for a sample of *Daime* (Liwszyc et al. 1992) and several Peruvian *ayahuasca* samples (McKenna et al. 1984), and twice as great as the amount reported for a sample of *Hoasca* from the Brazilian church *União do Vegetal* (Callaway et al. 1996). Similarly, the β-carboline concentrations found in the *ayahuasca* used in the present study were also higher than those reported in the previously mentioned samples. In view of the mild psychological effects reported from the 0.48 mg DMT/kg body weight dosage (Grob et al. 1996), and considering the total amounts of DMT consumed in what have been reported as typical doses (McKenna et al. 1984; Liwszyc et al. 1992), the following experimental doses were chosen for the present study: 0.5 mg DMT/kg body weight as the low dose and 0.75 and 1.0 mg DMT/kg body weight as the medium and high dose, respectively. The freeze-dried material was encapsulated in 00 gelatin capsules containing 0.5, 0.25, or 0.125 g, and stored at –20°C under nitrogen atmosphere and protected from light until administered to the volunteers. Placebo capsules consisted of 00 gelatin capsules with 0.75 g lactose. Each volunteer received his calculated individual dose by combination of these capsules. Placebo capsules were added when necessary, so that all volunteers received 20 capsules on each experimental day.

Study design and experimental procedure

The study was carried out in a single-blind fashion. Volunteers were informed that they would receive a single oral dose of encapsulated freeze-dried *ayahuasca* (one low, one medium, and one high dose) or placebo on each of 4 experimental days. In order to avoid subjective effects related to expectancy, the volunteers were also informed that administrations would be made in a double-blind balanced fashion. For security reasons, they were actually administered in increasing doses, i.e., placebo for the first session, the low dose containing 0.5 mg DMT/kg for the second session, the medium dose containing 0.75 mg DMT/kg for the third session, and the high dose containing 1.0 mg DMT/kg for the fourth and final session, in order to control for tolerability and the possible risk in elevations of cardiovascular parameters. Two weeks prior to the beginning of the experimental sessions, volunteers abstained from any medication or illicit drug and remained drug-free throughout the 4 study weeks. Urinalysis for illicit drug use was carried out for each experimental session. Additionally, volunteers abstained from alcohol, tobacco, and caffeinated drinks 24 h prior to each experimental day. Experimental days were a week apart.

The volunteers were admitted to the research unit on 4 separate experimental days. Upon arrival at 8:00 a.m. under fasting conditions, a urine sample was collected, a cannula was inserted in the cubital vein of their right arm for drawing blood samples, and capsules were administered by approximately 9:00 a.m. with 250 ml tap water. Throughout the experimental session the volunteers remained seated in a comfortable reclining chair in a quiet and dimly lit room. The experimenter remained beside the volunteer for most of the time, and no music was used during the sessions. Four hours after administration of the capsules, the volunteers left the room, answered subjective effect questionnaires, were able to have a light meal if they wished to, and were discharged after 5 h from the administration.

Measurements

Besides the measures described below, spontaneous verbally reported effects were also recorded. Additionally, blood samples were drawn at set time points in order to establish the alkaloids' pharmacokinetic profiles (not reported here). The time points selected for the measurements described below were based on field observations of duration of *ayahuasca* effects, and on the published pharmacokinetic and pharmacodynamic data by Callaway et al. (1999).

Psychological measures

The psychological effects elicited by *ayahuasca* were measured by means of visual analogue scales (VAS) and self-report questionnaires. VAS were 100-mm horizontal lines with the following labels: "any effect" indicated any effect, either physical or psychological, that the volunteer attributed to the administered dosage; "good effects" indicated any effect the volunteer valued as good; "liking" reflecting that the volunteer liked the effects of the administered substance; "drunken" indicating any dizziness or lightheadedness; "stimulated" indicating any increases in thought speed and/or content, or any increases in associations and/or insights; and "high" which reflected any positive psychological effect the volunteer attributed to the treatment. The volunteers were requested to answer the VAS immediately before administration and at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after administration.

Self-report questionnaires included Spanish adaptations of the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI). The HRS version, which had been previously translated from English and validated in Spanish (Riba et al. 2000), includes six subscales: "somaesthesia", reflecting somatic effects; "affect", sensitive to emotional and affective responses; "volition", indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; "cognition", describing modifications in thought processes or content; "perception", measuring visual, auditory, gustatory, and olfactory experiences; and finally "intensity", which reflects the strength of the overall experience (Strassman et al. 1994). The ARCI (Lamas et al. 1994) consists of five scales or groups: morphine-benzedrine group (MBG), measuring euphoria; pentobarbital-chlorpromazine-alcohol group (PCAG), measuring sedation; lysergic acid diethylamide scale (LSD), measuring somatic-dysphoric effects; and the benzedrine group (BG) and the A scale, for amphetamine, both sensitive to stimulants. The volunteers answered the ARCI immediately before drug administration and, 4 h after drug intake, they again answered the ARCI and the HRS.

Tolerability measures

Cardiovascular variables were recorded by means of a sphygmomanometer cuff (Dinamap Critikon, Tampa, Fla., USA) which was placed around the volunteer's left arm. Blood pressure [systolic (SBP) and diastolic (DBP)] and heart rate were measured immediately before administration (baseline) and at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after intake. Somatic-dysphoric effects were recorded by means of the questionnaires previously mentioned, and as spontaneous verbal reports. Finally, after each experimental session, a blood sample was taken for laboratory testing, which included blood cell counts, plasma bilirubin, creatinine, and liver enzymes.

Statistical analysis

Values from cardiovascular measures and ARCI scores were transformed to differences from baseline and differences from preadministration scores, respectively. Transformed values, HRS scores, and mean values obtained across time points for a given treatment (i.e., cardiovascular and VAS data) were analyzed by means of a non-parametric Friedman test. When a significant effect was observed, *post hoc* comparisons were performed using the Wilcoxon test. In all tests performed, differences were considered statistically significant for P values lower than 0.05.

Results

Psychological effects

Results for the statistical analyses performed on all subjective effect variables are presented in Table 1. A significant effect of treatment was observed for all seven VAS items, all HRS subscales except "volition", and the A, MBG, and LSD scales of the ARCI. The 0.5 mg DMT/kg body weight dosage chosen in the present study as the lower dose proved to be psychoactive in five of the six volunteers and subthreshold for the sixth volunteer, who mistook it for the placebo. At this dose, the Wilcoxon test showed significant effects for all VAS items except for "high". A significant effect was also found for the HRS "somaesthesia" subscale. Finally, the ARCI questionnaire showed a significant increase in the MBG scale.

When administered at 0.75 and 1.0 mg DMT/kg body weight, *ayahuasca* was correctly identified as an active substance by all participants. All VAS items and all HRS subscales, except for "volition", discriminated between each of these two doses and the placebo. At the medium

Table 1 Statistical analyses performed on the visual analogue scale (VAS) scores (mean values across ten time points) and on scores obtained for the Hallucinogen Rating Scale (HRS) subscales and Addiction Research Center Inventory (ARCI) (differences from predrug values) (n=5). *NS* Not significant, *A* amphetamine scale, *LSD* lysergic acid diethylamide scale, *BG* benzedrine group, *MBG* morphine-benzedrine group, *PCAG* pentobarbital-chlorpromazine-alcohol group

Variable	Friedman test	Wilcoxon test					
		Placebo			0.5 mg/kg		0.75 mg/kg
	P value	0.5	0.75	1.0	0.75	1.0	1.0
VAS							
Any effect	**	*	*	*	*	*	NS
Good effects	**	*	*	*	(*)	*	NS
Visions	*	*	*	*	NS	(*)	NS
Liking	**	*	*	*	(*)	*	NS
Drunken	**	*	*	*	*	*	NS
Stimulated	**	*	*	*	(*)	NS	NS
High	**	(*)	*	*	*	*	NS
HRS							
Somaesthesia	**	*	*	*	*	NS	NS
Perception	*	NS	*	*	NS	(*)	NS
Cognition	*	NS	*	*	(*)	*	NS
Volition	NS	–	–	–	–	–	–
Affect	**	(*)	*	*	*	(*)	NS
Intensity	**	(*)	*	*	*	NS	NS
ARCI							
MBG	**	*	*	*	(*)	NS	NS
BG	NS	–	–	–	–	–	–
A	**	(*)	*	*	NS	(*)	NS
LSD	*	(*)	(*)	*	NS	NS	NS
PCAG	NS	–	–	–	–	–	–

*P<0.05, **P<0.01, (*)P<0.1

dose (i.e., 0.75 mg DMT/kg) the ARCI MBG and A scales showed statistically significant differences from the placebo. At the high dose, the LSD, MGB, and A scales showed significant differences from the placebo. Regarding discrimination between the doses, five of the seven VAS items and the HRS "cognition" subscale were able to discriminate between the low and the high doses. None of the variables were able to discriminate between the medium and the high doses. Three VAS items, "any effects", "drunken", and "high", were discriminative between the low and medium doses. Discrimination between these two doses was also achieved by the HRS "somaesthesia", "affect", and "intensity" subscales.

Scores on the HRS subscales for the four experimental conditions are shown in Fig. 1. Pre- and post-treatment scores on the ARCI scales for the four experimental conditions are shown in Fig. 2. The time course of effects, as reflected by the seven VAS items, is presented in Fig. 3. The initial somatic effects of *ayahuasca* appeared between 15–30 min, which translated as increases in the "any effect" VAS. This was followed by an onset of psychological effects at around 30–60 min, which was reflected by the increases in the other six VAS items. Both somatic and psychic effects peaked between 60 and 120 min after drug intake and gradually decreased to baseline levels at approximately 240 min. It is worth noting that the "good effects" and "liking" items of the VAS remained elevated at 240 min after drug administration, when most of the perceptual, cognitive, and affective effects had disappeared. The volunteers verbally described this state as a lingering sensation of well-being after the resolution of the more intense psychotropic effects.

Tolerability

Cardiovascular effects

Mean values for SBP, DBP, and heart rate over time are presented in Fig. 4 as differences from their baseline values. All three *ayahuasca* doses produced increases in SBP and DBP when compared with placebo. Changes were not statistically significant, although a robust trend toward significance was observed for SBP (P=0.0503) at the high dose. The peak differences in SBP were 13.8 mm Hg between the high dose and placebo, 13.4 mm Hg between the medium dose and placebo, and 8.8 mm Hg between the low dose and placebo. The maximal increases in SBP were observed at 90 min after administration of all three *ayahuasca* doses. The peak differences in DBP were 10.4 mm Hg between the high dose and placebo, 9.8 mm Hg between the medium dose and placebo, and 8.6 mm Hg between the low dose and placebo. The maximal increases in DBP were observed at 60 min after administration of all three *ayahuasca* doses. Mean arterial pressures showed a 10.6 mm Hg maximum difference from placebo at 60 min. Heart rate was affected very little by *ayahuasca*. Increases above baseline values were only seen for the medium and high doses, with peak differences of 9.2 beats/min between the high dose and placebo, 8 beats/min between the medium dose and placebo, and 6.4 beats/min between the low dose and placebo at 45 min after drug administration. At no point did SBP reach 140 mm Hg, nor did heart rate reach 100 beats/min for any individual volunteer. On the other hand, two volunteers showed sporadic

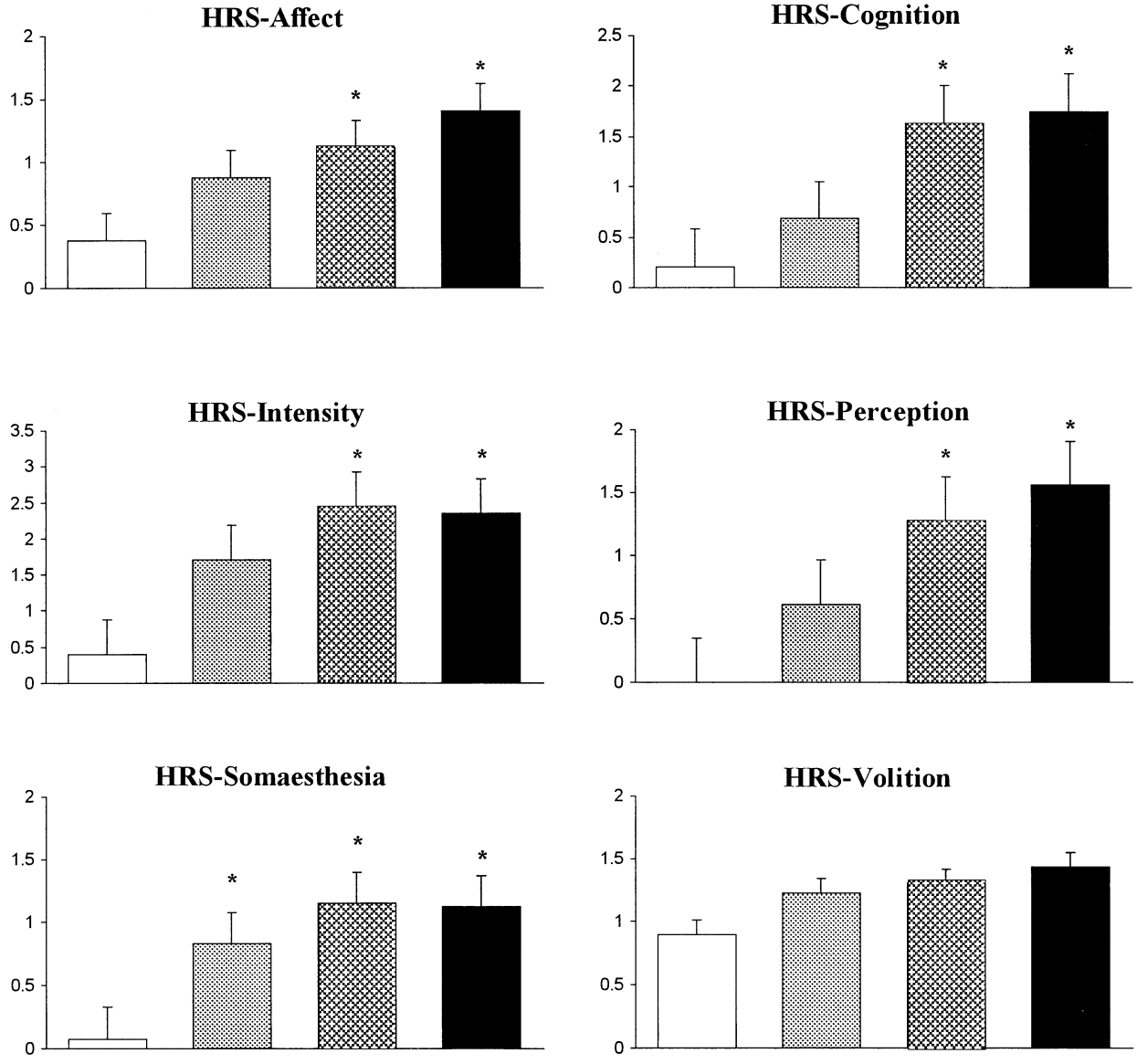


Fig. 1 Mean scores on the six Hallucinogen Rating Scale (HRS) subscales after administration of placebo (□), 0.5 mg *N,N*-dimethyltryptamine (DMT)/kg body weight *ayahuasca* (*lightly shaded*), 0.75 mg/kg (*shaded*), and 1.0 mg/kg (■). *Error bars* denote 1 standard error of mean (n=5). Significant differences from placebo (Wilcoxon test, P<0.05) are indicated by an *asterisk*

DBP values between 91–93 mm Hg after the medium and high doses, which lasted between 15 and 30 min.

Somatic-dysphoric effects

Table 2 lists the main somatic-dysphoric effects reported by the volunteers either spontaneously, or as positive responses to particular items in the HRS and ARCI questionnaires.

Blood analysis

No clinically relevant alterations were observed in the hematological or biochemical parameters tested after completion of each experimental session.

Verbal reports

The first effects noted by the volunteers were somatic modifications which included burning sensations in the stomach, tingling sensations, changes in perception of body temperature and skin sensitivity, and mild nausea. The onset of psychic effects was generally sudden and intense. Volunteers reported a certain degree of anxiety or fear at this initial stage, tending to decrease thereafter. Visual imagery was characteristic and dose-dependent. The images and visual modifications did not persist throughout the entire experience, but usually came and went in waves. These effects ranged from increases

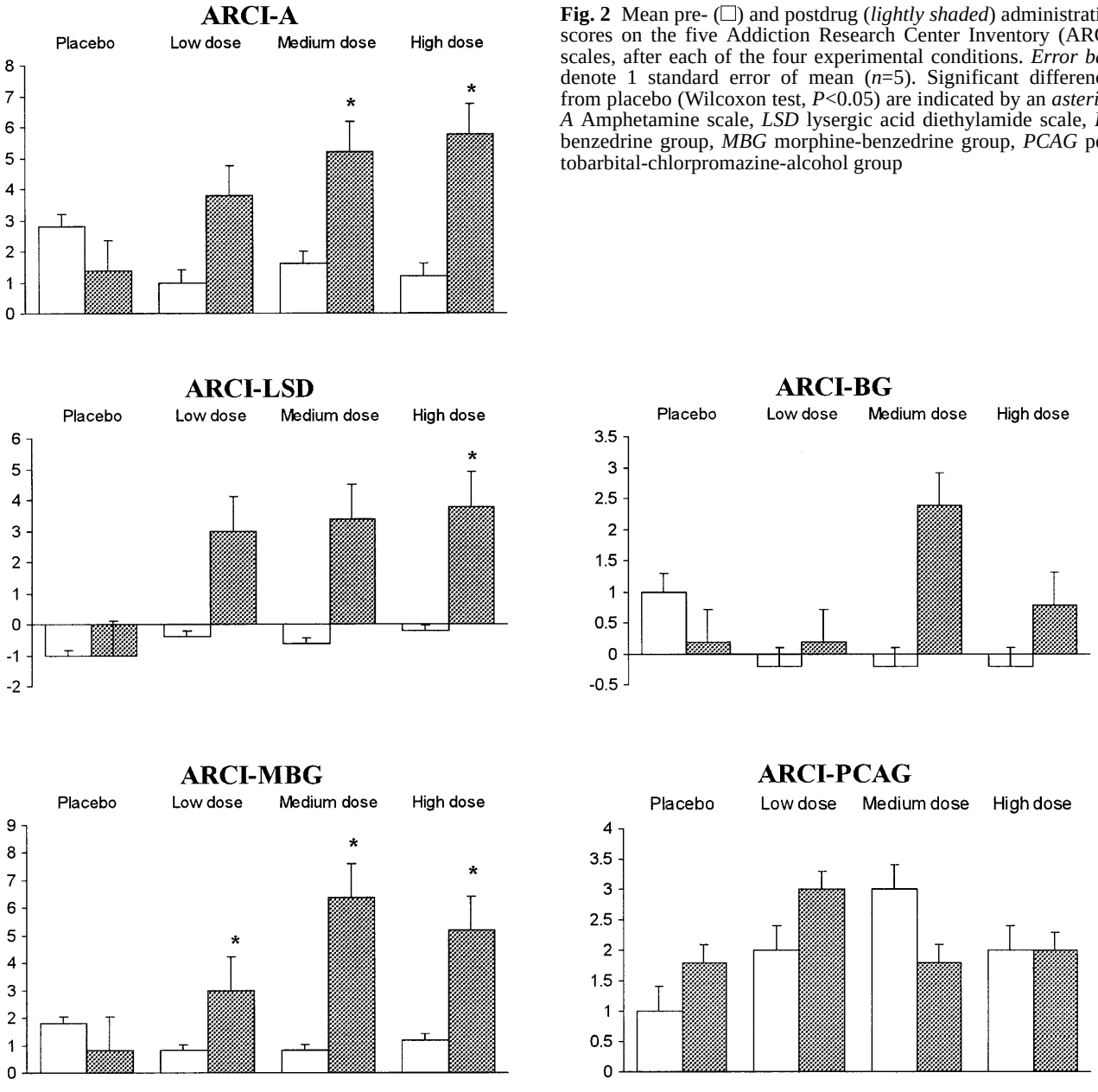


Fig. 2 Mean pre- (□) and postdrug (*lightly shaded*) administration scores on the five Addiction Research Center Inventory (ARCI) scales, after each of the four experimental conditions. *Error bars* denote 1 standard error of mean (n=5). Significant differences from placebo (Wilcoxon test, P<0.05) are indicated by an *asterisk*. *A* Amphetamine scale, *LSD* lysergic acid diethylamide scale, *BG* benzedrine group, *MBG* morphine-benzedrine group, *PCAG* pentobarbital-chlorpromazine-alcohol group

in an object's brightness and sharpness, or as vibrations in the visual field, to rapidly moving patterns, and scenes that were visible with eyes either closed or open at the medium and high doses. Changes in auditory perception were also reported and showed a dose-dependent effect. Hearing was perceived to be enhanced, with sounds becoming more clear and distinct. Although infrequent, transient auditory phenomena were reported in some subjects at the three doses. Thought processes were also modified, with the volunteers reporting an enhanced rate of thinking which generally centered on personal psychologic content. These thoughts were experienced as providing new insight into personal concerns. As the doses increased, emotional reactions were intensified, with the volunteers experiencing happiness, sadness, awe, amazement, and at times simultaneously contradictory feelings. At the medium and high doses, volunteers agreed on the similarity of the experience to dreaming. Memories were present, mostly related to recent personal matters. The sense of self and the passing of time were deeply affected at the medium and high doses. While sensations of closeness to others, happiness, and euphoria were similar at the medium and high doses, sensations of detachment from the body, oneness with the universe, and chaos, were more frequently reported with the latter. Five of the six volunteers were able to interact with the experimenter and the environment without major problems at all three doses. The sixth volunteer experienced a brief but intense disorientation state at the medium dosage. It is noteworthy that this volunteer had the least amount of experience with *ayahuasca*, having consumed it prior to the study on on-

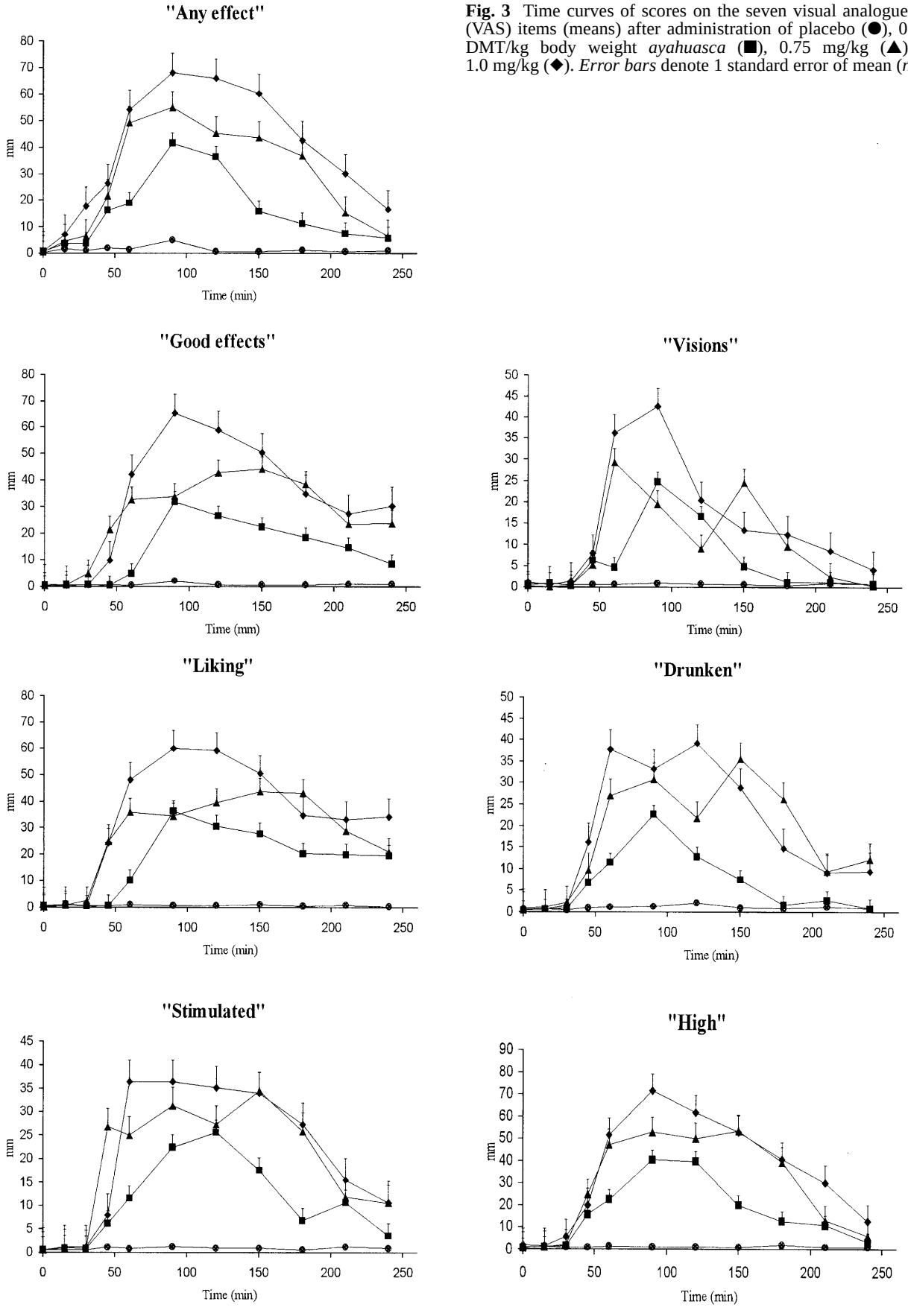


Fig. 3 Time curves of scores on the seven visual analogue scale (VAS) items (means) after administration of placebo (●), 0.5 mg DMT/kg body weight *ayahuasca* (■), 0.75 mg/kg (▲), and 1.0 mg/kg (◆). *Error bars* denote 1 standard error of mean (*n*=5)

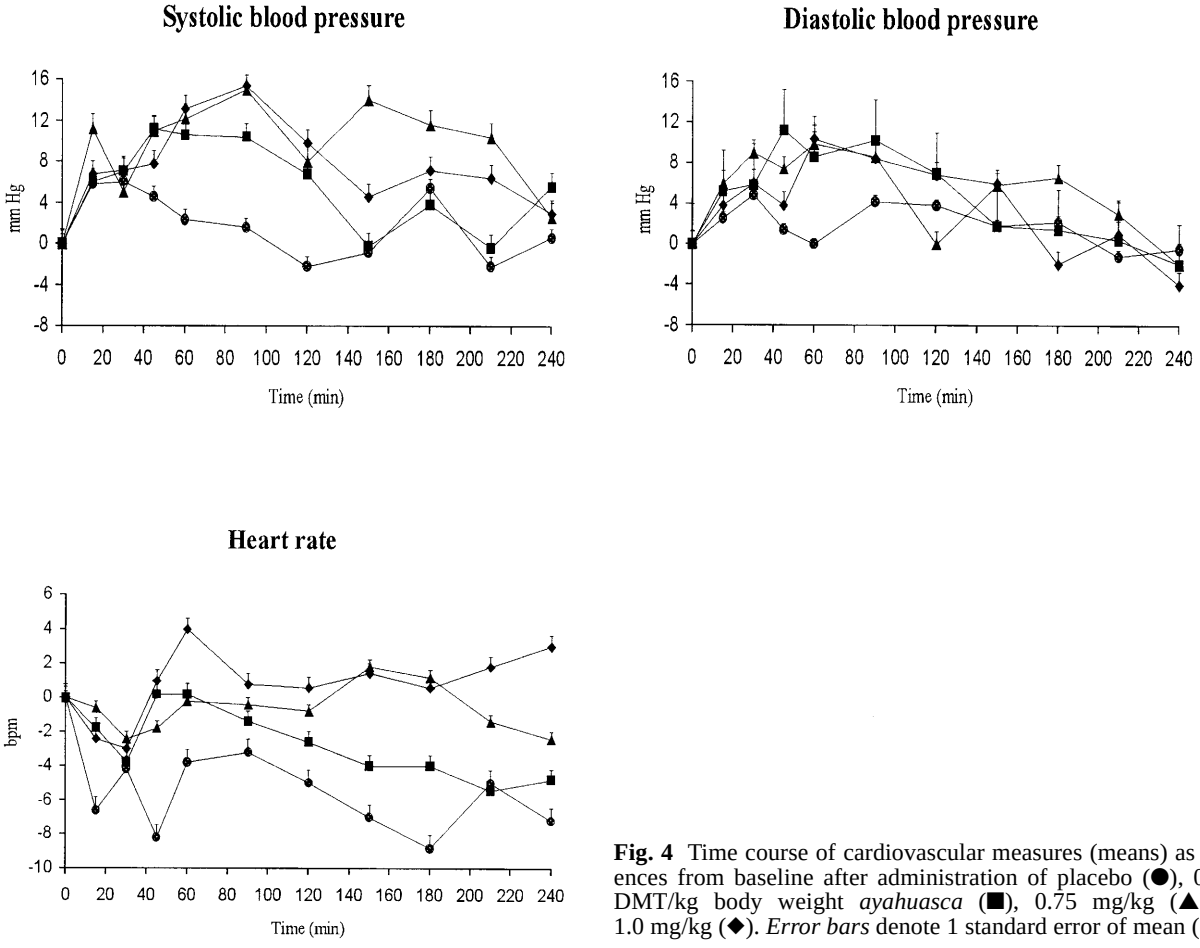


Fig. 4 Time course of cardiovascular measures (means) as differences from baseline after administration of placebo (●), 0.5 mg DMT/kg body weight *ayahuasca* (■), 0.75 mg/kg (▲), and 1.0 mg/kg (◆). *Error bars* denote 1 standard error of mean (n=5)

Table 2 Somatic-dysphoric effects spontaneously reported by the six volunteers, or as positive responses on particular items of the HRS and ARCI questionnaires on the 4 experimental days, presented as most to least frequently reported. Figures indicate the number of subjects who reported a specific effect, regardless of intensity, at the three different *ayahuasca* doses administered and placebo

	Somatic-dysphoric effect	Placebo	0.5 mg/kg	0.75 mg/kg	1.0 mg/kg
1	Body feels different[a]	1/6	5/6	6/6	5/5
2	Nausea[a]	0/6	4/6	5/6	3/5
3	Change in body temperature[a]	1/6	4/6	4/6	3/5
4	Electric/tingling feeling[a]	1/6	2/6	3/6	5/5
5	I have a disturbance in my stomach[b]	0/6	3/6	4/6	2/5
6	My hands feel clumsy[b]	1/6	2/6	3/6	3/5
7	My speech is slurred[b]	0/6	3/6	3/6	2/5
8	Urge to urinate[a]	1/6	1/6	3/6	3/5
9	Feel body shake/tremble[a]	0/6	1/6	3/6	2/5
10	Urge to move bowels[a]	0/6	2/6	0/6	3/5
11	I feel dizzy[b]	0/6	2/6	2/6	0/5
12	My head feels heavy[b]	0/6	2/6	2/6	0/5
13	Sweating[a]	0/6	1/6	2/6	1/5
14	A thrill has gone through me...[b]	0/6	0/6	1/6	0/5
15	Vomiting[c]	0/6	0/6	0/6	1/5
16	Disorientation[c]	0/6	0/6	1/6	0/5

[a]Item included in the HRS
[b]Item included in the ARCI
[c]Spontaneously reported

ly two occasions. Verbal support was sufficient to get him through this temporary crisis, but he was left with a general feeling of dissatisfaction toward the experience and withdrew from the study. Nevertheless, all volunteers, including this one, were well aware of the effects being caused by the administered drug and of their transient nature.

Discussion

The administration of *ayahuasca* to experienced healthy volunteers induced intense modifications of their conscious state, which was evaluated as dose-dependent elevations in all VAS items used, in five of the HRS subscales, and in the MBG, LSD, and A scales of the ARCI questionnaire. At no time of the study did any of the vol-

unteers lose consciousness or contact with reality. Compared with the effects of intravenous (IV) DMT (Strassman et al. 1994), clear differences were found in the intensity and duration of the experience. The slower onset and longer duration of effects seen for *ayahuasca* can be readily attributed to the oral route of administration for DMT and to the enzymatic blockade process (MAO inhibition) which mediates the drug's access to systemic circulation. Additionally, the competition between DMT and increasing levels of serotonin for available receptor sites may contribute to an overall attenuation of effects from *ayahuasca* vs IV administration of pure DMT. MAO inhibition not only allows for increased levels of serotonin and other monoamines but also temporarily blocks the immediate metabolism of DMT, thus extending its action relative to its IV administration. The cardiovascular effects observed were milder than those reported for IV DMT (Strassman and Qualls 1994). Peak increases of blood pressure and heart rate after *ayahuasca* were relatively delayed and comparable in magnitude to those brought about by a 0.1–0.2 mg/kg IV DMT dose. Our cardiovascular values are in line with those previously reported by Callaway and coworkers (1999) after an *ayahuasca* (*hoasca)* oral dose of 0.48 mg DMT/kg, though a direct comparison is not possible given the non placebo-controlled nature of this earlier study. Considering the fact that in the present study elevations were observed in cardiovascular parameter after placebo, it seems likely that the inclusion of a placebo control in the earlier study could have rendered lower increases of cardiovascular parameters for the 0.48 mg DMT/kg dose used. When compared with IV DMT, it is reasonable to assume that the reversible MAO-inhibiting properties of harmine and harmaline leads to a transient increase in endogenous monoamines, in addition to DMT's own cardiovascular effects. Nevertheless, the moderate nature of these increases could also be due to the simultaneous enhancement of vagal activity induced by decreased serotonin metabolism. Additionally, a*yahuasca* seemed to induce more somatic-dysphoric effects than IV DMT, the most frequently reported being the modifications in body feeling and nausea. These effects may be attributable to the β-carbolines present in the tea. A relationship between the nausea and other distressing effects on the digestive tract and increased 5-HT levels has been postulated (Callaway et al. 1999).

Scorings on the six HRS subscales and the nature of the effects elicited by *ayahuasca* at the present low dose resembled those reported by Strassman et al. (1994) after 0.1 mg/kg IV DMT. In both cases, somatic reactions predominated over perceptual or cognitive effects. Scores on the “affect”, “volition”, and “intensity” subscales were also close to those reported by Grob et al. (1996) after an *ayahuasca* dose equivalent to the low dose used in the present study. Except for the “perception” and “volition” subscales, which showed lower values, scores on the HRS at the medium dose were greater than those reported by Grob et al. (1996) and fell close to those described for 0.2 mg/kg IV DMT, a dosage known to be fully psychoactive for DMT (Strassman et al. 1994). These differences probably indicate less overwhelming perceptual effects and greater control over the experience after *ayahuasca*. Finally, the five volunteers who received the high dose (1.0 mg DMT/kg) identified it as being fully active and verbally described its effects as being very high in intensity. However, several subjective-effect variables showed a saturation relative to the 0.75 mg DMT/kg dose. This saturation, or ceiling effect, may indicate an “order” effect due to the exploratory nature of the study design, with doses being administered in an increasing order rather than in a randomized balanced manner. At the medium dose, scores on all HRS subscales were higher than those reported by Grob et al. (1996) in their single-dose study. The “cognition” subscale for the medium dose in the present study scored close to the value obtained by Strassman et al. (1994) at 0.4 mg/kg IV DMT, whereas scores on the other five subscales remained near those obtained after a 0.2 mg/kg IV DMT dose. Thus, not even at the 1.0 mg DMT/kg *ayahuasca* dose did the volunteers experience the overwhelming effects reported for the highest dose used in Strassman's study (0.4 mg/kg IV), probably reflecting the milder effects of DMT made orally active by means of MAO inhibition.

Results obtained for the ARCI-A scale are indicative of a subjective effect of increased activation. Despite the coexistence of marked somatic-dysphoric effects, as reflected by increases in the HRS-LSD scale, the administration of *ayahuasca* induced elevations in the ARCI-MBG scale, indicative of subjective feelings of well-being. The pleasant nature of the effects experienced by five of the six volunteers was also reflected as increases in the “good effects”, “liking”, and “high” VAS items, especially at the high dose. On the contrary, sedation ratings, as reflected by the ARCI-PCAG scale did not reach statistical significance and tended to decrease as the doses increased.

Regarding the similarities and differences of the *ayahuasca* experience with those elicited by other better characterized serotonergic psychedelics, important differences can be found in the time course of effects. A*yahuasca* effects are comparable in duration to those of psilocybin. On the other hand, mescaline and LSD are clearly longer-acting drugs, with peak effects at 3–5 h and an overall duration which can exceed 8 h (Strassman 1994). Psychological effects are difficult to compare between studies, due to the different psychometric instruments used. However, in a recent human study where the HRS was administered, psilocybin was found to induce increases in all the HRS subscales, including “volition”. This greater impairment of the subjects' capacity to interact with themselves and their surroundings was further corroborated by their verbal reports, which described sensations of loss of control and paranoid thoughts (Gouzoulis-Mayfrank et al. 1999a), neither of which were observed in the present study.

From a neurochemical perspective, data from preclinical studies strongly support the involvement of seroto-

nergic neurotransmission in the effects elicited by the classic psychedelics, which includes DMT. Such compounds containing an indole moiety bind with high affinity to both the 5-HT_{2A} and 5-HT_{1A} sites in the human brain. A close correlation has been found between psychotropic potency and binding at the 5-HT_{2A} site (Glennon et al. 1984) which is considered to be chiefly responsible for the behavioral effects elicited by these agents. The interaction with the 5-HT_{1A} site has recently been argued to modulate the intensity of the psychedelic experience (Strassman 1996). Additionally, evidence of a possible long-term modulation of serotonergic neurotransmission by *ayahuasca* has been reported in a previous study, in which an apparent upregulation of the platelet serotonin transporter was found in regular users of the tea (Callaway et al. 1994). Nevertheless, the role of dopaminergic involvement in the effects of the classic psychedelics has also been examined. A recent PET study found that the administration of psilocybin to human volunteers leads to the displacement of ^{11}C-raclopride in the striatum, an effect that may reflect an increase in dopamine release (Vollenweider et al. 1999). This secondary pro-dopaminergic activity may not be, however, the key to the perceptual and cognitive modifications induced by these agents, as in another study psilocybin's subjective effects were found to be increased rather than reverted by the D_2 receptor antagonist, haloperidol, while they were effectively counteracted by ketanserin and risperidone (Vollenweider et al. 1998).

Neuroimaging studies have revealed patterns of increased metabolism throughout the brain, and more specifically in the prefrontal cortices, particularly in the right hemisphere, in healthy volunteers after dosing with psilocybin (Vollenweider et al. 1997; Gouzoulis-Mayfrank et al. 1999b) and mescaline (Hermle et al. 1992). In this respect, recent electrophysiological studies have shown that 5-HT_{2A} receptor activation by serotonin mediates an increase of excitatory postsynaptic potentials (EPSPs) and currents (EPSCs) in pyramidal neurons of the neocortex and transitional cortex (Aghajanian and Marek 1997), an effect involving glutamate release and which is most pronounced in the medial prefrontal cortex (Marek and Aghajanian 1998a, b). These findings suggest an excitatory action of the classic psychedelics on the human frontal and parietal cortices and in the primary auditory and visual areas, which show very high densities of 5-HT_{2A} sites (Pazos et al. 1987). This excitatory effect may account for the enhancement and modifications of auditory and visual perception described by the volunteers. An analogous excitatory action on the somatosensory and visual association areas, both showing high 5-HT_{2A} densities, may also play a role in the peculiar modifications of perception brought about by *ayahuasca*. Finally, the activation of the anterior cingulate cortex (ACC), an area also showing dense serotonergic innervation and 5-HT_{2A} sites, could contribute to the emotional overtones of the *ayahuasca* experience. A recent PET study has implicated the ACC in normal emotional awareness (Lane et al. 1998), and psilocybin administration leads to increases in metabolism in this area, where 5-HT_{2A}-mediated EPSPs/EPSCs have also been recorded (Aghajanian and Marek 1997).

To summarize, *ayahuasca* induced a modified state of awareness in which stimulatory and psychedelic effects were present, and increased in a dose-dependent manner. The volunteers experienced modifications in perception and thought processes, such as rapid succession of thoughts, visions, and recollections of recent events, frequently having a marked emotional content. *Ayahuasca* was safely administered to the volunteers in this study and its effects were regarded as pleasant and desirable, except for one volunteer who experienced a dysphoric state that was characterized by transient disorientation and anxiety. Nevertheless, this adverse reaction was most likely related to the limited previous experience of that volunteer with the tea. Finally, the nature of the experience produced by *ayahuasca* resembled that of IV DMT, though it was less overwhelming, of longer duration, and displayed a greater variety of somatic-dysphoric effects. Moderate actions on blood pressure and heart rate were found and no clinically relevant changes were observed in biochemical parameters after any of the experimental sessions. Future studies will include measures of sensorimotor gating and brain imaging techniques in larger volunteer groups, using a double-blind balanced design, in order to obtain additional information on the mechanisms underlying the central effects of *ayahuasca.*

Acknowledgements We would like to express our gratitude to Esther Martínez, Félix González, and José María Fábregas for their continued support and help in contacting the people at CEFLURIS in Brazil, and to the latter for agreeing to submit their *ayahuasca* (*Daime*) for evaluation in a clinical trial. Thanks are also due to Sylvie Cotxet and David Martínez for their assistance during data collection. Finally, we greatly acknowledge the assistance of Ignasi Gich for his helpful comments on data analysis and revision of the manuscript. This work was supported by grant IR-97/034/980 from the Institut de Recerca HSCSP.

References

Aghajanian GK, Marek GJ (1997) Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. Neuropharmacology 36:589–599

Callaway JC, Grob CS (1998) Ayahuasca preparations and serotonin reuptake inhibitors: a potential combination for severe adverse interactions. J Psychoactive Drugs 30:367–369

Callaway JC, Airaksinen MM, McKenna DJ, Brito GS, Grob CS (1994) Platelet serotonin uptake sites increased in drinkers of *ayahuasca.* Psychopharmacology 116:385–387

Callaway JC, Raymon LP, Hearn WL, McKenna DJ, Grob CS, Brito GC, Mash DC (1996) Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with *Ayahuasca*. J Anal Toxicol 20:492–487

Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, Andrade EN, Andrade EO, Mash DC (1999) Pharmacology of hoasca alkaloids in healthy humans. J Ethnopharmacol 65:243–256

Deliganis A, Pierce P, Peroutka S (1991) Differential interactions of dimethyltryptamine (DMT) with 5-HT_{1A} and 5-HT_2 receptors. Biochem Pharmacol 41:1739–1744

Dobkin de Ríos M (1972) Visionary vine: hallucinogenic healing in the Peruvian Amazon. Chandler Publishing, San Francisco

Glennon RA, Titeler M, McKenney JD (1984) Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. Life Sci 35:2505–2511

Gouzoulis-Mayfrank E, Thelen B, Habermeyer E, Kunert HJ, Kovar KA, Lindenblatt H, Hermle L, Spitzer M, Sass H (1999a) Psychopathological, neuroendocrine and autonomic effects of 3,4-methylenedioxyethylamphetamine (MDE), psilocybin and *d*-methamphetamine in healthy volunteers. Psychopharmacology 142:41–50

Gouzoulis-Mayfrank E, Schreckenberger M, Sabri O, Arning C, Thelen B, Spitzer M, Kovar KA, Hermle L, Büll U, Sass H (1999b) Neurometabolic effects of psilocybin, 3,4-methylenedioxyethylamphetamine (MDE) and *d*-methamphetamine in healthy volunteers. A double-blind, placebo-controlled PET study with [^{18}F]FDG. Neuropsychopharmacology 20:565–581

Grob CS, McKenna DJ, Callaway JC, Brito GS, Neves ES, Oberlaender G, Saide OL, Labigalini E, Tacla C, Miranda CT, Strassman RJ, Boone KB (1996) Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. J Nerv Ment Dis 184:86–94

Hermle L, Fünfgeld M, Oepen G, Botsch H, Borchardt D, Gouzoulis E, Fehrenbach RA, Spitzer M (1992) Mescaline-induced psychopathological, neuropsychological, and neurometabolic effects in normal subjects: experimental psychosis as a tool for psychiatric research. Biol Psychiatry 32:976–991

Lamas X, Farré M, Llorente M, Camí J (1994) Spanish version of the 49-item short form of the Addiction Research Center inventory. Drug Alcohol Depend 35:203–209

Lane RD, Reiman EM, Axelrod B, Lang-Sheng Y, Holmes A, Schwartz GE (1998) Neural correlates of levels of emotional awareness. Evidence of an interaction between emotion and attention in the anterior cingulate cortex. J Cogn Neurosci 10:525–535

Liwszyc GE, Vuori E, Rasanen I, Issakainen J (1992) Daime – a ritual herbal potion. J Ethnopharmacol 36:91–92

López P (1999) *Ayahuasca*, el último alucine. Tiempo 910:26–28

Marek GJ, Aghajanian GK (1998a) The electrophysiology of prefrontal serotonin systems: therapeutic implications for mood and psychosis. Biol Psychiatry 44:1118–1127

Marek GJ, Aghajanian GK (1998b) Indoleamine and the phenethylamine hallucinogens: mechanisms of psychotomimetic action. Drug Alcohol Depend 51:189–198

Marshall J (1997) Daimismo, la comunión con la *ayahuasca*. Integral 1:16–23

McKenna DJ, Towers GHN, Abbott F (1984) Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of *ayahuasca*. J Ethnopharmacol 10:195–223

Metzner R (1999) Amazonian vine of visions. In: Metzner R (ed) Ayahuasca. Hallucinogens, consciousness and the spirit of nature. Thunder's Mouth Press, New York

Ott J (1999) Pharmahuasca: human pharmacology of oral DMT plus harmine. J Psychoactive Drugs 31:171–177

Pazos A, Probst A, Palacios JM (1987) Serotonin receptors in the human brain. IV. Autoradiographic mapping of serotonin-2 receptors. Neuroscience 21:123–139

Pierce P, Peroutka S (1989) Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. Psychopharmacology 97:118–122

Riba J, Rodríguez-Fornells A, Strassman RJ, Barbanoj MJ (2000) Psychometric assessment of the Hallucinogen Rating Scale. Drug Alcohol Depend (in press)

Rivier L, Lindgren J (1972) *Ayahuasca*, the South American hallucinogenic drink: ethnobotanical and chemical investigations. Econ Bot 29:101–129

Schultes RE, Hofmann A (1980) The botany and chemistry of hallucinogens. Thomas, Springfield, Illinois

Schultes RE, Hofmann A (1982) Plantas de los dioses: orígenes del uso de los alucinógenos. Fondo de Cultura Económica, México D.F.

Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the state-trait anxiety inventory. Consulting Psychologists Press, Palo Alto, California

Strassman RJ (1994) Human psychopharmacology of LSD, dimethyltryptamine and related compounds. In: Pletscher A, Ladewig D (eds) 50 years of LSD: current status and perspectives of hallucinogens. Parthenon, London

Strassman RJ (1996) Human psychopharmacology of *N*,*N*-dimethyltryptamine. Behav Brain Res 73:121–124

Strassman RJ, Qualls CR (1994) Dose response study of *N*,*N*-dimethyltryptamine in humans. I. Neuroendocrine, autonomic and cardiovascular effects. Arch Gen Psychiatry 51:98–108

Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R (1994) Dose response study of *N*,*N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch Gen Psychiatry 51:98–108

Suzuki O, Katsumata Y, Oya M (1981) Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. Biochem Pharmacol 30:1353–1358

Vollenweider FX, Leenders KL, Scharfetter C, Maguire P, Stadelmann O, Angst J (1997) Positron emission tomography and fluorodeoxyglucose studies of metabolic hyperfrontality and psychopathology in the psilocybin model of psychosis. Neuropsychopharmacology 16:357–372

Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via serotonin-2 agonist action. Neuroreport 9:3897–3902

Vollenweider FX, Vontobel P, Hell D, Leenders KL (1999) 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man. A PET study with [^{11}C]raclopride. Neuropsychopharmacology 20:424–433

Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage *ayahuasca* in healthy volunteers

Jordi Riba,[1] Peter Anderer,[2] Adelaida Morte,[1] Gloria Urbano,[1] Francesc Jané,[1] Bernd Saletu[2] & Manel J. Barbanoj[1]

[1]*Àrea d'Investigació Farmacològica, Institut de Recerca, Hospital de la Santa Creu i Sant Pau (HSCSP), Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Spain and* [2]*Division of Sleep Research and Pharmacopsychiatry, Department of Psychiatry, University of Vienna, Austria*

Aims *Ayahuasca* is a traditional South American psychoactive beverage used in Amazonian shamanism, and in the religious ceremonies of Brazilian-based syncretic religious groups with followers in the US and several European countries. This tea contains measurable amounts of the psychotropic indole *N*,*N*-dimethyltryptamine (DMT), and β-carboline alkaloids with MAO-inhibiting properties. In a previous report we described a profile of stimulant and psychedelic effects for *ayahuasca* as measured by subjective report self-assessment instruments. In the present study the cerebral bioavailability and time-course of effects of *ayahuasca* were assessed in humans by means of topographic quantitative-electroencephalography (q-EEG), a noninvasive method measuring drug-induced variations in brain electrical activity.

Methods Two doses (one low and one high) of encapsulated freeze-dried *ayahuasca*, equivalent to 0.6 and 0.85 mg DMT kg^{-1} body weight, were administered to 18 healthy volunteers with previous experience in psychedelic drug use in a double-blind crossover placebo-controlled clinical trial. Nineteen-lead recordings were undertaken from baseline to 8 h after administration. Subjective effects were measured by means of the Hallucinogen Rating Scale (HRS).

Results *Ayahuasca* induced a pattern of psychoactive effects which resulted in significant dose-dependent increases in all subscales of the HRS, and in significant and dose-dependent modifications of brain electrical activity. Absolute power decreased in all frequency bands, most prominently in the theta band. Mean absolute power decreases (95% CI) at a representative lead (P3) 90 min after the high dose were -20.20 ± 15.23 μV^2 and -2.70 ± 2.21 μV^2 for total power and theta power, respectively. Relative power decreased in the delta (-1.20 ± 1.31% after 120 min at P3) and theta (-3.30 ± 2.59% after 120 min at P3) bands, and increased in the beta band, most prominently in the faster beta-3 (1.00 ± 0.88% after 90 min at P3) and beta-4 (0.30 ± 0.24% after 90 min at P3) subbands. Finally, an increase was also seen for the centroid of the total activity and its deviation. EEG modifications began as early as 15–30 min, reached a peak between 45 and 120 min and decreased thereafter to return to baseline levels at 4–6 h after administration.

Conclusions The central effects of *ayahuasca* could be objectively measured by means of q-EEG, showing a time pattern which closely paralleled that of previously reported subjective effects. The modifications seen for the individual q-EEG variables were in line with those previously described for other serotonergic psychedelics and share some features with the profile of effects shown by pro-serotonergic and pro-dopaminergic drugs. The q-EEG profile supports the role of 5-HT_2 and dopamine D_2-receptor agonism in mediating the effects of *ayahuasca* on the central nervous system.

Correspondence: Dr Manel J. Barbanoj, Àrea d'Investigació Farmacològica, Institut de Recerca, Hospital de la Santa Creu i Sant Pau, St Antoni Maria Claret, 167, 08025 Barcelona, Spain. Tel.: 34 93 291 90 19; Fax: 34 93 291 92 86; E-mail: mbarbano@santpau.es

Received 24 May 2001, accepted 14 February 2002.

Keywords: ayahuasca, DMT, pharmaco-EEG, psychedelics, topography

Introduction

Ayahuasca is the Quechuan name for both the Amazon woody vine *Banisteriopsis caapi* (Malpighiaceae) and the sacred psychoactive beverage obtained from it. The beverage, also known by the names *Yajé*, *Natema*, *Santo Daime* and *Vegetal*, has been used throughout the Amazon Basin by shamans and healers since pre-Columbian times for medicinal purposes and as a means to contact the supernatural [1, 2]. More recently, syncretic religions combining the use of *ayahuasca* with Christian beliefs, particularly the *Santo Daime* and the *União do Vegetal*, have been established in Brazil, where they enjoy legal protection. Outside Brazil, smaller groups of followers have begun to consume the tea in the United States and in several European countries, including Germany, Great Britain, Holland, France and Spain [3]. Even though the number of users is still relatively small, adverse reactions associated with the simultaneous use of *ayahuasca* and other centrally active drugs have raised concern for public health [4], and extensive clinical data on its somatic, psychological and neurophysiological effects are warranted.

Banisteriopsis caapi, the basic ingredient of the beverage, is seldom found alone in *ayahuasca*. The tea is generally obtained by infusing the stems of the vine together with the leaves of other plants, namely *Psychotria viridis* (Rubiaceae) or *Diplopterys cabrerana* (Malpighiaceae) [5]. Chemical analyses have shown that *B. caapi* contains notable amounts of β-carboline alkaloids, mainly harmine and tetrahydroharmine (THH), followed by harmaline and trace amounts of harmol [5, 6]. *P. viridis* and *D. cabrerana* also contain indole alkaloids, mainly the potent short-acting psychedelic agent *N*,*N*-dimethyltryptamine (DMT) [5, 7].

This combination of *B. caapi* and *P. viridis* in a single oral preparation is a remarkable achievement of empirical ethnopharmacological knowledge, as psychoactivity arises from combining the pharmacodynamic actions of the β-carbolines and of DMT. Similarly to other indole and phenethylamine psychedelics such as LSD and mescaline [8], DMT shows affinity for the 5-$HT_{2A/2C}$ receptor sites in the central nervous system (CNS), where it displays agonist activity [9]. However, unlike most psychedelics, DMT is *a priori* only active when parenterally administered, because the oral ingestion of the drug alone leads to its metabolic breakdown by the enzyme monoamine oxidase (MAO) [10]. Interestingly, harmine and harmaline, and to a lesser extent THH, are potent MAO inhibitors [6]. Thus, it is widely accepted that the MAO-inhibiting action of the β-carbolines present in the tea allows the viable access of DMT to the systemic circulation and the CNS. In addition to facilitating a direct agonist action of DMT at the 5-$HT_{2A/2C}$ sites, the MAO-inhibiting properties of the β-carbolines may contribute to the overall effects of *ayahuasca*, firstly, by prolonging the effects of DMT due to its decreased metabolism, and secondly, by simultaneously enhancing the levels of endogenous catecholamines and serotonin [11].

In a previous study conducted to characterize the tolerability and psychological effect profile of *ayahuasca* [12], this tea was found to induce a pattern of psychostimulant and psychedelic effects, which qualitatively resembled those of other classical serotonergic agents, such as psilocybin, and parenteral DMT [13, 14]. *Ayahuasca* was able to induce dose-dependent perceptual, cognitive and affective modifications, with a milder intensity and longer duration than those previously described for intravenous DMT [14], but with an overall duration shorter than that of better characterized psychedelics such as LSD or mescaline [15].

The aim of the present study was to assess the central actions of *ayahuasca* by means of quantitative-electroencephalography (q-EEG), an objective noninvasive method used to evaluate drug effects on the CNS with high temporal resolution [16]. We intended thus to demonstrate its cerebral bioavailability and subsequent psychoactivity by means other than subjective self-report instruments, and implementing a double-blind randomised placebo-controlled design. Recordings of brain electrical activity were carried out before and at different time points after the administration of two different doses of encapsulated freeze-dried *ayahuasca* to a group of healthy volunteers with previous experience in the use of psychedelics.

Methods

Volunteers

Eighteen healthy volunteers (15 males and three females) with no current or previous history of neurological or psychiatric disorder and no family history of Axis-I psychiatric disorder in first degree relatives were included in the study. Eligibility criteria included prior experience with psychedelic drugs at least on five occasions without sequelae derived therefrom. The volunteers were given a structured psychiatric interview (DSM-III-R) and completed the trait-anxiety scale from the State-Trait Anxiety Inventory [17]. Exclusion criteria included a present or past history of Axis-I disorders and alcohol or other substance dependence, and high scores on trait anxiety. Volunteers were given a complete physical examination that included medical history, laboratory tests, ECG and urinalysis. All volunteers gave their written informed consent to participate. Mean age was 25.7 years (range: 19–38), mean weight 66.47 kg (range: 50.7–79.5) and

mean height 175.11 cm (range: 158–188). In addition to their prior intake of psychedelics, all volunteers had previous experience with cannabis and cocaine. Although prior exposure to *ayahuasca* was not required for participation, two of the volunteers had ingested this tea before inclusion. The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans, and was approved by the hospital's ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of *ayahuasca*, the general psychological effects of psychedelics and their possible adverse effects, as reported in the psychiatric literature.

Drug

The *ayahuasca* doses administered to the volunteers in the present study as the low and the high dose were the equivalent to 0.6 and 0.85 mg DMT kg^{-1} body weight. These doses were chosen based on tolerability and subjective effect data gathered in a previous study [12]. The *ayahuasca* was not administered in its original liquid form, but as a liophilizate. The DMT contents in the liophilizate had been determined by h.p.l.c., as described by Callaway and coworkers [18], and the β-carboline constituents following a modification of the method described therein. The concentrations found were: 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg THH per gram of freeze-dried material. These alkaloid contents corresponded to the following concentrations in the original tea: DMT 0.53 mg ml^{-1}, harmine 0.90 mg ml^{-1}, harmaline 0.06 mg ml^{-1} and THH 0.72 mg ml^{-1}. The calculated individual dose for each volunteer was administered by combining 00 gelatin capsules containing 0.5 g, 0.25 g or 0.125 g of freeze-dried *ayahuasca* and placebo capsules containing 0.75 g lactose. Placebo capsules were added when necessary, so that all volunteers took the same number of capsules on each experimental day.

Study design and experimental procedure

The volunteers participated in four experimental sessions. Volunteers were informed that they would randomly receive on each experimental day a single oral dose of encapsulated freeze-dried *ayahuasca* (one low and one high dose), a placebo and a random repetition of one of the three mentioned treatments. In actual fact they all received a placebo on the first experimental day in a single-blind fashion, followed by one of the three treatments from days 2 to 4 in a double-blind balanced fashion, according to a randomization table. The first nonrandomized placebo was administered in order to familiarize the volunteers with the experimental setting and to minimize the stress associated with the experimental interventions. Two weeks prior to the beginning of the experimental sessions, volunteers were requested to abstain from any medication or illicit drug until the completion of the study. Volunteers also abstained from alcohol, tobacco and caffeinated drinks 24 h prior to each experimental day. Urinalysis for illicit drug use was performed for each experimental session and was found negative for amphetamines, cocaine, opioids, benzodiazepines and alcohol. A 7 day washout period was established between experimental days.

On each experimental day participants arrived in the laboratory in the morning under fasting conditions. EEG electrodes were placed on the scalp and treatment capsules were administered at approximately 10.00 h with 250 ml tap water. EEG recordings were obtained at baseline and at regular intervals after treatment administration. The experimental sessions were undertaken in a quiet and dimly lit room with the volunteers seated in a reclining chair. The experimenter remained outside the room during the EEG recordings. At 4 h after administration of the capsules, when the most prominent subjective effects associated with the drug had disappeared, the volunteers answered subjective effect questionnaires, and had a meal. The last recording was performed at 8 h and volunteers were discharged approximately 9 h after drug administration.

Measurements

EEG acquisition and analysis

EEG recordings were obtained through 19 electrodes placed on the scalp according to the international 10/20 system on the following locations: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1 and O2, referenced to averaged mastoids by means of a Neuroscan SYNAMPS amplifier. Additionally, vertical and horizontal electrooculograms (EOG) were recorded. Vigilance controlled EEG (V-EEG) for 3 min with eyes closed was recorded at −15 (PRE-1), baseline (PRE-2), +15, +30, +45, +60, +90, +120, +150, +180, +210, +240, +360 and +480 min from drug administration. During the V-EEG recordings, the experimenter tried to keep the volunteers alert; as soon as drowsiness patterns appeared in the EEG they were aroused by acoustic stimulation. The EEG signal was recorded using high-pass and low-pass filters of 0.3 Hz and 30 Hz, respectively, and digitized online with a sampling frequency of 100 Hz.

A two-step artefact processing procedure was used. It included ocular artifact minimization based on regression analysis in the time domain, as described by Semlitsch *et al.* [19], and automatic artifact rejection based on a time and frequency domain approach as described by Anderer *et al.* [20]. Subsequently, validity of the artifact processing procedure was visually inspected [21].

After recomputation to average reference, spectral analysis was performed for artefact-free 5 s epochs, resulting in a frequency resolution of 0.2 Hz. The spectral density curves for all artifact-free EEG epochs were averaged for a particular experimental situation. These mean spectral curves, containing data from 1.3 to 30 Hz, were quantified into 34 target variables: total power, absolute and relative power in 11 different frequency bands (delta [1.3–3.5 Hz], theta [3.5–7.5 Hz], alpha-1 [7.5–10.5 Hz], alpha-2 [10.5–13 Hz], beta-1 [13–16 Hz], beta-2 [16–20 Hz], beta-3 [20–25 Hz], beta-4 [25–30 Hz], combined delta-theta, alpha and beta), the dominant frequency in Hz, absolute and relative power of the dominant frequency, the centre-of-gravity frequency (centroids) and the frequency variability (centroid deviations) of the combined delta-theta, alpha and beta bands as well as of the total activity. Additionally, the vigilance alpha/delta-theta index was also calculated.

Topographic maps were computed by cubic interpolation of the values of the four nearest electrodes.

Subjective ratings

Volunteers were requested to answer the Hallucinogen Rating Scale (HRS), a self-report questionnaire specifically developed to quantify different aspects of psychedelic-induced subjective effects. The questionnaire includes six subscales: *Somaesthesia*, reflecting somatic effects; *Affect*, sensitive to emotional and affective responses; *Volition*, indicating the volunteer's capacity to willfully interact with his/her 'self' and/or the environment; *Cognition*, describing modifications in thought processes or content; *Perception*, measuring visual, auditory, gustatory and olfactory experiences; and finally *Intensity*, which reflects the strength of the overall experience [14]. In the present study a Spanish adaptation of the questionnaire was used [22].

Statistical analysis

EEG recordings

Statistical analysis of EEG recordings was performed following the IPEG (International Pharmaco-EEG Group) guideline on statistical design and analysis of pharmacodynamic trials [23]. Accordingly, the inferential strategy of descriptive data analysis (DDA) [24], as proposed for application to the mapping situation [25], was applied. In short, descriptive tests, preferably of simple null hypotheses such as equality of two treatment effects, are performed at all observation times, locations and measurements (variables). A nominal α-level for each test is chosen at 5%, and all P values lower than 0.05 are clearly distinguished in the graphical demonstration of the results. Therefore, the formal P value is calculated for each test, leading to certain pattern of P values in the whole data structure, of which the 'small' P values are indicative of areas of potentially true drug-effect-differences. Rather than considering these P values (should they be smaller than α) as a decision criterion for rejecting local null hypotheses (a procedure which would not be indicated in the absence of an α-correction measure), in DDA these patterns of small P values are analysed in a descriptive way in order to interpret results. This interpretation should be done not just by looking at the P values alone but by simultaneously taking into account the biomedical expectations based on the structure of the study. Therefore, the calculated P values and their pharmacologically sound patterns are used as 'judgement criteria'. Statistics included multivariate methods such as Hotelling T^2 to test overall differences between drugs, and paired t-tests to evaluate changes and interdrug differences in detail at different hours post-administration. According to the experimental design used, pharmacologically sound patterns of P values <0.05 would be those showing: (a) spatial clustering (b) time courses, and (c) dose dependencies. These results were displayed as significance probability maps. Additionally, dose/treatment-effect and time-effect relationships were explored by means of a multivariate, nonparametric approach [20]. Friedman tests and multiple Wilcoxon tests based on sign-adjusted changes in 28 V-EEG variables were applied. In all tests performed (parametric and nonparametric) PRE-2-values were considered as the predrug baseline, and comparisons were conducted with the randomized placebo.

Subjective ratings

HRS scores were analysed by means of a one-way analysis of variance (ANOVA) with repeated measures, with treatment (randomized placebo, *ayahuasca* low dose, *ayahuasca* high dose) as factor. Greenhouse-Geisser epsilon was used to correct possible violations of the sphericity assumption and to reduce Type I errors. Differences were considered statistically significant for $P<0.05$. When ANOVA showed significant differences between treatments, pairwise comparisons were carried out by means of t-tests, followed by Bonferroni correction.

Results

EEG recordings

(1) Pharmaco-EEG maps: multivariate analysis

In order to test the hypothesis that *ayahuasca* exerts significant central effects which induce modifications in brain electrical activity as compared with placebo, a multiple analysis of variance (MANOVA) with repeated measures was performed for V-EEG for each of the 19 electrodes. Treatment (randomized placebo, *ayahuasca*),

time (PRE-2, post) and the following set of variables: log-transformed absolute power values in the delta, theta, alpha-1, alpha-2, beta-1, beta-2, beta-3 and beta-4 frequency bands were considered in the MANOVA. Hotelling T^2 values were used in the significance probability maps to indicate differences between *ayahuasca*-induced and placebo-induced changes in brain electrical activity from baseline through 8 h after drug administration.

As shown in Figure 1, *ayahuasca* administration induced dose-dependent central effects as measured by the derived EEG variables, which were greater and longer lasting after the high dose. Thus, after the low 0.6 mg DMT kg^{-1} body weight dose, statistically significant differences with placebo were obtained only at isolated electrode locations between 45 min and 2.5 h postadministration. After the high 0.85 mg DMT kg^{-1} body weight dose, however, EEG changes were found over extensive scalp areas. These effects first attained statistical significance at 1 h, showed a peak between 1.5 and 2 h and gradually decreased thereafter, to disappear at 6–8 h. At the peak of the pharmacodynamic effects, variations in brain electrical activity were measured all over the scalp, with the greatest intensity in the central and right temporo-occipital electrodes.

(2) Pharmaco-EEG maps: univariate analysis

Topographic brain maps based on *t*-tests are described to show detailed drug-induced changes in the individual EEG variables.

Total power As shown in Figure 2, *ayahuasca* produced a significant and dose-dependent reduction in total power in electrodes located all over the scalp, with a temporal peak at 90 min after administration of the high dose. Both the centroid of the total activity and its deviation showed significant and dose-dependent increases peaking at 120 and 90 min, respectively.

Slow activity The effects of *ayahuasca* on slow activity are shown in Figure 3. Absolute power of the combined delta-theta activity was decreased in a dose-dependent manner after dosing with *ayahuasca*, with the peak

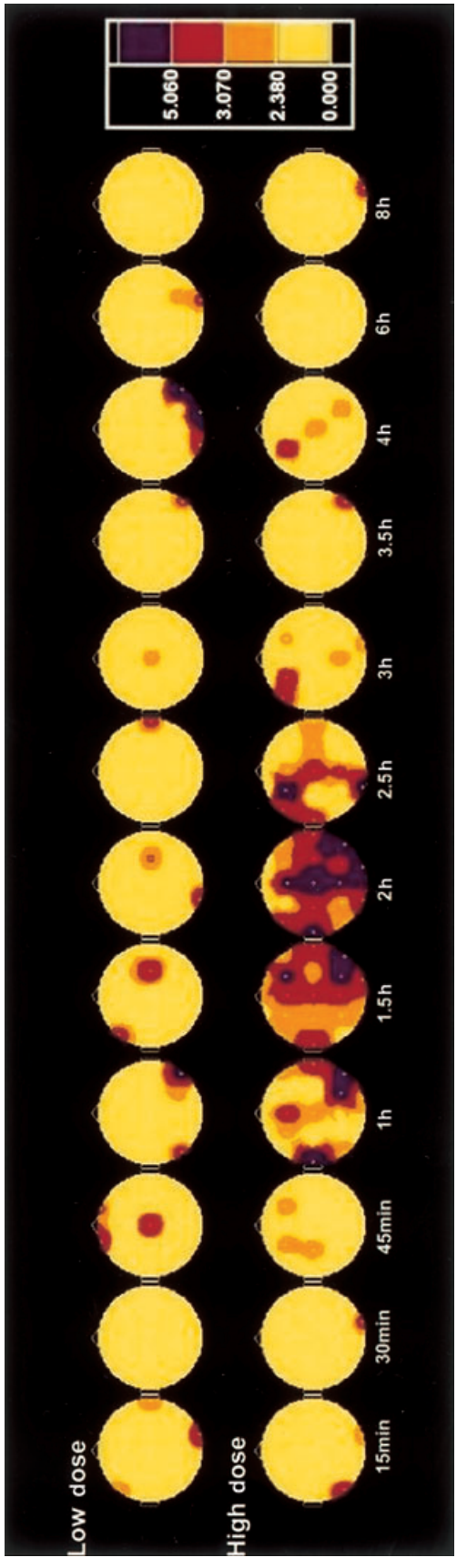
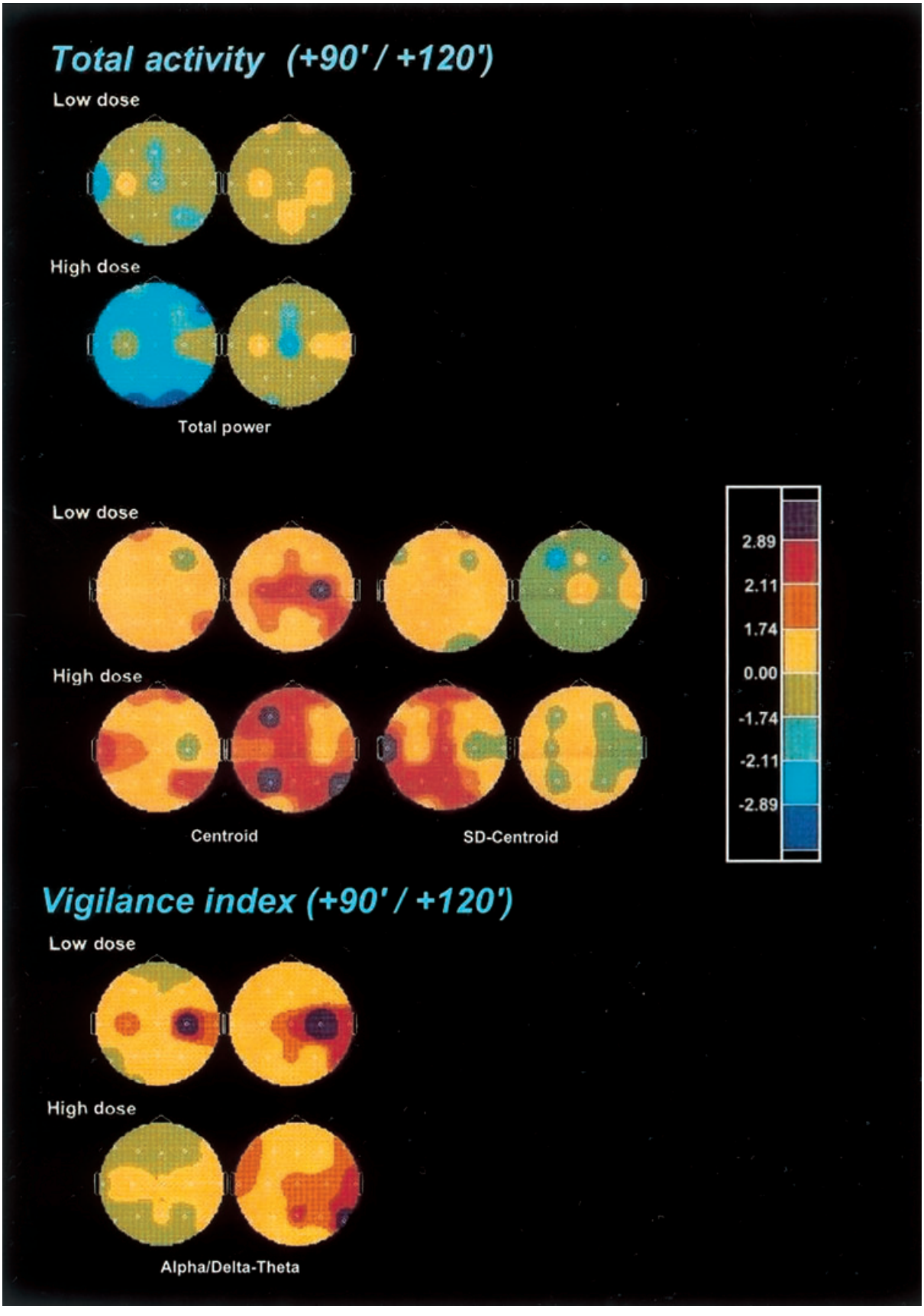


Figure 1 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced central effects at 12 time points *vs* baseline values (PRE-2) after low (upper row) and high (lower row) doses of *ayahuasca* ($n = 18$). The vertex view shows the nose at the top, the left ear to the left, the right ear to the right. Electrode positions are indicated by white dots. Maps are based on Hotelling T^2 obtained from multivariate tests in repeated measures ANOVAs on eight logarithmically transformed absolute power values in delta, theta, alpha-1, alpha-2, beta-1, beta-2, beta-3 and beta-4 frequency bands. The colour key shows T^2 values with hot/red colours indicating significant differences: $T^2 > 2.38 = P < 0.10$, $> 3.07 = P < 0.05$ and $> 5.06 = P < 0.01$.



decreases at 90 min for the low dose and between 90 and 120 min for the high dose. When examined separately, both the delta and theta frequency bands showed decreases in absolute power. However, the most dramatic decreases were found in the theta band, an effect which showed a dose-dependent pattern and peaked between 90 and 120 min.

Relative power of the combined delta-theta bands was also dose-dependently decreased, with the peak reductions at 120 min. Decreases in relative power were marginal for the delta band, while they were prominent and dose-dependent for the theta band. These reductions in relative power were maximal at 120 min, showing a widespread distribution all over the scalp.

The centroid of the combined delta-theta activity showed a significant though modest deceleration, with a significant increase of its deviation. Nevertheless, although dose-dependent, the deceleration of the centroid was not uniformly distributed over the scalp, showing the greatest decreases at C3, T4 and O1 at the high *ayahuasca* dose at 90 min after administration. At the high dose, the significant increase seen for the deviation of the centroid was obtained at 120 min and was restricted to the Pz and P3 leads.

Alpha activity The effects of *ayahuasca* on alpha activity are shown in Figure 4. Absolute alpha activity was significantly and dose-dependently decreased after *ayahuasca*. The decreases were more prominent at the high dose in the left-temporal and centro-parieto-occipital electrodes. The maximal decrease was observed at 90 min after administration. When separately examined, the alpha-2 band showed more significant and more widely distributed decreases than the alpha-1 band. Differently from the maximal total alpha and alpha-1 power decreases, the reductions in absolute power for the alpha-2 band peaked at 60 min after administration (not shown).

Relative alpha activity was significantly increased at 120 min after administration, showing an inverse dose-reponse pattern, with maximal increase after the low dose. While this increase was consistently observed in the alpha-1 sub-band, in the alpha-2 sub-band a decrease which reached the highest significance at 60 min after the intake was seen (not shown).

No consistent pattern of changes was observed after *ayahuasca* in the dominant frequency within the alpha band (not shown). A tendency towards statistical significance was seen in the absolute power of the dominant frequency (predominantly decreases) which reached significance marginally in some electrode sites between 45 and 120 min after administration of the high dose. Conversely, relative power of the dominant frequency did show statistically significant increases after the low and the high *ayahuasca* doses at 120 min after administration. Finally, no consistent drug-induced effects were found either for the centroid of the alpha activity or its deviation.

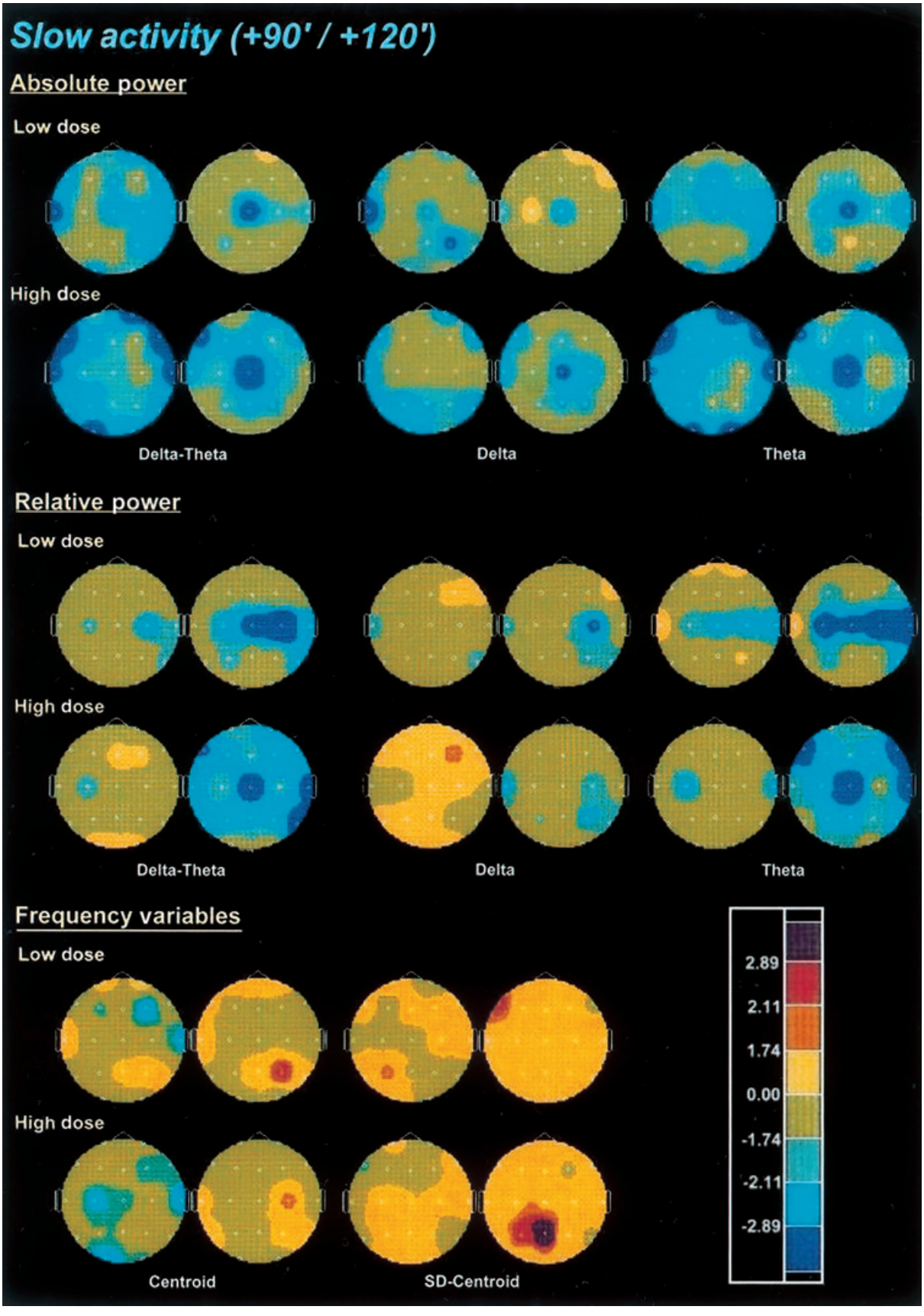
Fast activity The effects of *ayahuasca* on fast activity are shown in Figure 5. The absolute power of global beta activity was dose-dependently decreased by *ayahuasca*, with a maximal decrement at 90 min after administration. When split between the four frequency subbands, absolute power decreases were found to be more intense in the beta-1 range, with power decreases becoming less prominent as one moved to beta-2, beta-3 and beta-4. Peak decreases were observed at 90 min after administration, except for beta-3 which were more prominent at 45 min (not shown).

As far as relative power in the beta frequency range is concerned, statistically significant increases were found, these being more intense and longer-lasting at the high relative to the low *ayahuasca* dose. The maximal increments were obtained between 45 and 90 min after administration. Compared with absolute power values, the examination of relative power in the individual beta subbands rendered an inverse pattern of variation. Thus, relative power increases were marginally significant for the beta-1 band, became more widespread over the scalp for beta-2, more significant for beta-3 and were maximal for beta-4. Increases in the relative power of the beta-4 frequencies showed a predominant central and parieto-temporal distribution. Statistical significance for relative power increases for beta-2, beta-3 and beta-4 was obtained between 45 and 120 min after administration, with the maximal increase at 90 min.

The centroid of the beta frequency range showed a statistically significant and dose-dependent shift toward the higher values after *ayahuasca*, which also peaked at 90 min after administration. The deviation of the centroid was not significantly modified by the drug.

Table 1 lists 95% confidence intervals for changes in absolute (μV^2) and relative (%) power in all frequency bands at 90 and 120 min following the administration of

Figure 2 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced changes in total power and frequency variables of the EEG total activity (1.3–30 Hz), and in the alpha/delta-theta vigilance index, after low (upper rows) and high (lower rows) doses of *ayahuasca* ($n=18$) at 90 min (left) and 120 min (right) after administration *vs* baseline values (PRE-2). The vertex view shows the nose at the top, the left ear to the left, the right ear to the right. Electrode positions are indicated by white dots. Eight-colour scale represents drug-induced changes as compared with placebo based on *t*-values: lilac, increase at $P<0.01$; red, increase at $P<0.05$; ochre, increase at $P<0.10$; pale yellow, trend towards increase; pale green, trend towards decrease; bright green, decrease at $P<0.10$; light blue, decrease at $P<0.05$; dark blue, decrease at $P<0.01$.



the low and high *ayahuasca* doses in a single representative electrode (P3).

Vigilance index: alpha/delta-theta The alpha/delta-theta ratio (Figure 2) was also calculated for each of the recorded time points. This index showed a significant increase, relative to placebo, both after the low and the high *ayahuasca* doses between 90 and 150 min, with the maximal increase at 120 min.

(3) Non-parametric multilead EEG analysis

Dose/treatment-effect relationships were calculated using Friedman and multiple Wilcoxon tests of sign-adjusted changes from PRE-2-values in 28 V-EEG variables obtained in the 19 leads. As shown in Table 2, based on the rank-sums, administered at the low dose *ayahuasca* could only be differentiated from randomised placebo at 45 min and 60 min after dosing. At the high dose, however, statistically significant differences were found from 45 min through 120 min after administration. Pairwise comparisons considering the total rank-sum showed statistically significant differences between randomised placebo and each of the *ayahuasca* doses, and between the low and high *ayahuasca* doses.

Time-effect relationships were calculated using Friedman and multiple Wilcoxon tests for randomised placebo-corrected sign-adjusted changes from PRE-2-values in 28 V-EEG variables obtained in the 19 leads, as shown in Figure 6. After *ayahuasca* administration, changes on EEG variables were seen as early as 15–30 min, followed by a steep increase at 45 min in rank-sum values. At the high dose, *ayahuasca* showed the pharmacodynamic peak between 45 and 90 min, with rank-sum values gradually decreasing thereafter and approaching baseline at 4–6 h after administration. At the low dose, an analogous curve was found, with the pharmacodynamic peak between 45 and 90 min having an analogous subsequent decrease to that of the high dose. Compared to baseline values, at the low dose increases in rank-sum values did not reach statistical significance at any of the time points evaluated. At the high dose, statistically significant differences were found at 45, 60 and 90 min after administration.

Subjective ratings

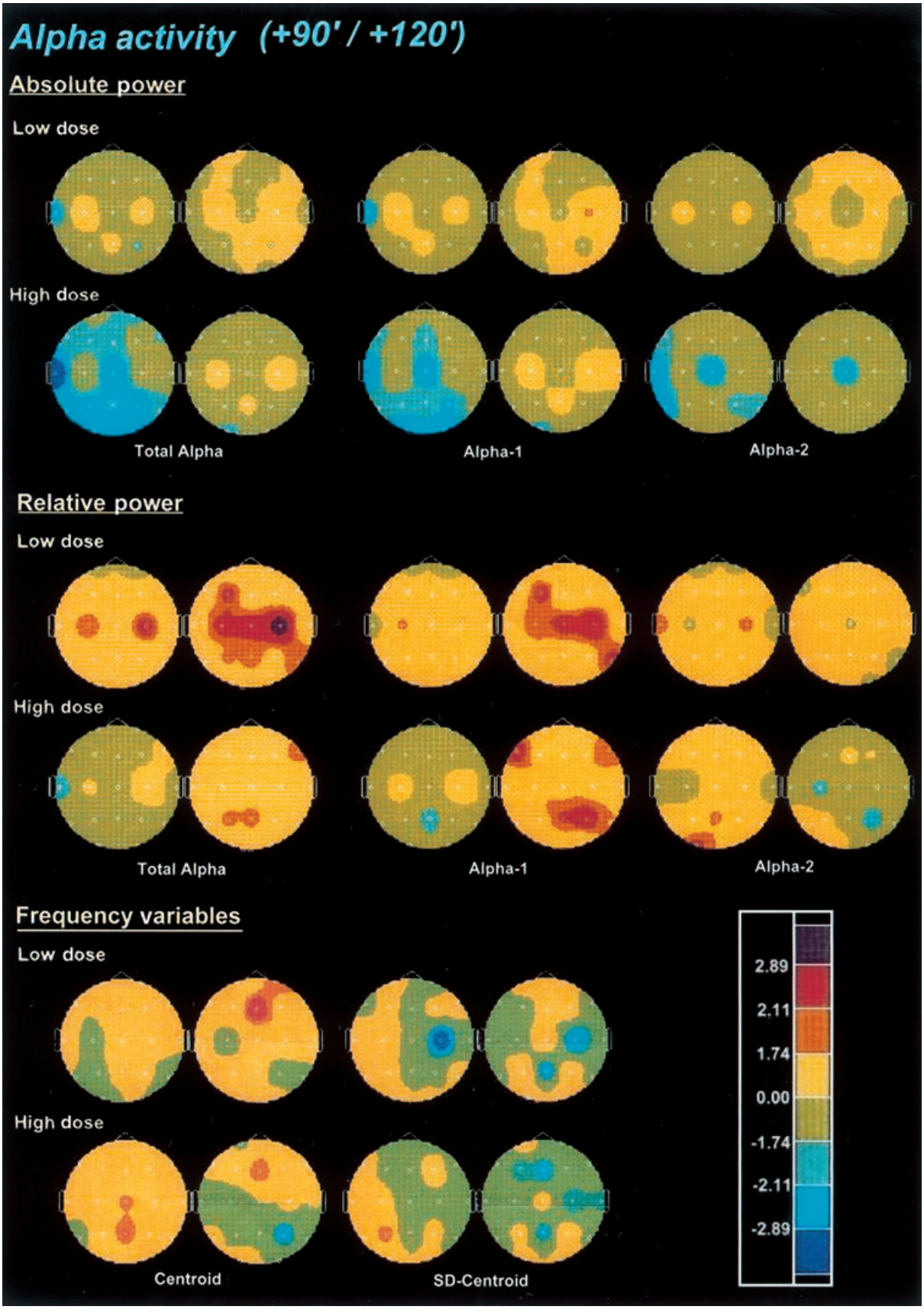
As shown in Table 3, *ayahuasca* induced significant dose-dependent increases in all subscales of the HRS, an instrument specifically designed to quantify the effects of psychedelic drugs. *Ayahuasca* was thus capable of inducing a modified state of awareness in which a psychedelic profile was prominent. At the low dose, all HRS subscales showed statistically significant increases relative to placebo, except for Volition, a measure of impairment in the capacity of the volunteer to interact with his/herself and his/her surroundings. This subscale however, reached statistical significance at the high dose, indicating that of the six aspects measured by the HRS, this was the least modified by *ayahuasca*. Qualitatively, the profile of effects induced by *ayahuasca* included paresthesias and perceptual modifications of predominantly visual, and to a lower extent, auditive nature. This coexisted with more elaborated modifications in thought, associations and emotion, in a global experience described as similar to dreaming activity.

Discussion

The administration of *ayahuasca* to a group of healthy volunteers induced a dose-dependent pattern of subjective effects typical of the psychedelics, replicating the profile obtained in a previous study [12]. In addition to results obtained by means of self-assessment instruments, the implementation of q-EEG demonstrated a significant effect of *ayahuasca*, as compared with placebo, on the human CNS. These effects consisted of an overall decrease in absolute power for all the frequency bands evaluated, and an acceleration of the centre-of-gravity frequency. Absolute power decreases were most prominent in theta, delta and slow beta bands, while the alpha and fast beta rhythms were less intensely affected. Relative power was found to be significantly decreased in the theta, and to a lower extent, delta band. In the alpha band, relative power showed an increase, predominantly in the alpha-1 subband, and significant increases were also obtained in relative power in the beta frequency band. These increases in relative fast activity were most prominent in the beta-3 and beta-4 subbands. Additionally, the alpha/delta-theta ratio, an index of activation, was found to be increased after *ayahuasca*.

The evaluation of the plots of the rank-sums of changes measured at the 19 leads over time showed the first increases between 15 and 30 min, which were followed by a steep rise at 45 min, reaching the maximum effects between 45 and 90 min EEG measures gradually declined thereafter to reach baseline values around 4–6 h after administration. Most remarkably, these objectively measured effects of the drug on the spontaneous brain electrical

Figure 3 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced changes in absolute power, relative power and frequency variables of the combined slow activity (1.3–7.5 Hz), delta (1.3–3.5 Hz) and theta (3.5–7.5 Hz) frequency bands after low (upper rows) and high (lower rows) doses of *ayahuasca* ($n = 18$), at 90 min (left) and 120 min (right) after administration *vs* baseline values (PRE-2). For technical description of the maps and explanation of the colour key see Figure 2.



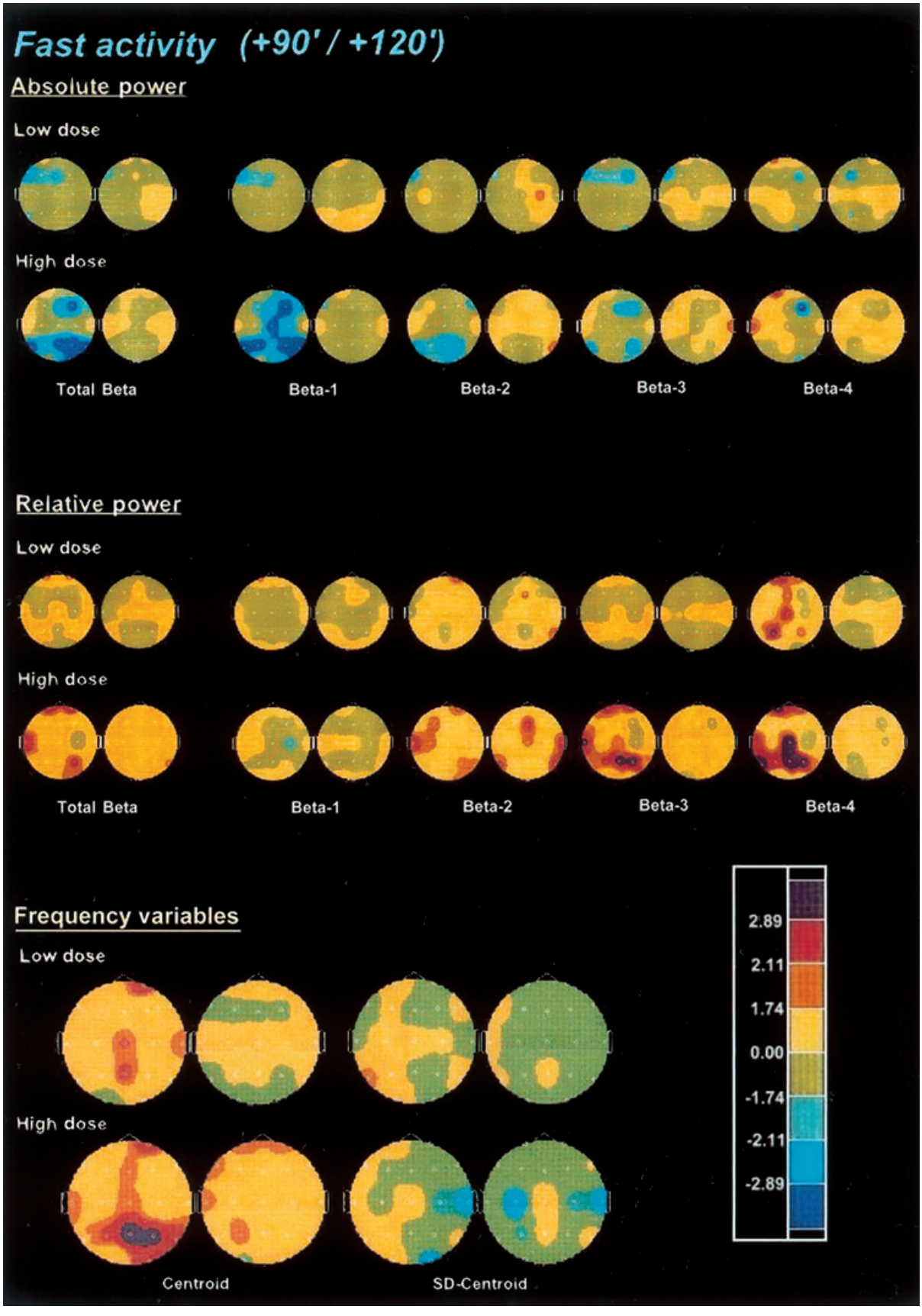
activity closely paralleled the time course of subjectively experienced effects, measured by means of self-report visual analogue scales, as previously reported [12].

To our knowledge, only one previous study has addressed the evaluation of EEG activity in humans after the ingestion of *ayahuasca*. A recent article reported the evaluation of the EEG effects of *ayahuasca* in a group of nine subjects in field conditions [26]. In the cited study, EEG recordings were obtained in the course of a ritual *Daime* session in Brazil. The study was conducted in the absence of a placebo control, and only with an approximate knowledge of the ingested *ayahuasca* dose, this being on average 0.67 mg DMT kg^{-1} body weight. These investigators reported significant changes after *ayahuasca* in relation to baseline values only in the 36–44 Hz band. Given that this frequency range was not evaluated in the present study, it is impossible to establish a comparison with the results obtained in the aforementioned study. Nevertheless, Don *et al.* also reported a pattern of changes in the classical frequency bands which did not reach statistical significance but which bore similarities to that observed in the present study. These nonsignificant variations included a 'slight increase in beta', and a 'slight decrease in theta and alpha'.

The changes in brain electrical activity observed in the present study are difficult to relate entirely to any pharmaco-EEG profile characteristic of the main psychotropic drug groups. Even a direct comparison with other psychedelics is far from easy. Virtually no studies have been conducted in the last 30 years regarding the effects of these drugs on the human EEG. The quantitative approach to the effects of psychedelics on the human EEG was absent at the time they attracted the greatest interest from psychiatry and psychopharmacology researchers in the 1950s and 1960s. Most of the information available from the early research conducted with these compounds is essentially qualitative. In these studies only marginal changes were described after the administration of psilocybin, mescaline or LSD on the visually inspected EEG trace, reporting at most an increase in fast rhythms and an overall decrease in signal amplitude [27]. Itil and coworkers, however, conducted a number of studies combining visual inspection and power spectrum analysis of the recordings obtained after administering anticholinergic compounds with true hallucinogenic properties, such as atropine, and serotonergic psychedelics like LSD. These researchers found almost opposite EEG patterns for these two groups of compounds. While atropine caused the alpha rhythm to disappear and the predominance of low-voltage slow waves, they concluded that the most characteristic effects of LSD were a reduction of theta activity and slow waves in general, as well as an increase in fast activity [27, 28]. In line with these observations, in the present study both absolute and relative power of slow activity decreased after *ayahuasca*, specially in the theta band. With regard to fast activity, while absolute power was decreased following *ayahuasca* administration, a marked enhancing effect was obtained for relative power. The milder increases were found for the slower beta-1 and beta-2 sub-bands and the most intense in the faster beta-3 and beta-4 sub-bands.

Ayahuasca shares the decremental effects seen on delta and theta power with both psychostimulants, such as amphetamine and methylphenidate, and serotonin releasers such as fenfluramine [29, 30]. Interestingly, psychostimulants act predominantly enhancing dopaminergic neurotransmission, in contrast with the serotonergic properties of psychedelics. However, a recent neuroimaging study in humans has shown that dopamine release takes place in the basal ganglia and the ventral striatum after the administration of psilocybin to humans [31], pointing to a role of dopaminergic neurotransmission in the effects of the classical psychedelics. Additional similarities are also to be found between the relative beta-3 and beta-4 band enhancing properties found for *ayahuasca*, and the analogous effect obtained after tricyclic antidepressants, which characterizes the group [29]. Drugs belonging to this pharmacological class inhibit the re-uptake of monoamines, which leads to increased levels of these endogenous compounds in the synapse [32]. (+)-Fenfluramine and the selective serotonin reuptake inhibitor fluoxetine also lead to increases in relative beta power [30, 33], an effect which is also shared by antidepressants showing MAOI properties [34]. It is consequently reasonable to assume that the blocking effects of the β-carbolines on MAO may have led to increased levels of monoamines, due to the blockade of their metabolism, which in turn may have contributed to the relative beta-promoting effect of *ayahuasca*. Regarding slow activity, the theta-dampening activity of psychostimulants and psychedelics is diametrically opposed to the theta-enhancing action of the classical neuroleptics such as haloperidol and chlorpromazine [30, 35]. This theta-enhancing action has also been observed in drugs with a mixed anti-D_2 and anti-5-HT_2 profile, such as risperidone [36], or the more selective 5-HT_2 blocker ketanserin [37], suggesting a

Figure 4 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced changes in absolute power, relative power and frequency variables of total alpha activity (7.5–13 Hz), alpha-1 (7.5–10.5 Hz), and alpha-2 (10.5–13 Hz) frequency bands after low (upper rows) and high (lower rows) doses of *ayahuasca* ($n = 18$), at 90 min (left) and 120 min (right) after administration *vs* baseline values (PRE-2). For technical description of the maps and explanation of the colour key see Figure 2.



pro-dopaminergic and pro-serotonergic activity for *ayahuasca*.

DMT, the main psychotropic agent in *ayahuasca*, not only binds to the 5-$HT_{2A/2C}$ receptors, located mainly at a postsynaptic level, but also shows affinity for the 5-HT_{1A} sites, which in certain brain regions correspond predominantly to somatodendritic autoreceptors [38]. Thus, DMT probably displays agonist activity also at the 5-HT_{1A} sites, a pattern shared by other indole psychedelics, in contrast with the phenethylamines like mescaline, which interact only with the 5-$HT_{2A/2C}$ receptors [39]. The pharmaco-EEG profile of drugs displaying selective agonist or partial agonist activity at the 5-HT_{1A} site has been described, allowing a more detailed discussion on the probable biochemical mechanisms involved in the EEG effects of *ayahuasca*. Indeed buspirone, a partial 5-HT_{1A} agonist, has been shown to produce marked increases in theta power, in the absence of other relevant EEG modifications [40]. As an opposed pattern was seen for the theta band after *ayahuasca*, one could postulate that 5-HT_{1A} agonism does not seem to be the predominant contribution at a molecular level to the EEG effects of *ayahuasca*. This is consistent with data from a previous study, in which increases in the intensity of the psychological effects elicited by intravenous DMT following blockade of the 5-HT_{1A} sites by pindolol were reported [41]. The observed increases suggest both that agonism at the 5-HT_{1A} site is not essential to obtain a psychedelic effect profile, and that a decreased binding of DMT at the 5-HT_{1A} sites leads to an increase in the amount of DMT available to interact with the 5-HT_2 receptors, and consequently to the enhanced subjective effects experienced by the volunteers. Thus, the present q-EEG findings would rather support a preponderant involvement of the 5-HT_2 receptor in the genesis of the central effects of the beverage.

To sum up, the cerebral bioavailability and psychoactivity of *ayahuasca* could be objectively measured by means of q-EEG, which evidenced a clear dose-dependent effect at the doses administered. Remarkably, the time

Table 1 95% confidence intervals for changes in absolute (μV^2) and relative (%) power in all frequency bands at 90 and 120 min, following the administration of the low 0.6 mg DMT kg^{-1} body weight, and high 0.85 mg DMT kg^{-1} body weight *ayahuasca* doses, in a single representative electrode (P3). All changes *vs* baseline (PRE-2) and randomized placebo. Data from 18 volunteers, showing mean change ±1.96 s.e.mean.

	Low dose		*High dose*	
	90 min	*120 min*	*90 min*	*120 min*
Absolute power (μV^2)				
Total power (1.3–30 Hz)	−5.70±18.62	−5.60±13.72	−20.20±15.23★	−8.30±18.07
Delta (1.3–3.5 Hz)	−1.20±1.57	−1.30±1.82	−1.40±1.10★	−1.70±1.84
Theta (3.5–7.5 Hz)	−1.10±2.70	−1.70±1.45★	−2.70±2.21★	−2.00±2.45
Alpha-1 (7.5–10.5 Hz)	−0.40±7.84	−3.00±8.41	−11.30±11.07★	−1.70±11.11
Alpha-2 (10.5–13 Hz)	−2.00±3.58	0.70±2.74	−2.60±3.65	−2.00±4.90
Beta-1 (13–16 Hz)	−0.30±0.53	0.01±1.96	−0.80±0.49★	−0.40±0.71
Beta-2 (16–20 Hz)	−0.50±0.82	−0.20±0.57	−1.00±0.98★	−0.30±0.84
Beta-3 (20–25 Hz)	−0.20±0.35	0.10±0.65	−0.40±0.53	−0.10±0.49
Beta-4 (25–30 Hz)	0.01±1.96	−0.10±0.12	−0.01±0.10	−0.01±0.06
Relative power (%)				
Delta (1.3–3.5 Hz)	−1.20±3.35	−1.80±2.70	0.50±1.63	−1.20±1.31
Theta (3.5–7.5 Hz)	−1.30±3.65	−3.20±2.98★	−1.40±2.12	−3.30±2.59★
Alpha-1 (7.5–10.5 Hz)	1.70±6.66	3.10±5.06	−2.70±5.88	4.40±5.39
Alpha-2 (10.5–13 Hz)	0.20±3.92	1.90±2.86	2.00±3.57	0.10±1.96
Beta-1 (13–16 Hz)	−0.20±0.65	0.01±1.96	−0.20±0.78	−0.40±0.61
Beta-2 (16–20 Hz)	0.30±0.59	0.10±0.39	0.40±0.57	0.30±0.53
Beta-3 (20–25 Hz)	0.20±0.49	−0.10±0.65	1.00±0.88★	0.20±0.65
Beta-4 (25–30 Hz)	0.20±0.14★	−0.10±0.16	0.30±0.24★	−0.10±0.27

Statistically significant differences *vs* placebo (★$P<0.05$) obtained after Student's *t*-test are indicated.

Figure 5 Significance probability maps showing differences between *ayahuasca*-induced and placebo-induced changes in absolute power, relative power and frequency variables of the combined fast activity (13–30 Hz), beta-1 (13–16 Hz), beta-2 (16–20 Hz), beta-3 (20–25) and beta-4 (25–30) frequency bands after low (upper rows) and high (lower rows) doses of *ayahuasca* ($n=18$), at 90 min (left) and 120 min (right) after administration *vs* baseline values (PRE-2). For technical description of the maps and explanation of the colour key see Figure 2.

Table 2 Dose/treatment-effect relationships after single oral doses of randomized placebo (A), low dose 0.6 mg DMT kg^{-1} body weight *ayahuasca* (B), high dose 0.85 mg DMT kg^{-1} body weight *ayahuasca* (C), and non-randomized placebo administered on the first (adaptation) experimental session (D). Data from 18 volunteers, based on sign-adjusted changes in 28 V-EEG variables (rank-sums, means of 19 electrodes, differences from PRE-2 baseline values).

Time (min)	*Randomized placebo (A)*	*Low dose (B)*	*High dose (C)*	*Adaptation placebo (D)*	χ^2	*Multiple Wilcoxon*
15	71.8	69.3	69.9	69.0	0.1	
30	63.5	76.8	79.9	59.8	6.3	
45	51.4	76.7	92.6	59.4	22.3**	A : B*, A : C**, D : C**
60	53.1	85.6	85.9	55.4	21.5**	A : B**, A : C** D : B**, D : C**
90	55.7	77.8	94.8	51.7	26.3**	A : C** D : B*, D : C**
120	62.0	72.5	90.3	55.2	15.2**	A : C* D : C*
150	62.1	74.8	86.3	56.8	11.3**	A : C(*) D : C*
180	65.9	74.7	74.4	64.9	1.5	
210	75.1	60.8	73.4	70.7	2.7	
240	76.3	62.1	71.4	70.3	2.6	
360	80.6	62.0	75.1	62.4	5.9	
480	70.2	62.7	83.7	63.3	5.9	
Total	787.7	855.8	977.7	738.9	57.7**	A : B*, C** D : B**, C** B : C**

(*) $= P<0.1$; *$P<0.05$; **$P<0.01$.

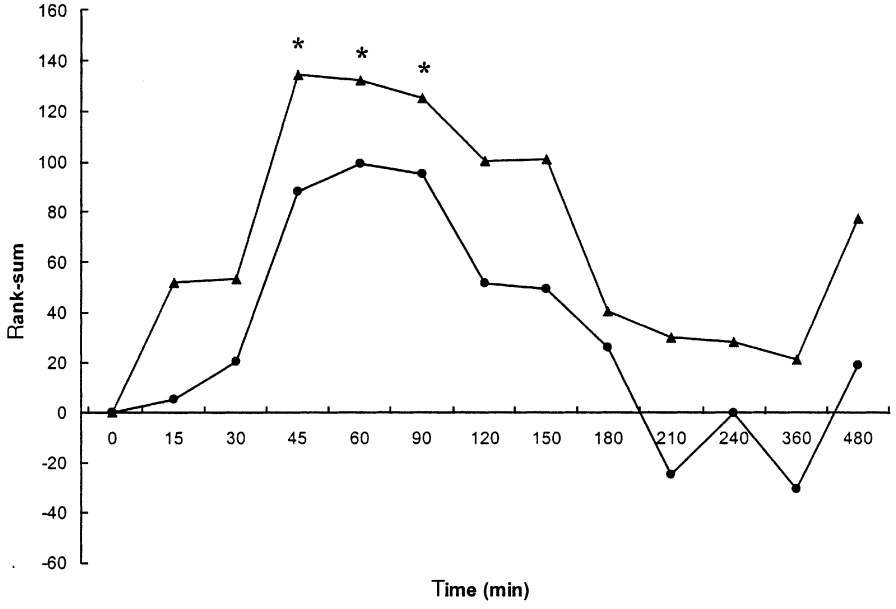


Figure 6 Time-effect relationships after single oral doses of 0.6 mg DMT kg^{-1} body weight *ayahuasca* (low dose) [●], and 0.85 mg DMT kg^{-1} body weight *ayahuasca* (high dose) [▲]. Plots show differences from baseline values (PRE-2) of sign-adjusted changes in 28 V-EEG variables (rank-sums, means of 19 electrodes, randomized placebo-corrected) from 18 volunteers. An asterisk indicates significant differences form baseline values obtained by means of multiple Wilcoxon.

pattern obtained for EEG effects closely paralleled that of previously reported subjective effects. The global reduction in total power and the shift toward higher frequencies after *ayahuasca* are in line with older reports on the classical serotonergic psychedelics, which described an amplitude reduction and a suppresion of slow activity in the human EEG. Finally, the detailed assessment of *ayahuasca* effects on the different EEG variables indicated common features

Table 3 Means (s.d.) of the scores obtained for the HRS questionnaire subscales ($n=18$) after single oral doses of randomized placebo, low dose 0.6 mg DMT kg^{-1} body weight *ayahuasca* and high dose 0.85 mg DMT kg^{-1} body weight *ayahuasca*, and results of the statistical analyses performed. Student's *t*-tests were followed by Bonferroni correction.

	ANOVA		*Student's* t-*test* vs *Placebo*		vs *Low dose*
Variable	P *value*	*Placebo*	*Low dose*	*High dose*	*High dose*
HRS					
Somaesthesia	***	0.07 (0.10)	0.50 (0.41)**	0.97 (0.40)**	**
Perception	***	0.09 (0.19)	0.55 (0.49)**	1.10 (0.67)**	**
Cognition	***	0.06 (0.16)	0.4 (0.45)**	0.96 (0.59)**	**
Volition	*	0.81 (0.79)	1.11(0.69)	1.35 (0.61)*	NS
Affect	***	0.32 (0.21)	0.65 (0.36)**	1.02 (0.38)**	**
Intensity	***	0.24 (0.45)	1.32 (0.73)**	1.85 (0.51)**	**

*$P<0.05$; **$P<0.01$; ***$P<0.001$; NS = not significant.

with the profile shown by pro-dopaminergic and pro-serotonergic drugs, and supports the involvement of serotonergic 5-HT_2 and dopaminergic D_2-receptor agonism in the central effects of *ayahuasca*.

We would like to thank Esther Martínez, Félix González and José María Fábregas for their continued support to our research project, and also CEFLURIS in Brazil for providing the *ayahuasca* (*Daime*) used in the present study. We are also greatful to James C. Callaway of the Department of Pharmaceutical Chemistry of the University of Kuopio, Finland, for quantifying the DMT in *ayahuasca*, and Maria Montero, Hospital de Sant Pau, Barcelona, for conducting the psychiatric interviews. Finally, our thanks to Rosa Antonijoan, Sylvie Cotxet, Llúcia Benito, Susanna Clos and David Martínez for their assistance during data-collection, and to Angeles Funes for editing the figures.

References

1 Dobkin de Rios M. *Visionary Vine: Hallucinogenic Healing in the Peruvian Amazon*. Prospect Heights, Illinois: Waveland Press, 1984.
2 Schultes RE, Hofmann A. *Plantas de los dioses: orígenes del uso de los alucinógenos*. México D.F. Fondo de Cultura Económica, 1982.
3 Anonymous. L'Ayahuasca: de l'Amazonie à la Jungle Urbaine. In *La Géopolitique Mondiale Des Drogues 1998/1999, Paris: Observatoire Géopolitique Des Drogues*. 2000; 102–106.
4 Callaway JC, Grob CS. Ayahuasca preparations and serotonin reuptake inhibitors: a potential combination for severe adverse interactions. *J Psychoactive Drugs* 1998; **30**: 367–369.
5 Rivier L, Lindgren JE. 'Ayahuasca', the South American hallucinogenic drink. An ethnobotanical and chemical investigation. *Econ Bot* 1972; **26**: 101–129.
6 McKenna DJ, Towers GHN, Abbott F. Monoamine oxidase inhibitors in South American hallucinogenic plants. Tryptamine and β-carboline constituents of *ayahuasca*. *J Ethnopharmacol* 1984; **10**: 195–223.
7 Schultes RE, Hofmann A. *The Botany and Chemistry of Hallucinogens*. Springfield, Illinois: Charles C. Thomas, 1980.
8 Marek GJ, Aghajanian GK. Indoleamine and phenethylamine hallucinogens: mechanisms of psychotomimetic action. *Drug Alcohol Depend* 1998; **51**: 189–198.
9 Smith RL, Canton H, Barrett RJ, Sanders-Bush E. Agonist properties of *N,N*-dimethyltryptamine at serotonin 5-HT_{2A} and 5-HT_{2C} receptors. *Pharmacol Biochem Behav* 1998; **61**: 323–330.
10 Suzuki O, Katsumata Y, Oya M. Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. *Biochem Pharmacol* 1981; **30**: 1353–1358.
11 Callaway JC, McKenna DJ, Grob CS, *et al*. Pharmacokinetics of *Hoasca* alkaloids in healthy humans. *J Ethnopharmacol* 1999; **65**: 243–256.
12 Riba J, Rodríguez-Fornells A, Urbano G, *et al*. Subjective effects and tolerability of the South American psychoactive beverage *Ayahuasca* in healthy volunteers. *Psychopharmacology* 2001; **154**: 85–95.
13 Gouzoulis-Mayfrank E, Thelen B, Habermeyer E, *et al*. Psychopathological, neuroendocrine and autonomic effects of 3,4-methylenedioxyethylamphetamine (MDE), psilocybin and *d*-methamphetamine in healthy volunteers. *Psychopharmacology* 1999; **142**: 41–50.
14 Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R. Dose–response study of *N,N*-dimethyltryptamine in humans, II. Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry* 1994; **51**: 98–108.
15 Strassman RJ. Human psychopharmacology of LSD, dimethyltryptamine and related compounds. In *50 Years of LSD. Current Status and Perspectives of Hallucinogens*, eds Pletscher A, Ladewig D. New York: Parthenon, 1994; 145–174.
16 Saletu B. The use of pharmaco-EEG in drug profiling. In *Human Psychopharmacology. Measures and Methods*, Vol. 1, eds Hindmarch I, Stonier PD. Chichester: John Wiley, Sons, 1987; 173–200.
17 Spielberger CD, Gorsuch RL, Lushene RE. *Manual for the State-Trait Anxiety Inventory*. Palo Alto: Consulting Psychologists Press, 1970.
18 Callaway JC, Raymon LP, Hearn WL, *et al*. Quantitation of *N,N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with *ayahuasca*. *J Anal Toxicol* 1996; **20**: 492–497.
19 Semlitsch HV, Anderer P, Schuster P, Presslich O. A solution for reliable and valid reduction of ocular artifacts, applied to the P300 ERP. *Psychophysiology* 1986; **23**: 695–703.

20 Anderer P, Saletu B, Kinsperger K, Semlitsch H. Topographic brain mapping of EEG in neuropsychopharmacology – Part I. Methodological aspects. *Meth Find Exp Clin Pharmacol* 1987; **9**: 371–384.
21 Anderer P, Semlitsch HV, Saletu B, Barbanoj MJ. Artifact processing in topographic mapping of electroencephalografic activity in neuropsychopharmacology. *Psychiatry Res Neuroimaging* 1992; **45**: 79–93.
22 Riba J, Rodríguez-Fornells A, Strassman RJ, Barbanoj MJ. Psychometric assessment of the Hallucinogen Rating Scale. *Drug Alcohol Depend* 2001; **62**: 215–223.
23 Ferber G, Abt K, Fichte K, Luthringer R. IPEG guideline on statistical design and analysis for pharmacodynamic trials. International Pharmaco-EEG group. *Neuropsychobiology* 1999; **39**: 92–100.
24 Abt K. Descriptive data analysis. A concept between confirmatory and exploratory data analysis. *Meth Inf Med* 1987; **26**: 77–78.
25 Abt K. Statistical aspects of neurophysiologic topography. *J Clin Neurophysiol* 1990; **7**: 519–534.
26 Don NS, McDonough BE, Moura G, *et al*. Effects of *Ayahuasca* on the human EEG. *Phytomedicine* 1998; **5**: 87–96.
27 Oughourlian JM, Rougeul A, Verdeaux J. Action des hallucinogènes sur l'électroencéphalogramme. *Thérapie* 1971; **26**: 953–968.
28 Itil T, Fink M. Klinische Untersuchungen und quantitative EEG-Daten bei experimentellen Psychosen. *Arzneimittelforschung* 1966; **16**: 237–239.
29 Herrmann WM, Schaerer E. Pharmaco EEG: computer EEG analysis to describe the projection of drug effects on a functional cerebral level in humans. In *Handbook of Electroencephalography and Clinical Neurophysiology*, Vol. 2, *Clinical application of computer analysis of EEG & other neurophysiological signals*, eds Lopes da Silva FH, Storm van Leeuwen W, Rémond A. Amsterdam: Elsevier, 1986; 385–445.
30 Saletu B, Barbanoj MJ, Anderer P, Sieghart W, Grünberger J. Clinical-pharmacological study with two isomers (*d*-, *l*-) of fenfluramine and its comparison with chlorpromazine and *d*-amphetamine: blood levels, EEG mapping and safety evaluation. *Meth Find Exp Clin Pharmacol* 1993; **15**: 291–312.
31 Vollenweider FX, Vontobel P, Hell D, Leenders KL. 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man. A PET study with [^{11}C]raclopride. *Neuropsychopharmacology* 1999; **20**: 424–433.
32 Baldessarini RJ. Drugs and the treatment of psychiatric disorders: depression and mania. In *The Pharmacological Basis of Therapeutics*, Ninth Edition, eds Hardman JG, Limbird LE. New York: McGraw-Hill, 1996; 431–459.
33 Barbanoj MJ, Antonijoan RM, Morte A, Riba J, Jané F. Study of human psychotropic drug interactions by means of q-EEG. In *Electrophysiological Brain Research in Preclinical, Clinical Pharmacology, Related Fields – An Update*, eds Saletu B, Krijzer F, Ferber G, Anderer P. Vienna: International Pharmaco-EEG Group, 2000; 164–172.
34 Saletu B, Grünberger J. On acute and chronic CNS effects of antidepressants in normals: neurophysiological, behavioral and pharmacokinetic studies with pirlindol. *Meth Find Exp Clin Pharmacol* 1985; **7**: 137–151.
35 McClelland GR, Cooper SM, Pilgrim AJ. A comparison of the central nervous system effects of haloperidol, chlorpromazine and sulpiride in normal volunteers. *Br J Clin Pharmacol* 1990; **30**: 795–803.
36 Lee DY, Lee KU, Kwon JS, *et al*. Pharmacokinetic-pharmacodynamic modeling of risperidone effects on electroencephalography in healthy volunteers. *Psychopharmacology* 1999; **144**: 272–278.
37 Reimann IW, Ziegler G, Ludwig L, Frölich JC. Central and autonomic nervous system side effects of ketanserin. *Arzneimittelforschung* 1986; **36**: 1681–1684.
38 Glennon RA, Dukat M. Serotonin receptor subtypes. In *Psychopharmacology, the Fourth Generation of Progress*, eds Bloom FE, Kupfer DJ. New York: Raven Press, 1995; 415–429.
39 Aghajanian GK. LSD and phenethylamine hallucinogens: common sites of neuronal action. In *50 Years of LSD. Current Status and Perspectives of Hallucinogens*, eds Pletscher A, Ladewig D. New York: Parthenon, 1994; 27–41.
40 Barbanoj MJ, Anderer P, Antonijoan RM, Torrent J, Saletu B, Jané F. Topographic pharmaco-EEG mapping of increasing doses of buspirone and its comparison with diazepam. *Hum Psychopharmacol Clin Exp* 1994; **9**: 101–109.
41 Strassman RJ. Human psychopharmacology of *N,N*-dimethyltryptamine. *Behav Brain Res* 1996; **73**: 121–124.

Psychopharmacology (2002) 165:18–28
DOI 10.1007/s00213-002-1237-5

ORIGINAL INVESTIGATION

Jordi Riba · Antoni Rodríguez-Fornells · Manel J. Barbanoj

Effects of *ayahuasca* on sensory and sensorimotor gating in humans as measured by P50 suppression and prepulse inhibition of the startle reflex, respectively

Received: 2 January 2002 / Accepted: 15 July 2002 / Published online: 12 October 2002

Abstract *Rationale: Ayahuasca*, a South American psychotropic plant tea, combines the psychedelic agent and 5-$HT_{2A/2C}$ agonist *N*,*N*-dimethyltryptamine (DMT) with β-carboline alkaloids showing monoamine oxidase-inhibiting properties. Current human research with psychedelics and entactogens has explored the possibility that drugs displaying agonist activity at the 5-$HT_{2A/2C}$ sites temporally disrupt inhibitory neural mechanisms thought to intervene in the normal filtering of information. Suppression of the P50 auditory evoked potential (AEP) and prepulse inhibition of startle (PPI) are considered operational measures of sensory (P50 suppression) and sensorimotor (PPI) gating. Contrary to findings in lower animals, unexpected increases in sensorimotor gating have been found in humans following the administration of the serotonergic psychedelic psilocybin and the serotonin releaser 3,4-methylenedioxymethamphetamine (MDMA). In addition, to our knowledge P50 suppression has not been assessed previously in humans following the administration of a 5-$HT_{2A/2C}$ agonist. *Objectives:* To assess the effects of the acute administration of *ayahuasca* on P50 suppression and PPI in humans, in order to evaluate the drug's modulatory actions on these measures of sensory and sensorimotor gating. *Methods:* Eighteen healthy volunteers with prior experience of psychedelic drug use participated in a clinical trial in which placebo or *ayahuasca* doses (0.6 mg and 0.85 mg DMT/kg body weight) were administered according to a double-blind, cross-over balanced design. P50 and startle reflex (pulse-alone and 60 ms, 120 ms, 240 ms and 2000 ms prepulse-to-pulse intervals) recordings were undertaken at 1.5 h and 2 h after drug intake, respectively. *Results: Ayahuasca* produced diverging effects on each of the two gating measures evaluated. Whereas significant dose-dependent reductions of P50 suppression were observed after *ayahuasca*, no significant effects were found on the startle response, its habituation rate, or on PPI at any of the prepulse-to-pulse intervals studied. *Conclusion:* The present findings indicate, at the doses tested, a decremental effect of *ayahuasca* on sensory gating, as measured by P50 suppression, and no distinct effects on sensorimotor gating, as measured by PPI.

Keywords *Ayahuasca* · DMT · Psychedelics · Prepulse inhibition of startle · P50 suppression · Sensory gating · Sensorimotor gating · Human

J. Riba · M.J. Barbanoj (✉)
Àrea d'Investigació Farmacològica, Institut de Recerca,
Hospital de la Santa Creu i Sant Pau (HSCSP),
Departament de Farmacologia i Terapèutica,
Universitat Autònoma de Barcelona, St. Antoni Maria Claret, 167,
08025 Barcelona, Spain
e-mail: mbarbanoj@hsp.santpau.es
Tel.: +34-93-2919019
Fax: +34-93-2919286

A. Rodríguez-Fornells
Department of Neuropsychology, Otto von Guericke University,
39112 Magdeburg, Germany

Introduction

Ayahuasca is a powerful psychotropic plant concoction, which contains the serotonergic psychedelic agent *N*,*N*-dimethyltryptamine (DMT) (Rivier and Lindgren 1972; Schultes and Hofmann 1980). This beverage, which is the shamanic inebriant par excellance in the Upper Amazon River Basin (Schultes and Hofmann 1982; Dobkin de Rios 1984), is obtained by infusing the stems of the woody vine *Banisteriopsis caapi* (malpighiaceae) together with the leaves of *Psychotria viridis* (rubiaceae) or *Diplopterys cabrerana* (malpighiaceae). *Banisteriopsis caapi*'s chief contribution to the infusion is a series of β-carboline alkaloids, namely harmine, tetrahydroharmine and, to a lesser degree, harmaline, while *Psychotria viridis* and *Diplopterys cabrerana* contribute varying amounts of DMT (Rivier and Lindgren 1972; Schultes and Hofmann 1980).

When administered parenterally, DMT is a potent ultra-short-acting psychedelic agent (Strassman et al. 1994), which binds to the 5-$HT_{2A/2C}$ receptor sites in the central nervous system (CNS), where it acts as an agonist

(Pierce and Peroutka 1989; Smith et al. 1998). Interestingly, this compound is entirely inactive after oral ingestion (Ott 1999), probably due to metabolic breakdown by gut and liver monoamine oxidase (MAO) (Suzuki et al. 1981). However, the β-carboline alkaloids present in *ayahuasca* display MAO inhibitory properties (McKenna et al. 1984). By combining both plants in a single oral preparation, the extensive first-pass effect on DMT can be diminished thanks to the reversible inhibition of MAO elicited by the β-carbolines, thus enabling DMT to reach the systemic circulation and the CNS.

Ayahuasca has attracted the interest of biomedical researchers as its use has spread in recent years, reaching the urban areas of South America, Europe, and North America, where it is used in the context of divination, traditional medicine, and syncretic religions (Dobkin de Rios 1996a, 1996b; Anonymous 2000). In previous studies we found that in a clinical setting *ayahuasca* was able to induce dose-dependent perceptual cognitive and affective modifications characteristic of the psychedelics, as measured by self-report, subjective-effect measures (Riba et al. 2001a) and a pattern of changes in spontaneous brain electrical activity analogous to that caused by other drugs displaying agonist activity at the 5-HT_2 and D_2 receptor sites (Riba et al. 2002).

Recently, the disruptive activity of psychedelics on the "gating" of sensory information has been postulated (Vollenweider 1994). This hypothesis is based on the assumption of the existence of brain mechanisms directed at filtering out, under normal conditions, the flow of sensory information reaching consciousness. Decreases in gating had been initially proposed as an underlying deficit common to a number of neuropsychiatric disorders, where a sensory overflow is postulated (Braff et al. 2001). According to this model, serotonergic psychedelics, dopaminergic agonists, and *N*-methyl-D-aspartate (NMDA) antagonists would interact with brain structures involved in the gating mechanisms, temporarily decreasing their functionality and giving rise to the characteristic perceptual and cognitive effects elicited by these agents (Vollenweider 1994).

Two neurophysiological measures have been developed to evaluate the functionality of neural gating mechanisms: suppression of the P50 auditory evoked potential (AEP) and prepulse inhibition of the startle reflex (PPI). The P50 AEP is a midlatency potential appearing about 50 ms after the presentation of an auditory stimulus (Picton et al. 1974). The consecutive administration of two identical stimuli, conditioning (C) and testing (T) stimuli, at a certain inter-stimulus interval, typically 500 ms, leads to a decrease in the amplitude of the second P50 wave (Adler et al. 1982). The amplitude decrement seen for the T stimulus is thought to obey active inhibitory mechanisms triggered by the C stimulus (Freedman et al. 1983). P50 suppression is regarded as a measure of sensory gating, and its neural substrates have been located in the hippocampus, in the mesial temporal lobe (Adler et al. 1998).

The second operational measure, PPI, is based on the inhibitory effect of a weak sensory stimulus (the prepulse) on the motor response caused by a stronger startle reflex-eliciting stimulus. The startle reflex is a brainstem reflex occurring after the presentation of intense and sudden sensory stimuli. PPI is obtained when the startling stimulus is preceded 15–400 ms by the prepulse, and it manifests as a decrease in the intensity of the reflex (Blumenthal 1999). In contrast to P50, PPI is considered a measure of sensorimotor gating, given that the response measured is the motor output to the presented stimulus. While the neural circuit mediating the startle reflex is located in the brainstem, PPI is regulated by descending projections from areas in the forebrain. These areas are interconnected in a complex circuitry combining excitatory and inhibitory synapses (Swerdlow et al. 2001).

Pharmacological challenge studies in humans have shown dopaminergic agents to disrupt PPI and P50 suppression (Adler et al. 1994a; Hutchinson and Swift 1999; Light et al. 1999), while unexpected increases in PPI have been observed after the administration of serotonergic psychedelics/entactogens, such as psilocybin and 3,4-methylenedioxymethamphetamine (MDMA) (Gouzoulis-Mayfrank et al. 1998; Vollenweider et al. 1999). To our knowledge no study has been carried out to date on the influence of serotonergic psychedelics/entactogens on the human P50 suppression paradigm.

The aim of the present study was to evaluate both P50 suppression and PPI in a single group of healthy volunteers after the acute administration of *ayahuasca* and to assess a possible differential drug modulation of these two measures.

Materials and methods

Volunteers

Eighteen healthy volunteers (15 males and 3 females) with no current or previous history of neurological or psychiatric disorder and no family history of axis-I psychiatric disorder in first degree relatives were included in the study. Eligibility criteria included prior experience with psychedelic drugs on at least five occasions without sequelae derived thereof. The volunteers were given a structured psychiatric interview [Diagnostic and Statistical Manual of Mental Disorders (DSM)-III-R] and completed the trait-anxiety scale from the State-Trait Anxiety Inventory (Spielberger et al. 1970). Exclusion criteria included a present or past history of axis-I disorders and alcohol or other substance dependence, and high scores on trait anxiety. Volunteers were given a complete physical examination that included a medical history, laboratory tests, electrocardiogram (ECG), and urinalysis. Mean age was 25.7 years (range 19–38 years), mean weight 66.47 kg (range 50.7–79.5 years) and mean height 175.11 cm (range 158–188 cm). In addition to their prior intake of psychedelics, all volunteers had previous experience with cannabis and cocaine. Although prior exposure specifically to *ayahuasca* was not required for participation, two of the volunteers had ingested the beverage before inclusion in this study. The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans and was approved by the hospital ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of *ayahuasca* and the general psycho-

logical effects of psychedelics and their possible adverse effects, as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drug

Two *ayahuasca* doses containing 0.6 mg and 0.85 mg DMT/kg body weight were chosen as the low and high doses, respectively, based on tolerability and subjective effects assessed in a previous study (Riba et al. 2001a). The *ayahuasca* was not administered in its original liquid form, but as a liophilizate. The freeze-dried homogenized material was obtained from a 9.6-l batch of *Daime* obtained from Cefluris, a Brazilian-based religious organization related to the *Santo Daime* church. The DMT contents had been determined by means of high-performance liquid chromatography (HPLC), as described by Callaway and coworkers (1996), and the β-carbolines according to a modified version of the method described therein. As reported in a previous paper, the 9.6-l batch yielded 611 g freeze-dried powder, containing 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg THH per gram. These alkaloid contents corresponded to the following concentrations in the original tea: DMT 0.53 mg/ml, harmine 0.90 mg/ml, harmaline 0.06 mg/ml, and THH 0.72 mg/ml (Riba et al. 2001a). The calculated individual dose for each volunteer was administered by combining 00 gelatin capsules containing 0.5, 0.25, or 0.125 g freeze-dried *ayahuasca* and placebo capsules containing 0.75 g lactose. Placebo capsules were added when necessary, so that all volunteers took the same number of capsules on each experimental day.

Study design and experimental procedure

The volunteers participated in four experimental sessions. Volunteers were informed that they would randomly receive on each experimental day a single oral dose of encapsulated freeze-dried *ayahuasca* (one low and one high dose) or placebo and a random repetition of one of the three mentioned treatments. In actual fact, they all received a placebo on the first experimental day in a single-blind fashion, followed by one of the three treatments from day 2 to day 4 in a double-blind balanced fashion, according to a randomization table. The first non-randomized placebo was administered in order to familiarize the volunteers with the experimental setting and to minimize the stress associated with the experimental interventions. The data obtained during the first session was not included in the statistical analysis performed and is not reported. Two weeks prior to the beginning of the experimental sessions, volunteers abstained from any medication or illicit drug and remained drug free throughout the four study weeks. Urinalysis for illicit drug use was made for each experimental session. Additionally, volunteers abstained from alcohol, tobacco, and caffeinated drinks 24 h prior to each experimental day. There was a 7-day washout period between experimental days.

On each experimental day, participants arrived at the laboratory in the morning under fasting conditions, and capsules were administered by approximately 10,00 hours with 250 ml tap water. The P50 and PPI sessions were begun at 1.5 h and 2 h after drug administration, respectively, coinciding with the peak of subjective effects (Riba et al. 2001a). The recordings were undertaken in a quiet room with the volunteers seated in a reclining chair. The experimenter remained in the neighboring room for the entire time of the recordings and monitored volunteers for alertness. Four hours after administration of the capsules, the volunteers answered subjective-effect questionnaires and had a meal. They remained in the research unit throughout the afternoon and were discharged approximately 9 h after administration.

Measurements

P50 elicitation and recording

One hundred and twenty pairs of auditory stimuli were delivered by means of air earphones. Auditory stimuli were 75-dB [A], 1000-Hz pure-tone pips of 4-ms duration, with a 500-ms inter-stimulus separation and a constant interval between pairs of 8 s. No background noise was presented during the session. Electroencephalogram (EEG) recordings were obtained by means of nineteen electrodes placed on the scalp according to the international 10/20 system, plus leads for horizontal and vertical eye-movement monitoring. All scalp electrodes were referenced to the averaged mastoids. Impedance was kept below 5 kΩ. Throughout the entire recording session, volunteers remained with eyes open with sight on a fixation point. High- and low-pass filters were set at 0.1 Hz and 100 Hz, respectively. The digitation rate was 250 Hz. The continuous recordings were epoched at an interval between 100 ms pre-stimulus and 1000 ms post-stimulus and baseline corrected (–100, 0). This was followed by rejection of any trial showing an activity exceeding $\pm$75 μV. All artifact-free epochs were averaged to obtain the average AEP including the first or C stimulus and the second or T stimulus. The obtained averages were re-filtered between 10 Hz and 50 Hz to facilitate P50 identification (Jerger et al. 1992). P50 identification and scoring was carried out on average individual waveforms at Cz as described by Adler et al. (1994b). The C peak was identified as the greatest positivity between 40 ms and 80 ms after stimulus presentation. If more than one peak of equal amplitude was detected, the later one was selected. Peak amplitude was assessed as the difference between this peak and the preceding negative N40 trough. In cases where no N40 could be identified, the P50 amplitude was measured to pre-stimulus baseline (Cardenas et al. 1997). The T peak was identified in the same way, with the further constraint that it had to appear at a latency between $\pm$10 ms of the latency value found to the P50 wave to the C stimulus (Adler et al. 1994b).

Startle reflex elicitation and recording

Startle stimuli were 1-KHz pure tones of 116 dB [A], with a 50-ms duration and an instantaneous rise/fall time. Acoustic stimuli were presented binaurally through air headphones. Prepulses were non-startling 1-KHz pure tones of 80 dB [A] and a 20-ms duration. No background noise was presented during the session. The electromyogram (EMG) signal was recorded bipolarly from the orbicularis oculi muscle by means of two 0.5-cm diameter silver surface disc electrodes, placed 1 cm below and 1 cm medial from the external canthus of the right eye (Fridlund and Carcioppo 1986). Two electrodes placed above and below the left eye were used to control spontaneous and voluntary blinking. The ground electrode was placed on the forehead. Impedance level was maintained below 5 KΩ. Amplifier filters were set at 10 Hz (high pass) and 500 Hz (low pass). The EMG signal was digitized at a 1000-Hz rate.

Each startle sequence was initiated with an acclimation phase comprising five pulse-alone startle stimuli, which were not used later in the calculation of PPI. These were followed by three blocks of trials comprising pulse-alone trials and prepulsed trials at the following prepulse-to-pulse intervals: 60, 120, 240, and 2000 ms. Each block included three pulse-alone trials and three prepulsed trials at each of the four intervals used. Thus, 45+5 startle stimuli were delivered in the course of a startle reflex recording session. The mean inter-trial interval was 20 s (range 10–29 s). Four different sequences of stimuli were used throughout the study, each subject receiving a different sequence on each experimental day. The order of the sequences was varied according to a randomization table and was counterbalanced across subjects. The order of presentation of each trial type was pseudo-random and varied across blocks and across sequences.

The recorded EMG signal was full-wave rectified off-line and smoothed using a five-point moving average filter. Peak eye-blink amplitude was defined as the highest point in the EMG response

within a time window of 120 ms after stimulus administration. Baseline EMG was computed as the mean EMG in the 30-ms preceding stimulus onset. Reactivity was defined as blink magnitude in the pulse-alone trials. Trials in which the apparent response had an onset latency of less than 20 ms after stimulus administration and/or a rise time greater than 95 ms were rejected. In those trials in which no response was detected, amplitude was scored as 0 μV. Epochs were screened and rejected if artifacts were present.

Subjective ratings

Volunteers were requested to answer two questionnaires measuring psychedelic-induced subjective effects. The first questionnaire was the Hallucinogen Rating Scale (HRS) (Strassman et al. 1994). The HRS includes six subscales: *somaesthesia*, reflecting somatic effects; *affect*, sensitive to emotional and affective responses; *volition*, indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; *cognition*, describing modifications in thought processes or content; *perception*, measuring visual, auditory, gustatory and olfactory experiences; and *intensity*, which reflects the strength of the overall experience. In the present study, a Spanish version of the questionnaire was used (Riba et al. 2001b).

The second questionnaire administered was a Spanish version of the Altered States of Consciousness Questionnaire ("Ausser-gewöhnliche Psychische Zustände", APZ) developed by Dittrich (1998). It includes 72 items distributed in three subscales: *oceanic boundlessness* ("Ozeanische Selbstentgrenzung", OSE), measuring changes in the sense of time, derealization and depersonalization phenomena subjectively experienced as positive; *dread of ego-dissolution* ("Angstvolle IchAuflösung", AIA), measuring thought disorder and decreased body and thought control associated with arousal and anxiety; and *visionary restructuralization* ("Visionäre Umstrukturierung", VUS), referring to visual phenomena, such as illusions, hallucinations and synesthesia and to changes in the significance of objects. This instrument has been extensively used in studies involving the administration of psychedelics to humans. Volunteers were requested to answer the HRS and the APZ 4 h after drug intake.

Statistical analysis

P50 auditory evoked potential

Three measures related to response amplitude were derived from average waveforms at Cz for each subject and drug condition: P50 AEP amplitude values after the C and T stimuli, difference amplitude calculated as C–T, and finally percentage suppression calculated as [1–(T/C)]×100. Latency to peak after the C stimulus was also assessed. Amplitude values for the C stimulus were analyzed by means of a repeated-measures one-way analysis of variance (ANOVA) with drug as factor, in order to test for drug actions on the amplitude of the C trial. A repeated-measures, two-way ANOVA was subsequently performed, with drug and stimulus type (C vs T) as factors on amplitude values. Finally, repeated-measures, one-way ANOVAs with drug as factor were performed on difference amplitude, percentage suppression, and latency to peak values.

Startle reflex measures

Blink magnitude values were obtained from the recordings and averaged for each trial type (i.e., nine trials for each of the five trial types: pulse-alone, 60 ms prepulse-to-pulse indicated as PP60, 120 ms prepulse-to-pulse indicated as PP120, 240 ms prepulse-to-pulse indicated as PP240 and 2000 ms prepulse-to-pulse indicated as PP2000). The following variables were calculated: reactivity (magnitude of the startle response in the pulse-alone trials), magnitude of the startle response in the prepulsed trials (PP60, PP120, PP240, and PP2000), percentage PPI (PP60, PP120, PP240, PP2000), and percentage habituation. Percentage PPI for each prepulse condition was calculated as follows: [1–(prepulsed trial magnitude/pulse-alone magnitude)]×100. Percentage habituation was calculated as the difference of the averaged magnitude of pulse-alone trials in the first block minus the averaged magnitude of pulse-alone trials in the third block divided by magnitude in the first block and multiplied by 100 (i.e., %Hab = [(first block–third block)/first block]×100).

Reactivity was analyzed by means of a repeated-measures, two-way ANOVA with drug and block as factors. Percentage habituation was analyzed by means of a repeated-measures, one-way ANOVA with drug as factor. Magnitude of the startle response in the prepulsed conditions was analyzed by means of a repeated-measures, two-way ANOVA with drug and prepulse condition as factors. Finally, PPI data were subjected also to a repeated-measures, two-way ANOVA with drug and prepulse condition as factors.

Subjective reports

Scores on HRS and APZ subscales were analyzed by means of a one-way, ANOVA with repeated measures, with drug as factor. In all ANOVAs performed, Greenhouse-Geisser epsilon was used to correct possible violations of the sphericity assumption and to reduce type-I errors. *P* values after correction are shown. When ANOVA showed statistically significant differences between drug conditions, pair-wise comparisons were carried out by means of *t*-tests. Results were considered statistically significant for $P<0.05$.

Correlations

The Pearson's *r* was used to evaluate correlations between drug-induced changes in neurophysiological measures and in subjective-effect scores, and also between drug-induced changes in PPI and in P50 measures.

Results

Usable recordings of both PPI and P50 in all three experimental sessions for a given volunteer were obtained for 15 of the total 18 volunteers enrolled in the study. The results presented below were obtained from analysis of data corresponding to this subgroup of 15 volunteers (13 males and 2 females).

P50 auditory evoked potential

Figure 1 shows grand average AEP waveforms at the Cz site after the C and T stimuli for the three drug conditions. Figure 2 presents mean P50 amplitude values for C and T, difference amplitude values (C–T), and percentage suppression [1–(T/C)]×100, under the three drug conditions. Amplitude values of the P50 response after the C stimulus showed a decrease with dose, which did not reach statistical significance in the ANOVA ($F_{2,28}=2.57$, $P=0.10$, $\varepsilon=0.906$). Mean P50 amplitude (μV)±SEM for the C stimulus under the three drug conditions was 2.93±0.42 for placebo, 2.56±0.28 for the low dose, and 2.05±0.22 for the high dose. The two-way ANOVA with drug and stimulus type (C vs T) as factors showed the following results: whereas no significant main effect of

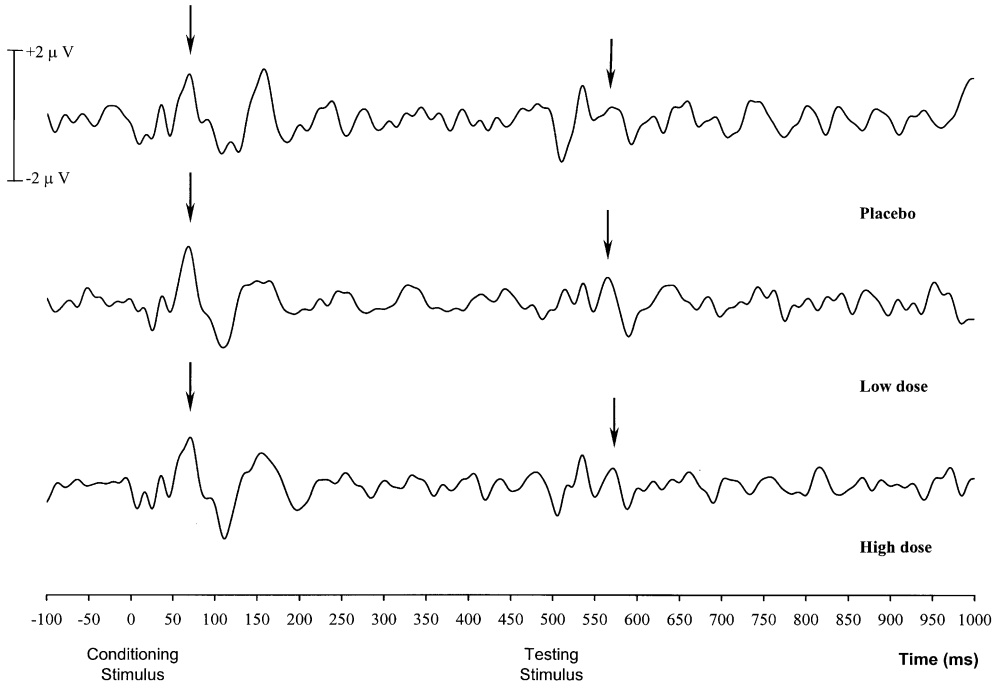


Fig. 1 Grand average band-pass filtered (10–50 Hz) auditory evoked potential (AEP) waveforms at the Cz site under the three drug conditions (n=15). The P50 component after the conditioning and testing stimuli are indicated with *arrowheads*

drug was seen on the overall amplitude of the P50 response ($F_{2,28}$=0.80), significant effects of stimulus type ($F_{1,14}$=38.49, P<0.001; linear contrast $F_{1,14}$=38.49, P<0.001; mean amplitude ±SEM: 2.53±0.21 μV for the C stimulus, 1.36±0.13 μV for the T stimulus), and the interaction drug × stimulus type ($F_{2,28}$=4.96, P<0.05, ε=0.856; linear contrast $F_{1,14}$=6.70, P<0.05) were obtained. An analogous significant effect was obtained for the difference amplitude variable (C–T), pointing out that *ayahuasca* reduced the P50 amplitude response difference to the C and T stimuli ($F_{2,28}$=4.96, P<0.05, ε=0.856; linear contrast $F_{1,14}$=6.70, P<0.05; mean difference amplitude ±SEM under the three drug conditions: 2.12±0.42 μV for placebo, 0.93±0.34 μV for the low dose, and 0.52±0.31 μV for the high dose). Pair-wise comparisons showed statistically significant differences from placebo both at the low (t_{14}=2.29, P<0.05) and the high (t_{14}=2.59, P<0.05) *ayahuasca* doses for difference amplitudes. A significant drug effect on percentage suppression was observed after *ayahuasca* ($F_{2,28}$=4.78, P<0.05, ε=0.844; linear contrast $F_{1,14}$=7.93, P<0.05; mean percentage suppression ±SEM under the three drug conditions: 71.86±8.41 for placebo, 24.57±17.17 for the low dose, and 6.00±18.10 for the high dose). Pair-wise comparisons showed statistically significant differences from placebo both at the low (t_{14}=2.83, P<0.05) and the high (t_{14}=2.82, P<0.05) *ayahuasca* doses for percentage suppression.

Finally, latency to peak of the P50 wave after the C stimulus decreased non-significantly after *ayahuasca* ($F_{2,28}$=2.76, P<0.1, ε=0.844; mean latency to peak ±SEM under the three drug conditions was 70.13± 1.91 ms for placebo, 68.53±1.17 ms for the low dose, and 65.20±2.14 ms for the high dose).

Startle reflex measures

Startle reactivity under the three drug conditions was analyzed by means of a two-way ANOVA with drug (placebo, *ayahuasca* low dose, *ayahuasca* high dose) and block of trials (first, middle and last block of the recording session) as factors. Figure 3, upper panel, shows pulse-alone startle magnitude values for each block of trials under the three drug conditions. A robust decrease of startle magnitude was observed as the recording session progressed, as evidenced by a significant effect of block ($F_{2,28}$=12.91, P<0.01, ε=0.687; linear contrast $F_{1,14}$=15.98, P<0.01; mean magnitude ±SEM for the first block was 104.96±19.63 μV, second block 66.97±13.39 μV, and third block 50.16±11.33 μV) in the ANOVA. Although mean magnitude values increased after the *ayahuasca* high dose, no significant effect of drug was seen in the ANOVA ($F_{2,28}$=1.97; mean magnitude ±SEM was 68.13±17.58 μV for placebo, 59.62±11.27 μV for the low dose, and 94.35±21.61 μV for the high dose). Finally, no significant drug × block interaction was observed ($F_{4,56}$=0.86). Similarly, a one-way ANOVA with drug as factor revealed no significant effect in percentage habituation ($F_{2,28}$=0.49; percentage habituation ±SEM was 41.74±13.25 for placebo, 37.64±11.51 for the low dose, and 36.65±46.06 for the high dose).

The effects of *ayahuasca* on global startle magnitude in the pulse-alone trials and in the prepulsed trials at the different prepulse-to-pulse intervals are shown in Fig. 3, lower panel. A two-way ANOVA with drug and prepulse condition as factors revealed a main effect of prepulse condition ($F_{3,42}$=15.02, P<0.001, ε=0.509; linear contrast $F_{1,14}$=18.95, P<0.01; mean magnitude ±SEM at the

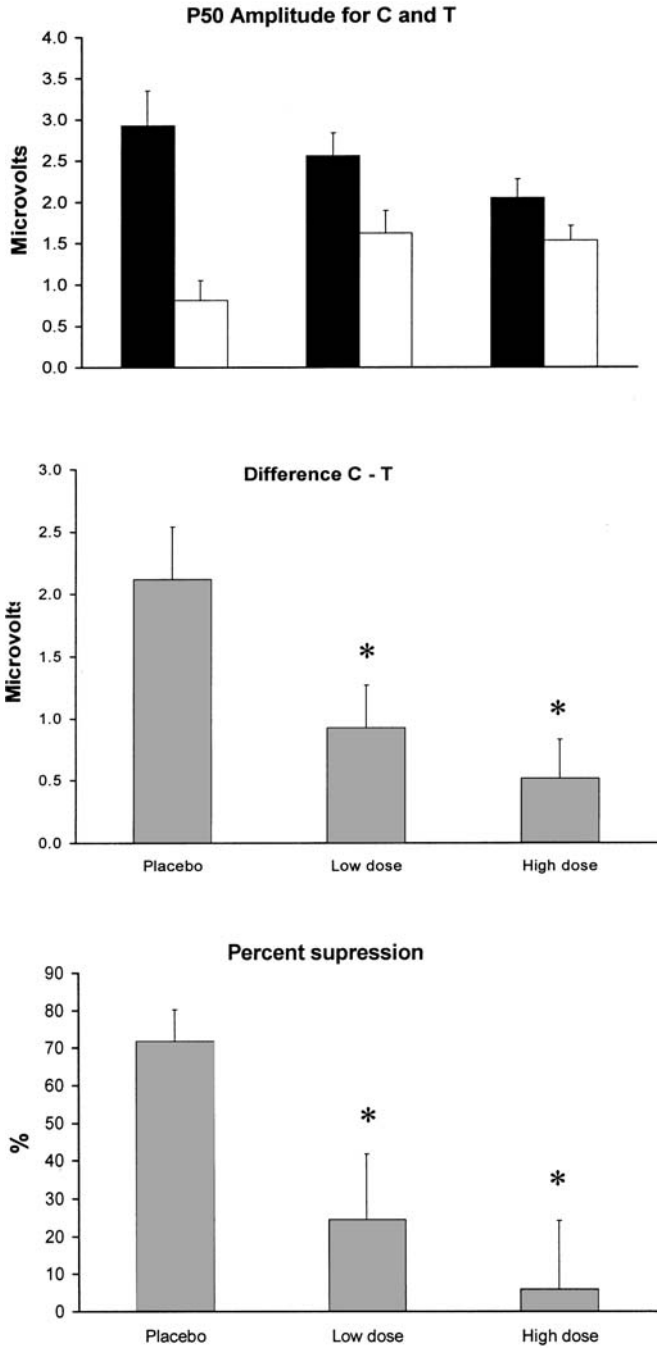


Fig. 2 *Upper panel* P50 amplitude to the conditioning (*closed square*) and testing (*open square*) stimuli under the three drug conditions. *Middle panel* Difference (conditioning–testing) of P50 amplitude values under the three drug conditions. *Lower panel* Percentage suppression values under the three drug conditions. In all three panels, *error bars* denote 1 SEM, and an *asterisk* indicates $P<0.05$ relative to placebo ($n=15$)

different prepulse-to-pulse intervals was: 74.03±13.79 μV pulse-alone, 21.14±3.80 μV PP60, 26.64±5.92 μV PP120, 48.65±11.45 μV PP240, and 80.79±16.96 μV PP2000). No significant effects of drug ($F_{2,28}=1.19$) or drug × prepulse condition ($F_{6,84}=0.65$) were observed.

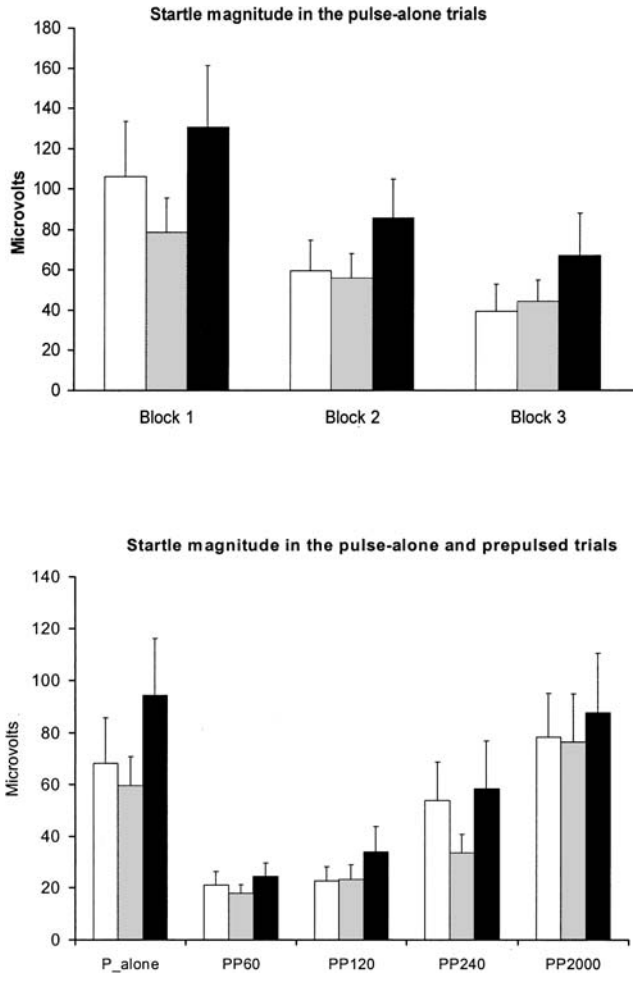


Fig. 3 *Upper panel* Mean startle magnitude values in the pulse-alone trials in each of the three blocks of trials comprising a recording session, after each of the three drug conditions. A main effect of block was found in the ANOVA ($F_{2,28}=12.91$, $P<0.01$), while no effects of drug or drug × block were observed. *Lower panel* Mean startle magnitude values after the pulse-alone and at each of the four prepulse-to-pulse intervals after each of the three drug conditions. In both panels (*open square*) placebo, (*shaded*) low dose, (*closed square*) high dose. *Error bars* denote 1 SEM ($n=15$). A main effect of prepulse condition was found in the ANOVA ($F_{3,42}=11.85$, $P<0.001$), while no effects of drug or drug × prepulse condition were observed

Figure 4 shows percentage inhibition (expressed as percentage facilitation for PP2000) values at the different prepulse-to-pulse intervals under the three drug conditions. A two-way ANOVA with drug and prepulse condition as factors revealed a main effect of prepulse condition ($F_{3,42}=11.85$, $P<0.001$, $\varepsilon=0.565$; linear contrast $F_{1,14}=36.35$, $P<0.001$; percentage inhibition in the four prepulse-to-pulse intervals ±SEM was: 59.16±5.93 PP60, 56.46±7.27 PP120, 21.13±20.87 PP240, and –19.89± 12.65 PP2000). No significant effect was seen for factor drug ($F_{2,28}=2.88$, $P<0.1$, $\varepsilon=0.938$; linear contrast $F_{1,14}=4.89$, $P<0.05$; percentage inhibition ±SEM across the four prepulse-to-pulse intervals for each drug condition was: 16.07±14.15 for placebo, 32.71±8.57 for the low dose,

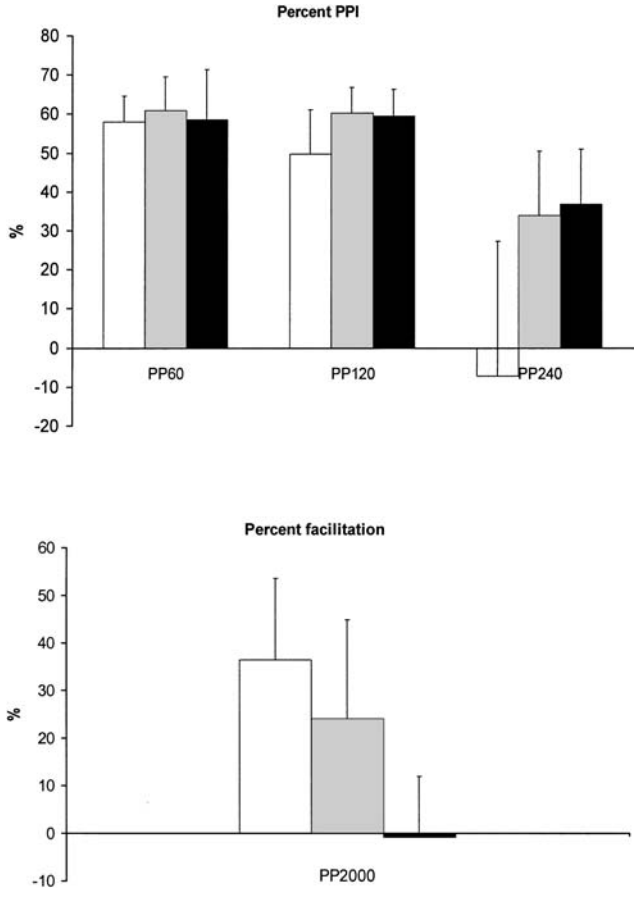


Fig. 4 *Upper panel* Mean values of percentage inhibition of the startle response at the 60, 120 and 240-ms prepulse-to-pulse intervals. *Lower panel* Mean values of percentage facilitation of the startle response at the 2000-ms prepulse-to-pulse interval. In both panels, (*open square*) placebo, (*shaded*) low dose, (*closed square*) high dose. *Error bars* denote 1 SEM (n=15). No effects of drug or drug × prepulse condition were observed

and 38.86±8.66 for the high dose). Finally, the interaction drug × prepulse condition was not found to be significant ($F_{6,84}$=1.42).

Subjective effects

The administration of the selected *ayahuasca* doses to a group of healthy volunteers with experience in the use of psychedelics induced a pattern of subjective effects that was reflected as increases in the scores of the HRS and APZ subscales, as shown in Table 1.

All HRS and APZ subscales showed statistically significant increases relative to placebo after *ayahuasca* administration, except for *volition*. The characteristic psychedelic pattern of effects reported by the volunteers had an overall duration of 4–6 h, reaching its maximum intensity between 90 min and 120 min. The most frequently reported perceptual effects were in the somatosensory and visual modalities. Somatosensory effects comprised altered bodily sensations, such as pins and needles, and increased skin sensitivity. Visual perception was characteristically modified, volunteers experiencing distortions of the visual field with eyes open, and more or less elaborate visions with eyes closed. Auditive phenomena were also present and consisted typically of alterations in external sounds, with true auditory hallucinations being less frequently reported. This modified state of awareness was also accompanied by changes in the cognitive sphere, with increased thought speed and associations, a reduction in the capacity to focus attention, and changes in mood, usually consisting of feelings of happiness and excitation. At the doses administered, *ayahuasca* did not induce full-blown psychotic symptoms and none of the participants lost insight into the

Table 1 Means (±SD) of the scores obtained for the Hallucinogen Rating Scale (HRS) and Spanish version of the Altered States of Consciousness (APZ) questionnaire subscales (n=15), and results of the statistical analysis performed. Student's t-tests were followed by Bonferroni correction. *ns* not significant

Variable	ANOVA		Student's t- test		
	P value	Placebo	vs Placebo		vs Low dose High dose
			Low dose	High dose	
HRS					
Somaesthesia	***	0.08±0.10	0.42±0.40*	0.93±0.36**	**
Perception	***	0.11±0.20	0.57±0.52**	1.11±0.68**	**
Cognition	***	0.07±0.18	0.44±0.48*	1.01±0.63**	**
Volition	(*)	0.93±0.81	1.23±0.68 ns	1.38±0.57 ns	ns
Affect	***	0.35±0.21	0.60±0.36*	1.02±0.38**	*
Intensity	***	0.22±0.44	1.27±0.79**	1.80±0.53**	**
APZ					
AIA	**	0.20±0.56	1.33±2.23 ns	3.40±2.77**	ns
OSE	***	0.20±0.41	2.53±2.90*	4.40±2.95**	ns
VUS	***	0.00±0.00	2.07±2.71*	4.07±3.33**	*

(*)P<0.1
*P<0.05
**P<0.01
***P<0.001

drug-induced nature of the psychological effects experienced.

Correlations

No significant correlations were found between drug-induced changes in P50 and PPI measures. Thus, the following results were obtained between drug-induced changes in (a) P50 difference values and drug-induced changes in PPI at the 60-ms (r=–0.253, P=0.362), 120-ms (r=0.212, P=0.449), 240-ms (r=0.151, P=0.590), and 2000-ms (r=0.412, P=0.127) intervals; and (b) P50 percentage suppression values and drug-induced changes in PPI at the 60-ms (r=–0.066, P=0.815), 120-ms (r=0.381, P=0.162), 240-ms (r=0.212, P=0.448), and 2000-ms (r=0.366, P=0.179) intervals.

Given that significant drug effects were found on P50 measures, these were correlated with subjective-effect scores. Again, no correlations were found between changes in (a) P50 difference values and drug-induced changes in HRS-somaesthesia (r=–0.244, P=0.382), HRS-perception (r=–0.313, P=0.255), HRS-cognition (r=–0.281, P=0.310), HRS-volition (r=–0.474, P=0.075), HRS-affect (r=–0.387, P=0.155), HRS-intensity (r=–0.225, P=0.421), APZ-AIA (r=–0.490, P=0.063), APZ-OSE (r=–0.319, P=0.246), and APZ-VUS (r=–0.393, P=0.147) scores; and (b) P50 percentage suppression values and drug-induced changes in HRS-somaesthesia (r=–0.207, P=0.458), HRS-perception (r=–0.321, P=0.243), HRS-cognition (r=–0.101, P=0.722), HRS-volition (r=–0.439, P=0.102), HRS-affect (r=–0.278, P=0.316), HRS-intensity (r=–0.235, P=0.400), APZ-AIA (r=–0.393, P=0.147), APZ-OSE (r=–0.247, P=0.374), and APZ-VUS (r=–0.186, P=0.507) scores.

Discussion

The results obtained in the present study indicate diverging effects for *ayahuasca* on P50 suppression and PPI. Whereas a statistically significant dose-dependent reduction of P50 suppression was observed following drug administration, no significant effects were seen on PPI values. Additionally, the rate of habituation of the startle reflex, another form of startle plasticity thought to reflect gating mechanisms, was not modified by *ayahuasca*. In addition, at the doses administered, *ayahuasca* induced a pattern of subjective effects, similar in nature to those reported in a previous study involving a smaller sample of volunteers (Riba et al. 2001a), as was evidenced by the self-report questionnaires administered.

The present results would argue for a disruptive effect of psychedelics on P50 suppression. Nevertheless, this conclusion should be regarded as preliminary and interpreted with caution, considering the presence of other pharmacologically active alkaloids in *ayahuasca*. The only studies that have evaluated the effects of pharmacological challenge on this measure in humans have concentrated mainly on cathecolaminergic drugs and NMDA antagonists. Thus, both D-amphetamine and the α_2-adrenoceptor antagonist yohimbine, a drug that increases noradrenaline release, have been shown to impair P50 suppression in healthy volunteers (Adler et al. 1994b; Light et al. 1999). Furthermore, while the dopamine agonist bromocriptine has also been found to disrupt P50 suppression (Adler et al. 1994a) in humans, a low dose of the NMDA antagonist ketamine failed to decrease P50 suppression (van Berckel et al. 1998).

Regarding data from animals, suppression of the N40 potential in rodents in a paired stimuli paradigm, homologous to that of the human P50, appears to be highly dependent on the integrity and functionality of cholinergic pathways (Adler et al. 1998). However, inhibition can be disrupted by amphetamine (Adler et al. 1986; Stevens et al. 1991) – analogously to data from humans – and by phencyclidine (Adler et al. 1986). This loss of N40 suppression has been found to depend on the noradrenergic and dopaminergic properties of these drugs, also in the case of phencyclidine (Stevens et al. 1991; Miller et al. 1992). The psychostimulant cocaine has also been found to cause a loss of N40 suppression (Boutros et al. 1994). Thus, increased catecholamine neurotransmission seems to exert the same disruptive effects on sensory gating in humans and lower animals. However, in the only study reported to date on the effects of 5-HT_2 modulation of N40 suppression, an unexpected disruptive effect was found for the 5-$HT_{2A/2C}$ antagonist ketanserin. Conversely, the 5-$HT_{2A/2C}$ agonist DOI increased filtering and was also capable of reverting the reductions in filtering caused by ketanserin and amphetamine (Johnson et al. 1998).

The effects of *ayahuasca* on PPI did not reach statistical significance at any of the prepulse-to-pulse intervals tested. In the only other human study performed to date involving serotonergic psychedelics, the administration of psilocybin provoked a mild though significant increase of PPI at a prepulse-to-pulse interval of 100 ms, with no significant effects on habituation (Gouzoulis-Mayfrank et al. 1998). Both in the present study and in that by Gouzoulis-Mayfrank and coworkers, the drug doses administered were moderate and, although causing modifications in thought processes and the sensorium, they did not induce a clear-cut psychotic syndrome. Vollenweider and coworkers (1999) administered the serotonin releaser MDMA to a group of healthy volunteers and found a significant increase in PPI at the prepulse-to-pulse interval of 120 ms, but no significant effects on habituation. Results in the present study replicate the absence of effects found for psychedelics and MDMA on the rate of habituation.

Recently, a mechanistic study has shown that pretreatment with the 5-$HT_{2A/2C}$ antagonist ketanserin has no effect on the PPI-enhancing activity of MDMA, even though the antagonist was able to attenuate some of the effects of the drug, fundamentally the MDMA-induced perceptual modifications (Liechti et al. 2001). Conversely, these authors reported a decrease in PPI after pretreatment with the serotonin re-uptake inhibitor citalo-

pram and concluded that the effects of MDMA on human PPI seem to be more dependent on serotonin release than on an interaction at the 5-$HT_{2A/2C}$ level. These results would question the role of the human 5-$HT_{2A/2C}$ site in the modulation of PPI, despite the fact that recent human data provide additional support to the role of these receptors in the genesis of the psychological effects of psychedelics (Vollenweider et al. 1998). Unfortunately, no studies to date have evaluated the effects of the blockade of this receptor on psychedelic-induced increases of PPI in humans. Interestingly, the pattern of effects shown by serotonergic drugs on the human PPI in the limited number of studies conducted to date is opposed to that by dopaminergic/noradrenergic agonists. Thus, D-amphetamine and bromocriptine have been shown to impair PPI in healthy volunteers (Abduljawad et al. 1998, 1999; Hutchinson and Swift 1999).

In contrast to the above data, a coincidental pattern of effects on startle habituation and PPI has been observed for dopaminergic and 5-$HT_{2A/2C}$ agonists in lower animals. Braff and Geyer (1980) demonstrated an impairment in habituation of tactile startle in rats after administration of the mixed serotonergic agonist LSD. PPI has also been found to be impaired in rats after the 5-$HT_{2A/2C}$ agonist DOI, an effect which can be prevented by mixed 5-$HT_{2A/2C}$ (Sipes and Geyer 1994) and selective 5-HT_{2A} antagonists (Sipes and Geyer 1995; Padich et al. 1996). In a recent article, LSD was found to disrupt PPI in rats, and this effect was prevented only by selective 5-HT_{2A} antagonists. Other antagonists with affinity for the 5-HT_{2C}, 5-$HT_{2B/2C}$, 5-HT_{1A}, and 5-HT_6 did not counteract LSD-induced disruptions (Ouagazzal et al. 2001). Similarly, in rats PPI is disrupted by systemic administration of dopamine agonists, such as apomorphine, amphetamine, or the D_2 agonist quinpirole, and reversed by antipsychotic agents showing anti-D_2 activity (Geyer et al. 2001). One aspect that may have been overlooked and that could be involved in the differences in PPI modulation found for indole psychedelics between species is the fact that these drugs interact with both the 5-$HT_{2A/2C}$ and 5-HT_{1A} sites. Activation of these receptors has been shown to mediate opposite behavioral effects (Krebs-Thomson and Geyer 1998) in animals, and 5-HT_{1A} activation has recently been found to increase PPI in mice (Dulawa et al. 2000). The degree to which either receptor is activated after indole psychedelics could vary between species, and, consequently, the overall drug-induced effects on PPI could also vary.

The diverging results obtained on PPI and P50 suppression after *ayahuasca* administration to humans seemingly indicate a differential drug action. In addition to differences in receptor-level interactions, P50 suppression and PPI may reflect different stages of information processing and involve different brain structures. While P50 suppression is essentially viewed as a hippocampal process (Freedman et al. 1996; Adler et al. 1998), based on data from animal studies, PPI is thought to be modulated by a complex circuit involving the limbic cortex, striatum, pallidum, and pontine tegumentum, (Swerdlow and Geyer 1999; Swerdlow et al. 2001), offering many targets for pharmacological modulation. Swerdlow et al. (2000) have postulated that P50 and PPI are interrelated to the extent that hippocampal circuitry participates in both processes. Thus, the sites of pharmacological action and the subsequent modulation of each gating measure by different neurotransmitter systems may consequently show considerable variation.

In conclusion, at the doses administered, *ayahuasca* induced a different pattern of effects on PPI and P50. The results obtained seemingly indicate no effect, or at best, a mild enhancing effect of the drug on PPI, a measure of sensorimotor gating. On the contrary, the observed significant dose-dependent decreases in P50 suppression after *ayahuasca* suggest a suppressing effect of the drug on normal sensory gating in humans. This differential modulation of sensorimotor and sensory gating by *ayahuasca* in humans could be due to differential drug effects on brain structures participating in each process. However, the fact that the subjective-effect profile induced by *ayahuasca*, which was typical of the psychedelics, did not resemble that of acute psychosis should also be taken into consideration. In addition, the pharmacological characteristics of the beverage, which combines MAO-inhibitors and DMT, precludes the generalization of the present findings to all 5-$HT_{2A/2C}$ agonists. Future studies with *ayahuasca* should examine wider dose ranges to better characterize the effects of this drug on gating mechanisms in the CNS.

Acknowledgements The authors wish to express their gratitude to José María Fábregas, Félix González and Esther Martínez, for their support in the initial stages of the research project. We are also indebted to Cefluris, in Brazil, who kindly provided the *ayahuasca* (*Daime*) used in the present study. We also wish to acknowledge the support of: James C. Callaway, Department of Pharmaceutical Chemistry, University of Kuopio, Finland, for quantifying the DMT in *ayahuasca*; Maria Montero, Hospital de Sant Pau, Barcelona, who conducted the psychiatric interviews with the volunteers; Michael Schlichting, European College for the Study of Consciousness, Göttingen, Germany, who facilitated the Spanish version of the APZ questionnaire; and Gloria Urbano, Adelaida Morte, Sylvie Cotxet, David Martínez and Llúcia Benito for their collaboration in data collection.

References

Abduljawad KAJ, Langley RW, Bradshaw CM, Szabadi E (1998) Effects of bromocriptine and haloperidol on prepulse inhibition of the acoustic startle reflex in man. J Psychopharmacol 12:239–245

Abduljawad KAJ, Langley RW, Bradshaw CM, Szabadi E (1999) Effects of bromocriptine and haloperidol on prepulse inhibition: comparison of the acoustic startle eyeblink response and the N1/P2 auditory-evoked response in man. J Psychopharmacol 13:3–9

Adler LE, Pachtman E, Franks RD, Pecevich M, Waldo MC, Freedman R (1982) Neurophysiological evidence for a defect in neuronal mechanisms involved in sensory gating in schizophrenia. Biol Psychiatry 17:639–654

Adler LE, Rose G, Freedman R (1986) Neurophysiological studies of sensory gating in rats: effects of amphetamine, phencyclidine, and haloperidol. Biol Psychiatry 21:787–798

Adler LE, Hope C, Hoffer LD, Stephen C, Young D, Gerhardt G (1994a) Bromocriptine impairs P50 auditory sensory gating in normal control subjects. Biol Psychiatry 35:630

Adler LE, Hoffer L, Nagamoto HT, Waldo MC, Kisley MA, Griffith JM (1994b) Yohimbine impairs P50 auditory sensory gating in normal subjects. Neuropsychopharmacology 10:249–257

Adler LE, Olincy A, Waldo M, Harris JG, Griffith J, Stevens K, Flach K, Nagamoto H, Bickford P, Leonard S, Freedman R (1998) Schizophrenia, sensory gating, and nicotinic receptors. Schizophr Bull 24:189–202

Anonymous (2000) L'Ayahuasca: de l'Amazonie à la Jungle Urbaine. In: La Géopolitique Mondiale des Drogues 1998/1999. Observatoire Géopolitique des Drogues, Paris, pp 102–106

Blumenthal TD (1999) Short lead interval startle modification. In: Dawson ME, Schell AM, Böhmelt AH (eds) Startle modification. Implications for neuroscience, cognitive science and clinical science. Cambridge University Press, Cambridge, pp 51–71

Boutros NN, Uretsky N, Berntson G, Bornstein R (1994) Effects of cocaine on sensory inhibition in rats: preliminary data. Biol Psychiatry 36:242–248

Braff DL, Geyer MA (1980) Acute and chronic LSD effects on rat startle: data supporting an LSD-rat model of schizophrenia. Biol Psychiatry 15:909–916

Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology 156:234–258

Callaway JC, Raymon LP, Hearn WL, McKenna DJ, Grob CS, Brito GC, Mash DC (1996) Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with *ayahuasca*. J Anal Toxicol 20:492–497

Cardenas VA, Gill P, Fein G (1997) Human P50 suppression is not affected by variations in wakeful alertness. Biol Psychiatry 41:891–901

Dittrich A (1998) The standardized psychometric assessment of altered states of consciousness (ASCs) in humans. Pharmacopsychiatry 31[Suppl 2]:80–84

Dobkin de Rios M (1984) Visionary vine: hallucinogenic healing in the Peruvian Amazon. Waveland Press, Prospect Heights

Dobkin de Rios M (1996a) Hallucinogens: cross-cultural perspectives. Waveland Press, Prospect Heights

Dobkin de Rios M (1996b) Commentary on "Human pharmacology of Hoasca": a medical anthropology perspective. J Nerv Ment Dis 184:95–98

Dulawa SC, Gross C, Stark KL, Hen R, Geyer MA (2000) Knockout mice reveal opposite roles for serotonin 1A and 1B receptors in prepulse inhibition. Neuropsychopharmacology 22:650–659

Freedman R, Adler LE, Waldo MC, Pachtman E, Franks RD (1983) Neurophysiological evidence for a defect in inhibitory pathways in schizophrenia: comparison of medicated and drug-free patients. Biol Psychiatry 18:537–551

Freedman R, Adler LE, Myles-Worsley M, Nagamoto HT, Miller C, Kisley M, McRae K, Cawthra E, Waldo M (1996) Inhibitory gating of an evoked response to repeated auditory stimuli in schizophrenic and normal subjects. Arch Gen Psychiatry 53:1114–1121

Fridlund AJ, Carcioppo JT (1986) Guidelines for human electromyographic research. Psychophysiology 23:567–589

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 156:117–154

Gouzoulis-Mayfrank E, Heekeren K, Thelen B, Lindenblatt H, Kovar KA, Sass H, Geyer MA (1998) Effects of the hallucinogen psilocybin on habituation and prepulse inhibition of the startle reflex in humans. Behav Pharmacol 9:561–566

Hutchinson KE, Swift R (1999) Effect of D-amphetamine on prepulse inhibition of the startle reflex in humans. Psychopharmacology 143:394–400

Jerger K, Biggins C, Fein G (1992) P50 suppression is not affected by attentional manipulations. Biol Psychiatry 31:365–377

Johnson RG, Stevens KE, Rose GM (1998) 5-Hydroxytryptamine$_2$ receptors modulate auditory filtering in the rat. J Pharmacol Exp Ther 285:643–650

Krebs-Thomson K, Geyer MA (1998) Evidence for a functional interaction between 5-HT$_{1A}$ and 5-HT$_2$ receptors in rats. Psychopharmacology 140:69–74

Liechti ME, Geyer MA, Hell D, Vollenweider FX (2001) Effects of MDMA (Ecstasy) on prepulse inhibition and habituation of startle in humans after pretreatment with citalopram, haloperidol, or ketanserin. Neuropsychopharmacology 24:240–252

Light GA, Malaspina D, Geyer MA, Luber BM, Coleman EA, Sackeim HA, Braff DA (1999) Amphetamine disrupts P50 supression in normal subjects. Biol Psychiatry 46:990–996

McKenna DJ, Towers GHN, Abbott F (1984) Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of *Ayahuasca*. J Ethnopharmacol 10:195–223

Miller CL, Bickford PC, Luntz-Leybman V, Adler LE, Gerhardt GA, Freedman R (1992) Phencyclidine and auditory sensory gating in the hippocampus of the rat. Neuropharmacology 31:1041–1048

Ott J (1999) Pharmahuasca: human pharmacology of oral DMT plus harmine. J Psychoactive Drugs 31:171–177

Ouagazzal AM, Grottick AJ, Moureau JL, Higgins GA (2001) Effect of LSD on prepulse inhibition and spontaneous behaviour in the rat: a pharmacological analysis and comparison between two rat strains. Neuropsychopharmacology 25:565–575

Padich RA, McCloskey TC, Kehne JH (1996) 5-HT modulation of auditory and visual sensorimotor gating. II. Effects of the 5-HT$_{2A}$ antagonist MDL 100,907 on disruption of sound and light prepulse inhibition produced by 5-HT agonists in Wistar rats. Psychopharmacology 124:107–116

Picton TW, Hillyard SA, Krausz HI, Galambos R (1974) Human auditory evoked potentials. I: Evaluation of components. Electroencephalogr Clin Neurophysiol 36:179–190

Pierce PA, Peroutka SJ (1989) Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. Psychopharmacology 97:118–122

Riba J, Rodríguez-Fornells A, Urbano G, Morte A, Antonijoan R, Montero M, Callaway JC, Barbanoj MJ (2001a) Subjective effects and tolerability of the South American psychoactive beverage *Ayahuasca* in healthy volunteers. Psychopharmacology 154:85–95

Riba J, Rodríguez-Fornells A, Strassman RJ, Barbanoj MJ (2001b) Psychometric assessment of the Hallucinogen Rating Scale. Drug Alcohol Depend 62:215–223

Riba J, Anderer P, Morte A, Urbano G, Jané F, Saletu B, Barbanoj MJ (2002) Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage *Ayahuasca* in healthy volunteers. Br J Clin Pharmacol 53:613–628

Rivier L, Lindgren JE (1972) "Ayahuasca", the South American hallucinogenic drink: an ethnobotanical and chemical investigation. Econ Bot 26:101–129

Schultes RE, Hofmann A (1980) The botany and chemistry of hallucinogens. Charles C. Thomas, Springfield

Schultes RE, Hofmann A (1982) Plantas de los dioses: orígenes del uso de los alucinógenos. Fondo de Cultura Económica, México D.F.

Sipes TA, Geyer MA (1994) Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. Neuropharmacology 33:441–448

Sipes TE, Geyer MA (1995) DOI disruption of prepulse inhibition of startle in the rat is mediated by 5-HT$_{2A}$ and not by 5-HT$_{2C}$ receptors. Behav Pharmacol 6:839–842

Smith RL, Canton H, Barrett RJ, Sanders-Bush E (1998) Agonist properties of *N*,*N*-dimethyltryptamine at serotonin 5-HT_{2A} and 5-HT_{2C} receptors. Pharmacol Biochem Behav 61:323–330

Spielberger CD, Gorusch RL, Lushene RE (1970) Manual for the state-trait anxiety inventory. Consulting Psychologists Press, Palo Alto

Stevens KE, Fuller LL, Rose GM (1991) Dopaminergic and noradrenergic modulation of amphetamine-induced changes in auditory gating. Brain Res 555:91–98

Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R (1994) Dose-response study of *N*,*N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch Gen Psychiatry 51:98–108

Suzuki O, Katsumata Y, Oya M (1981) Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. Biochem Pharmacol 30:1353-1358

Swerdlow NR, Geyer MA (1999) Neurophysiology and neuropharmacology of short lead interval startle modification. In: Dawson ME, Schell AM, Böhmelt AH (eds) Startle modification: implications for neuroscience, cognitive science and clinical science. Cambridge University Press, Cambridge, pp 114–133

Swerdlow NR, Braff DL, Geyer MA (2000) Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon. Behav Pharmacol 11:185–204

Swerdlow NR, Geyer MA, Braff DL (2001) Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology 156:194–215

van Berckel BN, Oranje B, Van Ree JM, Verbaten MN, Kahn RS (1998) The effects of low dose ketamine on sensory gating, neuroendocrine secretion and behavior in healthy human subjects. Psychopharmacology 137:271–281

Vollenweider FX (1994) Evidence for a cortical–subcortical imbalance of sensory information processing during altered states of consciousness using positron emission tomography and [18F]fluorodeoxyglucose. In: Pletscher A, Ladewig D (eds) 50 years of LSD: current status and perspectives of hallucinogens. Parthenon, London, pp 67–86

Vollenweider FX, Vollenweider-Scherpenhuyzen MFI, Bäbler A, Vogel H, Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 9:3897–3902

Vollenweider FX, Remensberger S, Hell D, Geyer MA (1999) Opposite effects of 3,4-methylenedioxymethamphetamine (MDMA) on sensorimotor gating in rats versus healthy humans. Psychopharmacology 143:365–372

0022-3565/03/3061-73–83$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 306:73–83, 2003

Vol. 306, No. 1
49882/1069698
Printed in U.S.A.

Human Pharmacology of Ayahuasca: Subjective and Cardiovascular Effects, Monoamine Metabolite Excretion, and Pharmacokinetics

JORDI RIBA, MARTA VALLE, GLORIA URBANO, MERCEDES YRITIA, ADELAIDA MORTE, and MANEL J. BARBANOJ

Àrea d'Investigació Farmacològica, Institut de Recerca, Hospital de la Santa Creu i Sant Pau; and Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Barcelona, Spain

Received February 3, 2003; accepted March 13, 2003

ABSTRACT

The effects of the South American psychotropic beverage ayahuasca on subjective and cardiovascular variables and urine monoamine metabolite excretion were evaluated, together with the drug's pharmacokinetic profile, in a double-blind placebo-controlled clinical trial. This pharmacologically complex tea, commonly obtained from *Banisteriopsis caapi* and *Psychotria viridis*, combines *N,N*-dimethyltryptamine (DMT), an orally labile psychedelic agent showing 5-hydroxytryptamine$_{2A}$ agonist activity, with monoamine oxidase (MAO)-inhibiting β-carboline alkaloids (harmine, harmaline, and tetrahydroharmine). Eighteen volunteers with prior experience in the use of psychedelics received single oral doses of encapsulated freeze-dried ayahuasca (0.6 and 0.85 mg of DMT/kg of body weight) and placebo. Ayahuasca produced significant subjective effects, peaking between 1.5 and 2 h, involving perceptual modifications and increases in ratings of positive mood and activation. Diastolic blood pressure showed a significant increase at the high dose (9 mm Hg at 75 min), whereas systolic blood pressure and heart rate were moderately and nonsignificantly increased. C_{max} values for DMT after the low and high ayahuasca doses were 12.14 ng/ml and 17.44 ng/ml, respectively. T_{max} (median) was observed at 1.5 h after both doses. The T_{max} for DMT coincided with the peak of subjective effects. Drug administration increased urinary normetanephrine excretion, but, contrary to the typical MAO-inhibitor effect profile, deaminated monoamine metabolite levels were not decreased. This and the negligible harmine plasma levels found suggest a predominantly peripheral (gastrointestinal and liver) site of action for harmine. MAO inhibition at this level would suffice to prevent first-pass metabolism of DMT and allow its access to systemic circulation and the central nervous system.

Ayahuasca, also known by the names Daime, Yajé, Natema, and Vegetal, is a psychotropic plant tea used by shamans throughout the Amazon Basin in traditional medicine, rites of passage, and magico-religious practices (Schultes and Hofmann, 1982; Dobkin de Rios, 1984). This ancient pattern of use has given way to a more widespread and frequent consumption by members of a number of modern Brazilian-based syncretic religious groups, mainly the Santo Daime and the Uniao do Vegetal, which have incorporated the use of the beverage in their rituals (Dobkin de Rios, 1996). In recent years, groups of followers of these Brazilian religions have become established in the United States and in several European countries, including Germany, Great Britain, Holland, France, and Spain (Anonymous, 2000). As a larger number of people have come into contact with ayahuasca, the tea has begun to attract the attention of biomedical researchers (Callaway et al., 1999; Riba et al., 2001b).

Ayahuasca is obtained by infusing the pounded stems of the malpighiaceous vine *Banisteriopsis caapi* either alone or, more frequently, in combination with the leaves of *Psychotria viridis* (rubiaceae) in Brazil, Peru, and Ecuador or *Diplopterys cabrerana* (malpighiaceae), used mainly in Ecuador and Colombia (Schultes and Hofmann, 1980; McKenna et al., 1984). *P. viridis* and *D. cabrerana* are rich in the psychedelic indole *N,N*-dimethyltryptamine (DMT; Rivier and Lindgren, 1972; Schultes and Hofmann, 1980), whereas *B. caapi* contains substantial amounts of β-carboline alkaloids, mainly harmine and tetrahydroharmine (THH), and to a lesser extent harmaline and traces of harmol and harmalol (Rivier and Lindgren, 1972; McKenna et al., 1984).

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
DOI: 10.1124/jpet.103.049882.

ABBREVIATIONS: DMT, *N,N*-dimethyltryptamine; THH, tetrahydroharmine; LSD, D-lysergic acid diethylamide; CNS, central nervous system; MAO, monoamine oxidase; COMT, catechol-*O*-methyltransferase; VMA, vanillylmandelic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; MDMA, methylenedioxymethamphetamine; HPLC, high-performance liquid chromatography; VAS, visual analog scale(s); HRS, Hallucinogen Rating Scale; ARCI, Addiction Research Center Inventory; MBG, morphine-benzedrine group; PCAG, pentobarbital-chlorpromazine-alcohol group; BG, benzedrine group; AUC, area under the concentration-time curve; CL/F, total plasma clearance; V_z/F, apparent volume of distribution; ANOVA, analysis of variance; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

DMT is structurally related to the neurotransmitter serotonin and, like better-characterized psychedelics such as LSD and mescaline, binds to 5-hydroxytryptamine $_{2A}$ receptors in the central nervous system (CNS), where it acts as an agonist (Pierce and Peroutka, 1989; Smith et al., 1998). Studies in humans have shown that when administered parenterally, DMT provokes dramatic modifications in perception, the sense of self and reality that can be very intense but relatively short in duration (Strassman et al., 1994). The drug also exerts marked autonomic effects elevating blood pressure, heart rate, and rectal temperature, and causes mydriasis (Strassman and Qualls, 1994). Unlike the vast majority of known psychedelic phenethylamines, tryptamines, and ergolines, DMT is orally inactive (Ott, 1999), apparently due to metabolism by monoamine oxidase (MAO; Suzuki et al., 1981). Interestingly, harmine and harmaline, and, to a lesser extent, THH, are potent MAO inhibitors (Buckholtz and Boggan, 1977; McKenna et al., 1984). In 1968, Agurell and coworkers (cited in Ott, 1999, p. 172) postulated that the interaction between β-carbolines and DMT in ayahuasca "might result in specific pharmacological effects". It is now a widely accepted hypothesis that following ayahuasca ingestion, MAO inhibition brought about by harmine, given that it is more potent than THH and is present in the tea in larger amounts than harmaline (McKenna et al., 1984), prevents the enzymatic degradation of DMT, allowing its absorption. It has also been speculated that β-carbolines may contribute to the overall central effects of ayahuasca by blocking brain MAO and weakly inhibiting serotonin reuptake, which combined would lead to enhanced neurotransmitter levels and modulate the effects of DMT (Callaway et al., 1999).

In the present paper we report a double-blind placebo-controlled crossover clinical trial conducted with ayahuasca, in which subjective and cardiovascular effects, and alkaloid pharmacokinetics were assessed in a group of healthy volunteers experienced in psychedelic drug use. Additionally, urine monoamine metabolites were studied to measure in vivo the MAO-inhibitory effects of ayahuasca. In this respect, the neurotransmitters norepinephrine, epinephrine, and dopamine are physiologically degraded by MAO and catechol-*O*-methyltransferase (COMT) to produce deaminated and methylated metabolites, respectively. Serotonin, on the other hand, is exclusively metabolized by MAO to produce a deaminated compound. In vivo and in vitro studies have shown that when MAO is pharmacologically inhibited, the levels of MAO-dependent deaminated metabolites decrease and those of COMT-dependent methylated metabolites increase. In humans, MAO inhibitors decrease, after acute administration, the urinary excretion of vanillylmandelic acid (VMA), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA), the deaminated metabolites of norepinephrine/epinephrine, dopamine, and serotonin, respectively, while increasing that of metanephrine and normetanephrine, the methylated metabolites of epinephrine and norepinephrine, respectively (Pletscher, 1966; Koulu et al., 1989). Monoamine metabolites have both a CNS and a non-CNS origin, and their assessment in urine does not give information regarding the organ in which MAO was inhibited. Nevertheless, this approach can identify dose-response relationships after drug administration and allows for the study of the time course of MAO inhibition.

Materials and Methods

Volunteers

A total of 18 volunteers (15 males and 3 females) with experience in psychedelic drug use were recruited by word of mouth. Eligibility criteria required prior use of psychedelics on at least five occasions without sequelae derived thereof, i.e., psychedelic-related disorders as described in the DSM-III-R. Participants had used psychedelics from six to hundreds of times. The most commonly used psychedelic was LSD (17 of 18), followed by psilocybian mushrooms (15 of 18) and ketamine (10 of 18). The least commonly used were peyote (3 of 18), *Salvia divinorum* (3 of 18), mescaline (2 of 18), *Amanita muscaria* (2 of 18), and *Datura stramonium* (1 of 18). Although prior exposure to ayahuasca was not required for participation, two of the volunteers had ingested this tea before inclusion. Besides psychedelics, volunteers had consumed cannabis (18 of 18), cocaine (17 of 18), and MDMA (17 of 18). Volunteers were in good health, confirmed by medical history, laboratory tests, ECG, and urinalysis. Prior to physical examination, volunteers were interviewed by a psychiatrist (structured interview for DSM-III-R) and completed the trait-anxiety scale from the State-Trait Anxiety Inventory (Spielberger et al., 1970). Exclusion criteria included current or previous history of psychiatric disorder and/or family history of Axis-I psychiatric disorder in first degree relatives, alcohol or other substance dependence, and high scores on trait anxiety (over 1 standard deviation above normative mean). Participants had a mean age of 25.7 years (range 19–38), mean weight 66.47 kg (range 50.7–79.5), and mean height 175.11 cm (range 158–188). The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans, and was approved by the hospital's ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of ayahuasca, and the general psychological effects of psychedelics and their possible adverse effects, as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drug

To administer ayahuasca in accurate dosings and masked in a double-blind, double-dummy design, a 9.6-liter batch of Brazilian Daime was subjected to a freeze-drying process that yielded 611 g of powder, which was subsequently homogenized and analyzed. The DMT content was determined by HPLC, as described by Callaway et al. (1996), and the β-carbolines were determined according to a modified version of the method described therein. One gram of freeze-dried material contained 8.33 mg of DMT, 14.13 mg of harmine, 0.96 mg of harmaline, and 11.36 mg of THH, which corresponded to the following alkaloid concentrations in the original tea: DMT, 0.53 mg/ml; harmine, 0.90 mg/ml; harmaline, 0.06 mg/ml; and THH, 0.72 mg/ml. The ayahuasca doses administered to the volunteers in the present study were chosen based on tolerability and subjective effect data gathered in a previous study (Riba et al., 2001b). The low and the high dose contained, per kilogram of body weight: 0.6/0.85 mg of DMT, 1.0/1.4 mg of harmine, 0.07/0.09 mg of harmaline, and 0.82/1.16 mg of THH. The average (range) alkaloid content in milligrams administered in each dose (low dose/high dose) was: 39.8 (30.4–47.9)/57.4 (43.7–67.7) for DMT, 67.4 (51.6–81.2)/95.8 (74.2–114.8) for harmine, 4.6 (3.5–5.5)/6.5 (5.0–7.8) for harmaline, and 54.2 (41.5–65.3)/77.0 (59.6–92.3) for THH. The calculated individual dose for each volunteer was administered by combining 00 gelatin capsules containing different amounts of freeze-dried ayahuasca, i.e., 0.5 g, 0.25 g, or 0.125 g, and placebo capsules containing 0.75 g of lactose. Placebo capsules were added when necessary, so that all volunteers took the same number of capsules on each experimental day. It is interesting to note that although the amount of DMT administered with the present low dose was similar to that administered in the only other published study on the human pharmacology of ayahuasca (Callaway et al., 1999), the amounts of β-car-

bolines administered in this work were much lower. This was due to the different alkaloid proportions present in the tea samples used in each study. Thus, the average amounts (range) in milligrams administered by Callaway et al. (1999) were: 35.5 (28.8–43.2) for DMT, 252.3 (204.0–306.0) for harmine, 29.7 (24.0–36.0) for harmaline, and 158.8 (128.4–196.6) for THH.

Study Design

Each volunteer participated in four experimental sessions at least 1 week apart. Volunteers were informed that on each experimental day they would randomly receive a single oral dose of encapsulated freeze-dried ayahuasca (one low and one high dose), a placebo, and a random repetition of one of the three mentioned treatments. In actual fact, they all received a placebo on the first experimental day in a single-blind fashion, followed by one of the three treatments from days 2 to 4 in a double-blind balanced fashion, according to a randomization table. The first nonrandomized placebo was administered to familiarize the volunteers with the experimental setting and to minimize the stress associated with the experimental interventions. Volunteers were requested to abstain from any medication or illicit drug use 2 weeks before the beginning of the experimental sessions until the completion of the study. Volunteers also abstained from alcohol, tobacco, and caffeinated drinks 24 h before each experimental day. Urinalysis for illicit drug use was performed for each experimental session. The volunteers were admitted to the research unit on four separate experimental days. Upon arrival at 8:00 AM under fasting conditions, a cannula was inserted in the cubital vein of their right arm for drawing blood samples, and capsules were administered at approximately 10:00 AM with 250 ml of tap water. Throughout the experimental session, the volunteers remained seated in a comfortable reclining chair in a quiet, dimly lit room. At 4 h after administration of the capsules, when the most prominent subjective effects associated with the drug had disappeared, the volunteers had a meal. The last experimental time point was at 8 h, and volunteers were discharged approximately 9 h after administration.

Study Methods

Subjective Effect Measures. The subjective effects elicited by ayahuasca were measured by means of visual analog scales (VAS) and self-report questionnaires. VAS were 100-mm horizontal lines with the following labels: "any effect," indicating any effect, either physical or psychological, that the volunteer attributed to the administered drug; "good effects," indicating any effect the volunteer valued as good; "liking," reflecting that the volunteer liked the effects of the administered substance; "drunken," indicating any dizziness or lightheadedness; "stimulated," indicating any increases in thought speed and/or content, or any increases in associations and/or insights; "visions," indicating modifications in visual perception, including any variations in object shape, brightness, or color and any illusion, abstract or elaborate, seen with either eyes closed or open; and "high," which reflected any positive psychological effect the volunteer attributed to the drug. Except for the "visions" item, the other VAS items administered had been used in human studies by other researchers assessing the subjective effects of a variety of psychoactive drugs (Farré et al., 1993, 1998; Camí et al., 2000). The volunteers were requested to answer the VAS immediately before administration (baseline) and at 15, 30, 45, 60, and 75 min, and 1.5, 2, 2.5, 3, 3.5, 4, 6, and 8 h after administration.

Self-report questionnaires included the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI). The HRS (Strassman et al., 1994) measures psychedelic-induced subjective effects and includes six scales: Somaesthesia, reflecting somatic effects; Affect, sensitive to emotional and affective responses; Volition, indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; Cognition, describing modifications in thought processes or content; Perception, measuring visual, auditory, gustatory, and olfactory experiences; and, finally, Intensity, which reflects the strength of the overall experience. In the present study, a Spanish adaptation of the questionnaire was used (Riba et al., 2001a). The range of scores for all HRS scales is 0 to 4. The short version of the ARCI (Martin et al., 1971) consists of five scales or groups: MBG, morphine-benzedrine group, measuring euphoria and positive mood; PCAG, pentobarbital-chlorpromazine-alcohol group, measuring sedation; LSD, lysergic acid diethylamide scale, measuring somatic-dysphoric effects; BG, the benzedrine group, measuring intellectual energy and efficiency; and the A scale, an empirically derived scale measuring amphetamine-like effects. Both the A and BG scales are sensitive to psychostimulants. The range of scores is 0 to 16 for MBG, −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG, and 0 to 11 for A. The questionnaire had been translated into Spanish and validated by Lamas et al. (1994). Volunteers answered the ARCI immediately before drug administration and 4 h after drug intake, whereas the HRS was only answered at 4 h postadministration.

Cardiovascular Measures. Systolic and diastolic blood pressure and heart rate were measured with the volunteer seated, immediately before administration (baseline), and at 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min after intake using a sphygmomanometer cuff (Dinamap; Critikon, Tampa, FL) placed around the volunteer's left arm. No measurements were made after 240 min, the time point when subjects had their meal and after which they were allowed to move and leave the room.

Urine Samples. Urine was collected in fractions of 0 to 8 h, 8 to 16 h, and 16 to 24 h in plastic containers with 3 ml of 6 N HCl and kept in the refrigerator during the 0- to 24-h collection period. Volunteers took home the two plastic containers corresponding to the 8- to 16-h and 16- to 24-h periods. Volume of each fraction was recorded and pH was adjusted to 2 to 4 with 6 N HCl, and two 50-ml aliquots were frozen at −20°C and stored at −80°C until analysis. The following monoamine metabolites, VMA, HVA, 5-HIAA, metanephrine, and normetanephrine were quantified by means of HPLC with coulometric detection following previously validated procedures (Soldin and Hill, 1980; Parker et al., 1986; Gamache et al., 1993). The limit of quantification was 3 mg/l for VMA, HVA, and 5-HIAA, 0.05 mg/l for metanephrine, and 0.10 mg/l for normetanephrine.

Blood Samples. Blood samples (10-ml EDTA tubes) were drawn at baseline, 30, 60, 90, 120, and 150 min, and 3, 4, 6, 8, and 24 h after administration for analysis of DMT, harmine, harmaline, and THH concentrations in plasma and those of the *O*-demethylated metabolites harmol and harmalol. Samples were centrifuged at 2000 rpm for 10 min at 4°C and plasma was immediately frozen at −20°C. The frozen plasma samples were stored at −80°C until analysis. DMT was quantified by gas chromatography with nitrogen-phosphorus detection and the β-carbolines by means of HPLC with fluorescence detection following previously reported methods (Yritia et al., 2002). The limit of quantification was 1.6 ng/ml for DMT, 0.5 ng/ml for harmine, 0.3 ng/ml for harmaline, 1.0 ng/ml for THH, and 0.3 ng/ml for harmol and harmalol. The intraday and interday coefficients of variation were lower than 10.9% and 13.4%, respectively, for all determined compounds.

Pharmacokinetic Analysis

After quantification of the different compounds in plasma, the following pharmacokinetic parameters were calculated using a noncompartmental approach by means of WinNonlin software (version 3.0; Pharsight, Mountain View, CA): maximum concentration (C_{max}), time taken to reach the maximum concentration (T_{max}), and area under the concentration-time curve from 0 to 8 h (AUC_{0-8h}), calculated by means of the trapezoidal rule. AUC was extrapolated to infinity ($AUC_{0-\infty}$) by addition of the residual area calculated by the last plasma concentration/terminal elimination rate constant. Terminal half-life ($t_{1/2\lambda z} = \ln 2/\lambda_z$) was obtained by linear regression analysis of the terminal log-linear portion of the plasma-concentration curve. Clearance (CL/F) was determined as dose/$AUC_{0-\infty}$. Appar-

ent volume of distribution (V_z/F) was calculated as dose/($\lambda_z \cdot AUC_{0-\infty}$). The $AUC_{0-\infty}$ normalized by dose ($AUC_{0-\infty}$/D) was also calculated. All data are expressed as mean ± S.D. except for T_{max}, where median and range are given.

Statistics

Prior to statistical analysis, ARCI scores were transformed to differences from preadministration values, and the following parameters were calculated for VAS items: peak effect (maximum absolute change from baseline values), time taken to reach the maximum effect (t_{max}), and the 8-h area under the curve (AUC_{0-8h}) of effect versus time calculated by the trapezoidal rule. For cardiovascular variables, peak effect (maximum absolute change from baseline values) and the 4-h area under the curve (AUC_{0-4h}) of effect versus time were calculated. The obtained parameters, transformed ARCI scores, and raw HRS scores were analyzed by means of a one-way repeated measures ANOVA with drug (placebo, ayahuasca low dose, ayahuasca high dose) as factor. When a significant effect was observed, post hoc comparisons were performed using Tukey's multiple comparisons test. The time course of subjective effects was explored using repeated measures two-way ANOVAs with drug and time (13 time points) as factors. When a drug by time interaction was significant, multiple comparisons were performed at each time point by means of Tukey's test.

Monoamine metabolite levels in urine were analyzed by means of a one-way repeated measures ANOVA with drug (placebo, ayahuasca low dose, ayahuasca high dose) as factor. When a significant effect was observed, post hoc comparisons were performed using Tukey's test. The time course of effects was explored using repeated measures two-way ANOVAs with drug and time (three time points) as factors. Pharmacokinetic parameter comparisons between doses were performed by means of Student's t test, except for T_{max}, which was compared by means of a nonparametric Wilcoxon test.

To explore possible differences in the time-to-peak of DMT plasma concentrations and time-to-peak of subjective effects (for each of the administered VAS), nonparametric Wilcoxon tests were performed comparing T_{max} for DMT and t_{max} for each VAS. These tests were performed for data obtained after each of the two administered ayahuasca doses. In all tests performed, differences were considered statistically significant for p values lower than 0.05.

Results

Subjective Effects. Subjective effects results are shown in Tables 1 and 2 and Figs. 1 and 2. Ayahuasca administration induced significant increases in all six HRS scales, both after the low and the high dose, except for Volition, which showed statistically significant differences from placebo only after the 0.85 mg of DMT/kg dose. The ARCI questionnaire showed statistically significant dose-dependent increases after ayahuasca in measures of stimulatory effects (A scale), euphoria (MBG scale), and somatic symptoms (LSD scale).

TABLE 1

Results of the statistical analyses performed on raw HRS scores, transformed ARCI scores (differences from predrug values), VAS measures (peak values and AUC_{0-8h}), and cardiovascular parameters (peak values and AUC_{0-4h})

For all measures $n = 18$.

Variable		ANOVA (2,34)		Tukey's Multiple Comparison Test		
				Placebo		Low Dose/High
		F	p Value	Low Dose	High Dose	
HRS						
Affect		29.35	<0.001	**	**	**
Cognition		31.66	<0.001	*	**	**
Somaesthesia		39.62	<0.001	**	**	**
Perception		38.76	<0.001	**	**	**
Volition		4.68	0.016	N.S.	*	N.S.
Intensity		77.35	<0.001	**	**	**
ARCI						
A		23.10	<0.001	*	**	**
BG		3.62	0.058			
LSD		10.05	<0.001	*	**	N.S.
MBG		11.22	<0.001	N.S.	**	N.S.
PCAG		0.91	0.412			
VAS						
Any Effect	Peak	39.62	<0.001	**	**	**
	AUC	18.06	<0.001	*	**	**
Good Effects	Peak	26.64	<0.001	**	**	*
	AUC	18.69	<0.001	**	**	*
Liking	Peak	29.82	<0.001	**	**	*
	AUC	15.10	<0.001	**	**	N.S.
Visions	Peak	16.28	<0.001	**	**	*
	AUC	7.25	0.002	N.S.	**	N.S.
Drunken	Peak	6.26	0.005	N.S.	**	N.S.
	AUC	4.83	0.014	N.S.	*	N.S.
Stimulated	Peak	16.62	<0.001	**	**	*
	AUC	11.57	<0.001	N.S.	**	*
High	Peak	33.97	<0.001	**	**	**
	AUC	22.33	<0.001	*	**	**
Cardiovascular						
SBP	Peak	2.91	0.068			
	AUC	1.90	0.166			
DBP	Peak	15.54	<0.001	**	**	N.S.
	AUC	5.59	0.008	*	*	N.S.
HR	Peak	1.79	0.183			
	AUC	3.12	0.057			

* $p < 0.05$; ** $p < 0.01$.

TABLE 2
Positive responses on particular items of the HRS questionnaire given by at least 75% of the 18 volunteers after the high ayahuasca dose
Each column indicates the number of subjects who reported the effect, regardless of intensity, at the two different ayahuasca doses administered and placebo. The letter in parentheses indicates the HRS scale in which the item belongs.

	Item	Placebo	0.6 mg/kg	0.85 mg/kg
1	High (I)	1/18	15/18	17/18
2	Body feels different (S)	4/18	12/18	17/18
3	Visual effects (P)	2/18	10/18	17/18
4	A "rush" (S)	0/18	9/18	17/18
5	Change in rate of time passing (C)	2/18	12/18	16/18
6	Eyes open visual field vibrating or jiggling (P)	2/18	10/18	15/18
7	Electric/tingling feeling (S)	1/18	9/18	15/18
8	Change in quality of thinking (C)	2/18	8/18	15/18
9	Change in visual distinctiveness of objects in room (P)	4/18	7/18	15/18
10	Sounds in room sound different (P)	2/18	5/18	15/18
11	Urge to close eyes (V)	5/18	8/18	14/18
12	Change in distinctiveness of sounds (P)	2/18	7/18	14/18
13	Change in rate of thinking (C)	1/18	7/18	14/18
14	Excited (A)	1/18	7/18	14/18

A, Affect; C, Cognition; I, Intensity; P, Perception; S, Somaesthesia; V, Volition.

Scores on the BG and PCAG scales were not significantly different from placebo.

Scorings on all seven VAS items showed significant drug effects (peak values and AUC) and significant drug by time interactions. Initial effects appeared between 30 and 45 min, reflected as rises in the VAS any effect item, and were followed by a prominent increase at around 60 min, as indicated by steep rises in all seven VAS items. In general terms, the maximum scorings were observed between 90 and 120 min after drug administration. A gradual return to baseline levels followed thereafter and was complete at 360 min. Regarding effect magnitude, the largest scores were obtained for the VAS any effect, liking, and high, followed by VAS good effects, visions, and stimulated items. The least modified VAS after ayahuasca administration was the drunken item.

More qualitative information on the nature of the effects brought about by ayahuasca is provided in Table 2, which lists the most frequently reported positive responses to specific items of the HRS questionnaire.

Cardiovascular Effects. Mean values for systolic (SBP) and diastolic blood pressure (DBP) and heart rate (HR) over time are presented in Fig. 3, and results of the statistical analysis performed are shown in Table 1.

Ayahuasca administration produced only moderate elevations of cardiovascular parameters. Statistically significant changes relative to placebo were only found for DBP, both for peak values and AUC. The largest difference in DBP between the low dose and placebo was 9 mm Hg and occurred at 75 min after dosing. Between the high dose and placebo, differences of 10 and 9 mm Hg were observed at 15 and 75 min, respectively. A maximal increase of 7 mm Hg from baseline values was observed at 60 min for the low dose. After the high dose, a maximal increase of 9 mm Hg was observed at 15 min. For SBP, the largest differences with placebo were observed at 75 min and corresponded to 4 and 6 mm Hg increases for the low and high dose, respectively. Similarly, the maximal increase in SBP relative to baseline values was observed at 75 min and corresponded to 6 and 8 mm Hg for the low and high dose, respectively. Finally, HR showed the largest differences with placebo at 60 min and corresponded to 5 and 4 beats/min increases for the low and the high ayahuasca doses, respectively. The maximal increase from baseline values observed for HR was 4 beats/min and occurred at 60 min after administration of both the low and high ayahuasca doses.

Only two volunteers showed SBP values equal to or above 140 mm Hg at any time point: volunteer 1 at 75 and 90 min (140 mm Hg) after receiving the low dose, and at 60 (146 mm Hg) and 75 min (140 mm Hg) after receiving the high dose; and volunteer 6 as early as 15 min after administration of the high ayahuasca dose (146 mm Hg). Two volunteers showed DBP above 90 mm Hg: volunteer 1 at 30 min (93 mm Hg) after the low dose, and at 15 min (96 mm Hg) after the high dose; and volunteer 15 at 120 and 150 min (95 and 92 mm Hg, respectively) after administration of the high dose. Regarding HR, volunteer 1 also showed values above 100 beats/min (101 beats/min) at 60 min after the high dose.

Urine Monoamine Metabolites. Urine samples were successfully collected for 15 of the 18 volunteers enrolled in the study, and results are given for this subgroup only. Statistical analyses showed a significant effect of drug only for normetanephrine. No significant drug by time interaction was found for any of the metabolites studied. In view of this, the total monoamine metabolite amounts excreted during the 0- to 24-h period after placebo and the two ayahuasca doses are presented in Table 3. As shown therein, rather than the expected decreases in deaminated metabolites (VMA, HVA, 5-HIAA), drug administration increased the excretion of these compounds nonsignificantly. Similarly, levels of the *O*-methylated metabolites metanephrine and normetanephrine increased with dose, although only the latter showed statistically significant differences with placebo.

Pharmacokinetics. The time course of plasma concentrations and the calculated pharmacokinetic parameters for DMT, harmaline, THH, harmol, and harmalol are shown in Fig. 4 and Table 4. The graphs correspond to 15 of the total 18 participants enrolled in the study. To avoid the miscalculation of pharmacokinetic parameters, data from three volunteers were excluded from the analysis due to vomiting occurring after administration of the low dose (volunteer 6) and the high dose (volunteers 4 and 18). An additional subject (volunteer 12) was excluded from the calculation of harmalol parameters. Plasma levels for this volunteer after the high dose showed a plateau between 6 and 24 h, precluding parameter assessment.

As shown in Table 4, C_{max} and AUC values increased with

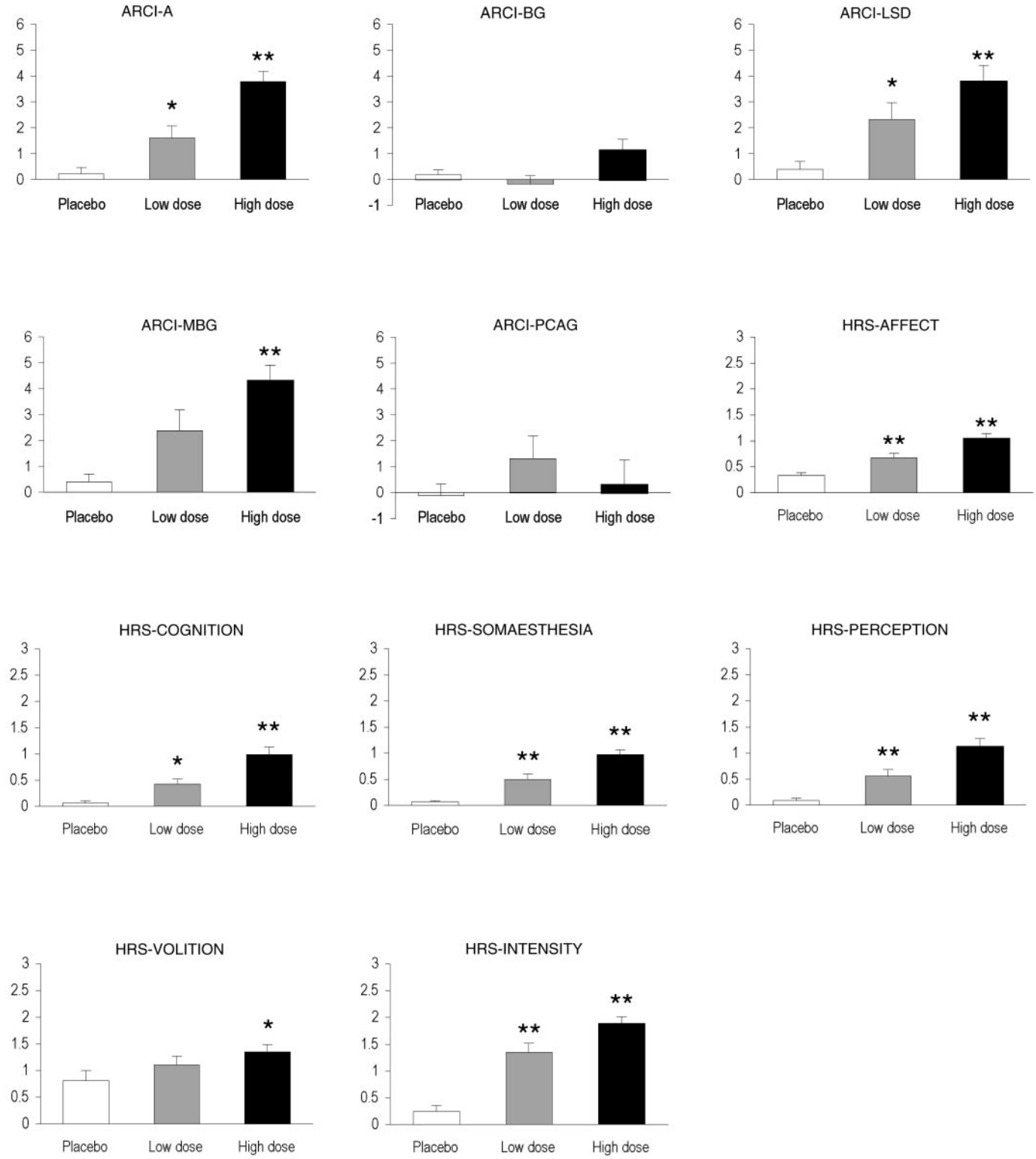


Fig. 1. Mean scores on the ARCI and HRS scales after administration of placebo (white), 0.6 mg of DMT/kg of body weight ayahuasca (shaded), and 0.85 mg of DMT/kg of body weight ayahuasca (black). Error bars denote 1 S.E.M. ($n = 18$). Significant differences from placebo are indicated by one ($p < 0.05$) or two ($p < 0.01$) asterisks.

dose for all measured compounds. DMT showed a T_{max} of 1.5 h (median) after both the low and high doses. Nevertheless, the upper end of the range of T_{max} values increased with dose, and the Wilcoxon test indicated a statistically significant difference between doses. A larger T_{max} after the high ayahuasca dose is evident also in the DMT concentration-time curve included in Fig. 4. Both harmaline and THH plasma concentrations peaked later than DMT, and their T_{max} values were larger after the high relative to the low ayahuasca dose. An unexpected finding was the absence of

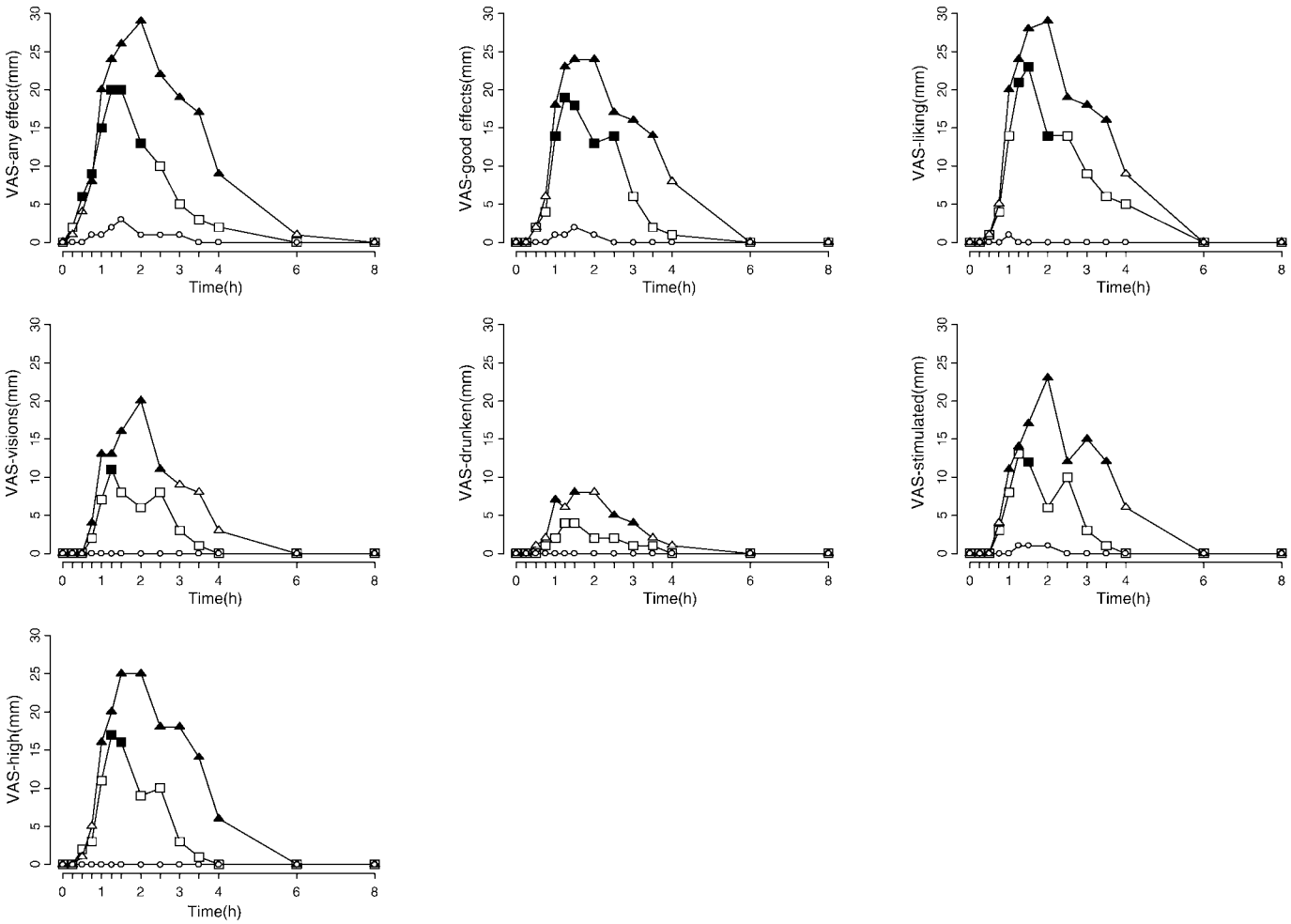


Fig. 2. Time curves of scores on the seven VAS items (means from 18 volunteers) after administration of placebo (circle), 0.6 mg of DMT/kg of body weight ayahuasca (square), and 0.85 mg of DMT/kg of body weight ayahuasca (triangle). Filled symbols indicate a significant difference from placebo.

measurable harmine plasma levels except for a few time points in 4 of 18 volunteers, precluding the calculation of pharmacokinetic parameters for this alkaloid.

Interestingly, all volunteers showed measurable levels of harmol, the *O*-demethylated analog of harmine. Plasma concentrations showed dose-dependent increases and peaked at 1.5 and 2 h after the low and high doses, respectively. Harmalol, the *O*-demethylated analog of harmaline, could also be quantified. Maximum concentrations were attained later than for harmaline, with T_{max} observed at 2.5 and 2.75 h after the low and high dose, respectively.

The AUC normalized by dose was calculated for each parent alkaloid, and these values were compared between doses by means of a paired Student's *t* test. A statistically significant difference was found for DMT, suggesting a possible nonproportional increase of plasma levels between doses. In line with this possibility, mean V_z/F and CL/F values calculated for DMT decreased with dose. These decreases were statistically significant for V_z/F and showed a tendency for CL/F ($t(14) = 1.94$, $p = 0.073$).

In support of a parallel evolution of DMT plasma levels and subjective effects, no significant differences were found between DMT T_{max} values and any of the seven VAS t_{max} values at any of the two administered ayahuasca doses.

Discussion

The psychotropic effects of ayahuasca could be demonstrated in a group of experienced psychedelic users who, in their vast majority, had reported no prior exposure to the tea. Oral administration of the freeze-dried material induced feelings of increased activation (ARCI-A, VAS-stimulated), euphoria and well being (ARCI-MBG, VAS-high, VAS-liking, VAS-good effects), and somatic effects (ARCI-LSD), in addition to perceptual modifications (HRS-Perception, VAS-visions) and changes in thought content (HRS-Cognition) and increased emotional lability (HRS-Affect). Increases in VAS-high have been observed after a great variety of drugs including MDMA (Camí et al., 2000), cocaine (Farré et al., 1993), and the sedative flunitrazepam (Farré et al., 1998). The VAS-stimulated item reflects more specifically the effects of psychostimulants such as amphetamine and MDMA (Camí et al., 2000). Increases in VAS-drunken, which was the least modified VAS item by ayahuasca, have been observed mainly after sedatives, such as flunitrazepam (Farré et al., 1998), and alcohol (Farré et al., 1993), but also after 125 mg of MDMA (Camí et al., 2000). Regarding the HRS, our findings are in line with results by other researchers who have demonstrated statistically significant increases in all HRS

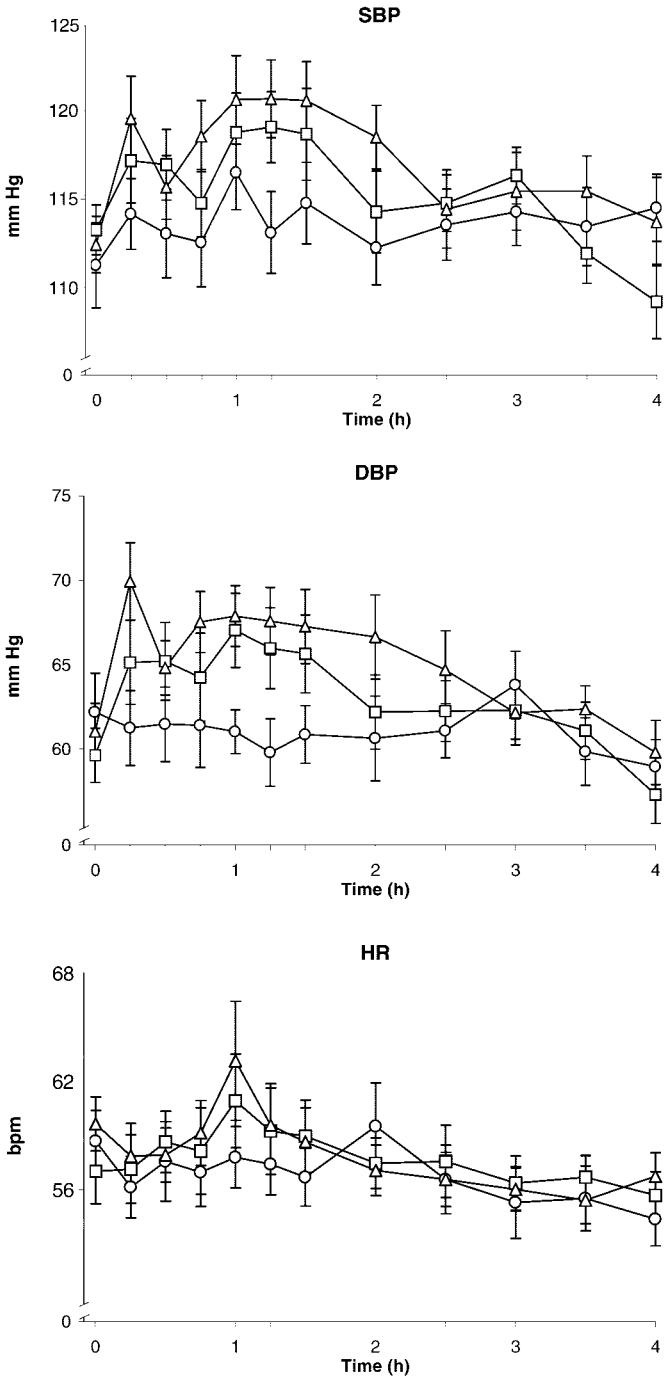


Fig. 3. Time course of cardiovascular measures (means from 18 volunteers) after administration of placebo (circle), 0.6 mg of DMT/kg of body weight ayahuasca (square), and 0.85 mg of DMT/kg of body weight ayahuasca (triangle). Error bars denote ± 1 S.E.M. (n = 18).

scales after the administration of various psychedelics, such as i.v. DMT and oral psilocybin (Strassman et al., 1994; Gouzoulis-Mayfrank et al., 1999). However, ayahuasca differed from these drugs in the time course of effects. The overall duration was longer than that of i.v. DMT, but shorter than that of mescaline or LSD (Strassman, 1994). Finally, regarding the ARCI questionnaire, increases in the ARCI-A, ARCI-BG, and ARCI-MBG scales are a common feature of psychostimulants (Martin et al., 1971; Lamas et al., 1994). However, in contrast, with drugs like amphetamine, methamphetamine, ephedrine, and methylphenidate (Martin et al., 1971), ayahuasca did not induce significant increases in the ARCI-BG scale, a measure of subjectively perceived improvement in intellectual efficiency. The coexistence of drug-induced stimulation with a wide range of modifications in the sensorium places ayahuasca among the psychedelics, a drug class which shares arousing properties with psychostimulants (Brawley and Duffield, 1972).

The present results on the subjective effects induced by ayahuasca in a clinical research setting replicate those obtained in a preliminary study involving a smaller sample of volunteers with prior experience with ayahuasca, and with a single-blind nonrandomized design (Riba et al., 2001b). In the previous study, statistically significant increases were observed in all HRS items, except volition, and in the ARCI-MBG, ARCI-LSD, and ARCI-A scales. In the present study, however, scores on these measures at the 0.6 and 0.85 mg of DMT/kg doses tended to be lower than those obtained after 0.5 and 0.75 mg of DMT/kg doses, respectively. Several factors such as sample size, study design, and prior exposure to ayahuasca could account for these differences. Scores on the HRS items at the present low dose were also lower than those reported by Grob et al. (1996), except for the somaesthesia and perception items, after the administration of an equivalent ayahuasca dose, in terms of DMT content, to a group of experienced long-term ritual users. Nevertheless, scores on all HRS items after the present high dose were higher than those reported by these researchers. Compared with i.v. DMT as described by Strassman et al. (1994), ayahuasca evokes effects of milder intensity, which show a slower onset and a longer overall duration. Scorings on the six HRS scales after the present high dose fell between those reported after 0.1 and 0.2 mg/kg i.v. DMT.

In our previous study on ayahuasca (Riba et al., 2001b), we failed to observe statistically significant modifications of cardiovascular parameters in a five-subject sample. In the present work, only modifications in DBP reached statistical significance. Increases in DBP, SBP, and HR were milder than those reported for other more prototypical sympathomimetics, such as amphetamine or MDMA, at doses showing psychotropic properties (Mas et al., 1999; de la Torre et al., 2000). DBP increases from baseline values after both ayahuasca doses were somewhat lower than the elevations from baseline values reported by Callaway et al. (1999) after an ayahuasca dose containing 0.48 mg of DMT/kg but larger amounts of β-carbolines.

The time course of DMT plasma concentrations closely paralleled that of subjective effects. The steep rise in DMT plasma levels observed at 1 h coincided with an analogous rise in VAS scores, and peak DMT concentrations and peak effects were obtained between 1.5 and 2 h. In the present study, quantifiable plasma levels were observed for DMT and THH. T_{max} values for DMT and THH were similar to those reported by Callaway et al. (1999). However, C_{max} values for DMT and THH in the present study were lower than expected, even after taking into account the smaller amounts administered in the case of THH. This could be due to a lower alkaloid bioavailability from the lyophilizate compared with the aqueous solution administered by Callaway et al. (1999). The calculated V_z/F values are similar in both studies, but Callaway et al. (1999) reported higher $t_{1/2}$ and lower CL/F values. In the case of DMT, these differences may be associ-

TABLE 3
Urinary excretion of monoamine metabolites pooled from 0 to 24 h after placebo, 0.6 mg, and 0.85 mg of DMT/kg of body weight ayahuasca
Figures indicate mean values (95% confidence interval), expressed in micromoles, from 15 volunteers.

Metabolite	ANOVA		Placebo	Tukey's Multiple Comparison test		
				Placebo		Low Dose/High
	F	*p* Value		Low Dose	High Dose	
VMA	0.61	0.552	20.21 (15.58–32.91)	22.54 (15.11–33.41)	23.31 (14.76–33.30)	
HVA	0.17	0.843	30.32 (23.22–49.14)	32.73 (20.83–46.90)	34.36 (19.44–45.72)	
5-HIAA	1.21	0.313	35.73 (27.11–57.53)	37.30 (28.64–60.55)	43.07 (34.25–71.63)	
MN	1.94	0.163	0.52 (0.41–0.87)	0.56 (0.46–0.96)	0.62 (0.51–1.06)	
NMN	12.56	<0.001	1.06 (0.86–1.79)	1.18 (1.03–2.09)	1.40** (1.22–2.48)	*

MN, metanephrine; NMN, normetanephrine.
* $p < 0.05$; ** $p < 0.01$.

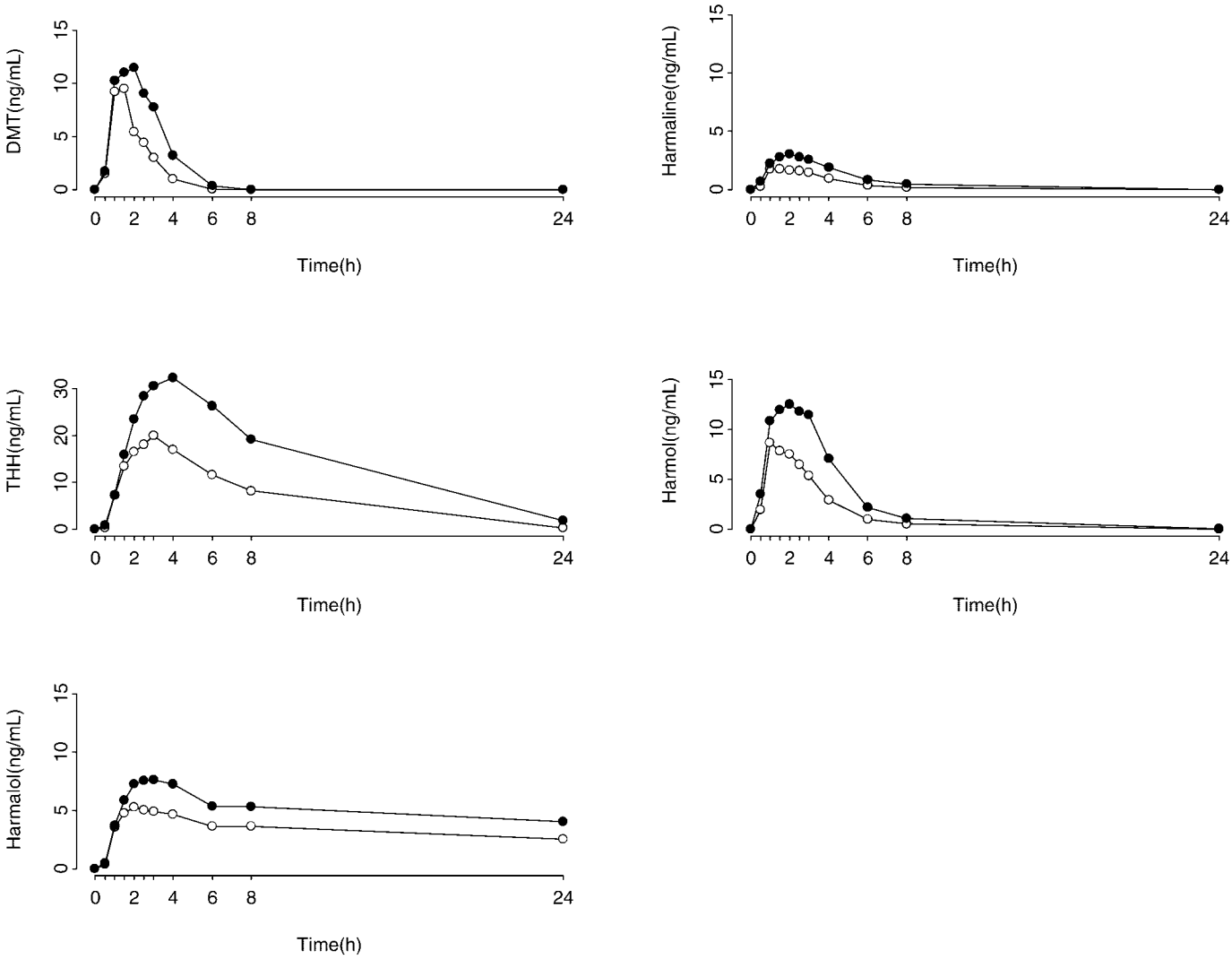


Fig. 4. Plasma concentration-time curves ($n = 15$) for three of the four main alkaloids present in ayahuasca (DMT, harmaline, and THH) and the *O*-demethylated analogs of harmine (harmol) and harmaline (harmalol). Open circles, low 0.6 mg of DMT/kg dose of ayahuasca; filled circles, high 0.85 mg DMT/kg dose of ayahuasca.

ated with the lower levels of harmala alkaloids present in our ayahuasca and the consequent lower degree of MAO inhibition. In addition to these interstudy differences, it is interesting to note that the normalized AUC calculated for DMT in the present study showed a statistically significant increase between the low and the high ayahuasca doses. This is suggestive of a nonlinear increment of DMT levels following the administration of increasing doses of ayahuasca. Considering that both V_z/F and CL/F decreased in a similar proportion between doses, these data could be interpreted as indicating a greater DMT bioavailability following the high dose, probably related to the higher amounts of harmala alkaloids ingested, leading to more effective MAO inhibition.

Another relevant difference from the study by Callaway et al. (1999) is the lack of measurable concentrations of harmine in plasma and the presence of significant levels of harmol and

TABLE 4

Pharmacokinetic parameters for DMT, harmaline, THH, harmol, and harmalol calculated for each of the two administered ayahuasca doses

Values indicate mean (S.D.), except for T_{max}, where median (range) is given. Fifteen volunteers were included in the analysis except for harmalol, where parameters were calculated from 14 volunteers.

	C_{max}	T_{max}	AUC_{0-8h}	$AUC_{0-\infty}$	$AUC_{0-\infty}/D$	$t_{1/2\lambda z}$	CL/F	V_z/F
	ng/ml	*h*	*ng/ml · h*$^{-1}$	*ng/ml · h*$^{-1}$		*h*	*l/h*	*liters*
Low Dose								
DMT	12.14* (9.09)	1.5* (1–2.5)	18.84* (10.67)	21.55* (9.93)	0.0005* (0.0003)	1.07 (0.58)	2281.41 (1054.7)	3509.86* (2158.08)
Harmaline	2.48* (1.28)	1.5 (1–3)	7.02* (4.02)	8.13* (4.39)	0.0017 (0.0009)	2.01 (0.56)	745.76 (379.68)	2040.75* (1044.47)
THH	23.06* (11.45)	2.5* (1.5–3)	100.83* (58.20)	172.07* (123.75)	0.0030 (0.0021)	4.78 (3.45)	559.84 (408.74)	3069.87 (2551.81)
Harmol	10.95* (6.04)	1.5 (1–2.5)	27.08* (12.51)	28.33* (12.78)		1.64 (0.29)		
Harmalol	6.74* (3.52)	2.5* (1–4)	31.14* (15.91)	206.93 (165.97)		30.33 (20.53)		
High Dose								
DMT	17.44 (10.49)	1.5 (1–4)	33.17 (14.68)	38.33 (17.53)	0.0007 (0.0003)	1.06 (0.77)	1812.65 (803.66)	2505.97 (1529.11)
Harmaline	4.32 (2.43)	2 (1–4)	12.80 (5.75)	14.87 (7.34)	0.0023 (0.0012)	1.95 (0.81)	596.78 (370.42)	1439.23 (567.18)
THH	39.40 (20.63)	3 (1.5–6)	180.89 (106.51)	351.89 (255.44)	0.0046 (0.0034)	4.68 (1.52)	364.94 (291.34)	2072.70 (1044.60)
Harmol	17.57 (7.72)	2 (1–3)	49.97 (16.88)	52.27 (17.30)		1.49 (0.28)		
Harmalol	9.59 (4.17)	2.75 (1.5–4)	46.79 (20.60)	333.54 (304.94)		48.64 (77.09)		

* $p < 0.05$.

harmalol. Differences in ayahuasca harmine content alone cannot entirely explain the absence of this alkaloid in plasma, considering that THH was present in the lyophilizate in amounts similar to those of harmine and was later measurable in plasma. Thus, harmine was either not absorbed in the gastrointestinal tract or was extensively degraded by first-pass metabolism before reaching systemic circulation. The presence of harmol in plasma would support the second hypothesis. Harmol glucuronide and harmol sulfate have been described as the main urine metabolites of harmine following its i.v. administration in humans (Slotkin et al., 1970). A very recent study has found cytochrome P450 to catalyze the *O*-demethylation of harmine and harmaline, and has identified CYP2D6 and CYP1A1 as the major isoenzymes involved in the process (Yu et al., 2003). Nevertheless, we cannot conclude that harmine was completely metabolized to render harmol, because very small amounts of harmol and harmalol have been detected in *B. caapi* and ayahuasca (Rivier and Lindgren, 1972; McKenna et al., 1984). Thus, it cannot be entirely ruled out that at least part of the amounts found in plasma could have been ingested with the tea.

The low plasma levels found for harmine in the present study could explain the absence of a clear-cut MAO inhibitor effect on the urinary excretion of monoamine metabolites. The acute administration of a MAO-A inhibitor induces a decrease in the levels of oxidized deaminated monoamine metabolites and an increase in the levels of COMT-dependent methylated compounds (Pletscher, 1966; Koulu et al., 1989). Whereas in the present study normetanephrine, a methylated breakdown product of norepinephrine, showed statistically significant increases after dosing with ayahuasca, the levels of the deaminated metabolites measured, i.e., VMA, HVA, and 5-HIAA, did not show decreases but, rather, were nonsignificantly increased. It is thus unclear whether the observed neurotransmitter metabolite profile was secondary to MAO inhibition. An alternative explanation would be an increase in norepinephrine release induced by DMT, which would fit well the observed sympathomimetic properties of this compound. However, this assumption is not supported by the limited available evidence from related compounds. Results obtained in two studies involving LSD administration to humans found no drug effects on monoamine metabolite excretion (Hollister and Moore, 1967; Messiha and Grof, 1973), and to our knowledge, no data are available on the effects of parenteral DMT on these measures. In any case, MAO inhibition by ayahuasca alkaloids effectively facilitated the access of DMT to systemic circulation but may have been insufficiently potent or insufficiently prolonged to modify the profile of deaminated monoamine metabolites in the 8-h urine collection periods used.

To conclude, the present findings indicate that following ayahuasca administration to humans, measurable DMT plasma levels are obtained together with distinct psychedelic effects. Psychoactivity is attained with negligible levels of circulating harmine. These results and the lack of a clear-cut systemic MAO inhibitor effect are suggestive of a harmine-DMT interaction predominantly taking place in the gastrointestinal tract and possibly in the liver. Harmine effects at a peripheral level would appear to suffice to prevent first-pass metabolism of DMT and allow its access to the CNS in amounts able to evoke psychotropic effects.

Acknowledgments

We express gratitude to José María Fábregas, Félix González, and Esther Martínez for their support in the initial stages of the research project. We are also indebted to CEFLURIS in Brazil, who kindly provided the ayahuasca (Daime) used in the present study. We also acknowledge the support of James C. Callaway, Department of Pharmaceutical Chemistry, University of Kuopio, Finland, for quantifying the DMT in ayahuasca; María Montero, who conducted the psychiatric interviews with the volunteers; Josep Maria Queraltó, Biochemistry Department, Hospital de Sant Pau, Barcelona, for quantifying urine monoamine metabolites; and Sylvie Cotxet, David Martínez, and Llúcia Benito for collaboration in data collection, and Pablo Ayesta for editing the figures.

References

Anonymous (2000) L'Ayahuasca: de l'Amazonie à la Jungle Urbaine, in *La Géopolitique Mondiale des Drogues 1998/1999*, pp 102–106, Observatoire Géopolitique des Drogues, Paris.

Brawley P and Duffield JC (1972) The pharmacology of hallucinogens. *Pharmacol Rev* **24:**31–66.

Buckholtz NS and Boggan WO (1977) Monoamine oxidase inhibition in brain and liver produced by β-carbolines: structure-activity relationships and substrate specificity. *Biochem Pharmacol* **26:**1991–1996.

Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, Andrade EN, Andrade EO, and Mash DC (1999) Pharmacokinetics of *hoasca* alkaloids in healthy humans. *J Ethnopharmacol* **65:**243–256.

Callaway JC, Raymon LP, Hearn WL, McKenna DJ, Grob CS, Brito GC, and Mash DC (1996) Quantitation of *N,N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with *Ayahuasca*. *J Anal Toxicol* **20:**492–497.

Camí J, Farré M, Mas M, Roset PN, Poudevida S, Mas A, San L, and de la Torre R (2000) Human pharmacology of 3,4-methylenedioxymethamphetamine ("ecstasy"): psychomotor performance and subjective effects. *J Clin Psychopharmacol* **20:**455–466.

de la Torre R, Farré M, Roset PN, Hernández López C, Mas M, Ortuño J, Menoyo E,

Pizarro N, Segura J, and Camí J (2000) Pharmacology of MDMA in humans. *Ann NY Acad Sci* **914:**225–237.
Dobkin de Rios M (1984) *Visionary Vine: Hallucinogenic Healing in the Peruvian Amazon*. Waveland Press, Prospect Heights, IL.
Dobkin de Rios M (1996) Commentary on "Human pharmacology of Hoasca": a medical anthropology perspective. *J Nerv Ment Dis* **184:**95–98.
Farré M, de la Torre R, Llorente M, Lamas X, Ugena B, Segura J, and Camí J (1993) Alcohol and cocaine interactions in humans. *J Pharmacol Exp Ther* **266:**1364–1373.
Farré M, Terán MT, Roset PN, Mas M, Torrens M, and Camí J (1998) Abuse liability of flunitrazepam among methadone-maintained patients. *Psychopharmacology* **140:**486–495.
Gamache PH, Kingery ML, and Acworth IN (1993) Urinary metanephrine and normetanephrine determined without extraction by using liquid chromatography and coulometric array detection. *Clin Chem* **39:**1825–1830.
Gouzoulis-Mayfrank E, Thelen B, Habermeyer E, Kunert HJ, Kovar KA, Lindenblatt H, Hermle L, Spitzer M, and Sass H (1999) Psychopathological, neuroendocrine and autonomic effects of 3,4-methylenedioxyethylamphetamine (MDE), psilocybin and *d*-methamphetamine in healthy volunteers. *Psychopharmacology* **142:**41–50.
Grob CS, McKenna DJ, Callaway JC, Brito GS, Neves ES, Oberlaender G, Saide OL, Labigalini E, Tacla C, Miranda CT, et al. (1996) Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *J Nerv Ment Dis* **184:**86–94.
Hollister LE and Moore F (1967) Urinary catecholamine excretion following lysergic acid diethylamide in man. *Psychopharmacologia* **11:**270–275.
Koulu M, Scheinin M, Kaarttinen A, Kallio J, Pyykkö K, Vuorinen J, and Zimmer RH (1989) Inhibition of monoamine oxidase by moclobemide: effects on monoamine metabolism and secretion of anterior pituitary hormones and cortisol in healthy volunteers. *Br J Clin Pharmacol* **27:**243–255.
Lamas X, Farré M, Llorente M, and Camí J (1994) Spanish version of the 49-item short form of the Addiction Research Center Inventory. *Drug Alcohol Depend* **35:**203–209.
Martin WR, Sloan JW, Sapira JD, and Jasinski DR (1971) Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine and methylphenidate in man. *Clin Pharmacol Ther* **12:**245–258.
Mas M, Farré M, de la Torre R, Roset PN, Ortuño J, Segura J, and Camí J (1999) Cardiovascular and neuroendocrine effects and pharmacokinetics of 3,4-methylenedioxymethamphetamine in humans. *J Pharmacol Exp Ther* **290:**136–145.
McKenna DJ, Towers GHN, and Abbott F (1984) Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of *ayahuasca*. *J Ethnopharmacol* **10:**195–223.
Messiha FS and Grof S (1973) D-Lysergic acid diethylamide (LSD)—effect on biogenic amines excretion in man. *Biochem Pharmacol* **22:**2352–2354.
Ott J (1999) Pharmahuasca: human pharmacology of oral DMT plus harmine. *J Psychoact Drugs* **31:**171–177.
Parker NC, Levtzow CB, Wright PW, Woodard LL, and Chapman JF (1986) Uniform chromatographic conditions for quantifying urinary catecholamines, metanephrines, vanillylmandelic acid, 5-hydroxyindoleacetic acid, by liquid chromatography, with electrochemical detection. *Clin Chem* **32:**1473–1476.
Pierce PA and Peroutka SJ (1989) Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. *Psychopharmacology* **97:**118–122.
Pletscher A (1966) Monoamine oxidase inhibitors. *Pharmacol Rev* **18:**121–129.
Riba J, Rodríguez-Fornells A, Strassman RJ, and Barbanoj MJ (2001a) Psychometric assessment of the Hallucinogen Rating Scale. *Drug Alcohol Depend* **62:**215–223.
Riba J, Rodríguez-Fornells A, Urbano G, Morte A, Antonijoan R, Montero M, Callaway JC, and Barbanoj MJ (2001b) Subjective effects and tolerability of the South American psychoactive beverage *ayahuasca* in healthy volunteers. *Psychopharmacology* **154:**85–95.
Rivier L and Lindgren JE (1972) "Ayahuasca", the South American hallucinogenic drink: an ethnobotanical and chemical investigation. *Econ Bot* **26:**101–129.
Schultes RE and Hofmann A (1980) *The Botany and Chemistry of Hallucinogens*. Charles C. Thomas, Springfield, IL.
Schultes RE and Hofmann A (1982) *Plantas de los dioses: orígenes del uso de los alucinógenos*. Fondo de Cultura Económica, México D. F.
Slotkin TA, DiStefano V, and Au WYW (1970) Blood levels and urinary excretion of harmine and its metabolites in man and rats. *J Pharmacol Exp Ther* **173:**26–30.
Smith RL, Canton H, Barrett RJ, and Sanders-Bush E (1998) Agonist properties of *N,N*-dimethyltryptamine at serotonin 5-HT_{2A} and 5-HT_{2C} receptors. *Pharmacol Biochem Behav* **61:**323–330.
Soldin SJ and Hill JG (1980) Simultaneous liquid-chromatographic analysis for 4-hydroxy-3-methoxymandelic acid and 4-hydroxy-3-methoxyphenylacetic acid in urine. *Clin Chem* **26:**291–294.
Spielberger CD, Gorsuch RL, and Lushene RE (1970) *Manual for the State-Trait Anxiety Inventory*. Consulting Psychologists Press, Palo Alto.
Strassman RJ (1994) Human psychopharmacology of LSD, dimethyltryptamine and related compounds, in *50 Years of LSD: Current Status and Perspectives of Hallucinogens* (Pletscher A and Ladewig D eds) pp 145–174, Parthenon, London.
Strassman RJ and Qualls CR (1994) Dose-response study of *N,N*-dimethyltryptamine in humans. I. Neuroendocrine, autonomic and cardiovascular effects. *Arch Gen Psychiatry* **51:**85–97.
Strassman RJ, Qualls CR, Uhlenhuth EH, and Kellner R (1994) Dose-response study of *N,N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry* **51:**98–108.
Suzuki O, Katsumata Y, and Oya M (1981) Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. *Biochem Pharmacol* 30:1353–1358.
Yritia M, Riba J, Ortuño J, Ramirez A, Castillo A, Alfaro Y, de la Torre R, and Barbanoj MJ (2002) Determination of *N,N*-dimethyltryptamine and β-carboline alkaloids in human plasma following oral administration of *Ayahuasca*. *J Chromatogr Biomed Appl* **779:**271–281.
Yu A, Idle JR, Krausz KW, Kupfer A, and Gonzalez FJ (2003) Contribution of individual cytochrome P450 isoenzymes to the *O*-demethylation of the psychotropic β-carboline alkaloids harmaline and harmine. *J Pharmacol Exp Ther* **305:**315–322.

Address correspondence to: Manel J. Barbanoj, Àrea d'Investigació Farmacològica, Institut de Recerca, Hospital de la Santa Creu i Sant Pau., St. Antoni Maria Claret, 167, Barcelona 08025, Spain. E-mail: mbarbanoj@hsp.santpau.es

Pharmacopsychiatry

Neuropsychobiology

Original Paper

Neuropsychobiology 2004;50:89–101
DOI: 10.1159/000077946

Effects of the South American Psychoactive Beverage *Ayahuasca* on Regional Brain Electrical Activity in Humans: A Functional Neuroimaging Study Using Low-Resolution Electromagnetic Tomography

Jordi Riba[a] Peter Anderer[b] Francesc Jané[a] Bernd Saletu[b]
Manel J. Barbanoj[a]

[a]Àrea d'Investigació Farmacològica, Institut de Recerca, Hospital de la Santa Creu i Sant Pau, Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Barcelona, Spain; [b]Division of Sleep Research and Pharmacopsychiatry, Department of Psychiatry, University of Vienna, Vienna, Austria

Key Words

Ayahuasca · N,N-Dimethyltryptamine · Psychedelics · Electroencephalography · Low-resolution electromagnetic tomography · Humans

Abstract

Ayahuasca, a South American psychotropic plant tea obtained from *Banisteriopsis caapi* and *Psychotria viridis*, combines monoamine oxidase-inhibiting β-carboline alkaloids with N,N-dimethyltryptamine (DMT), a psychedelic agent showing 5-HT_{2A} agonist activity. In a clinical research setting, *ayahuasca* has demonstrated a combined stimulatory and psychedelic effect profile, as measured by subjective effect self-assessment instruments and dose-dependent changes in spontaneous brain electrical activity, which parallel the time course of subjective effects. In the present study, the spatial distribution of *ayahuasca*-induced changes in brain electrical activity was investigated by means of low-resolution electromagnetic tomography (LORETA). Electroencephalography recordings were obtained from 18 volunteers after the administration of a dose of encapsulated freeze-dried *ayahuasca* containing 0.85 mg DMT/kg body weight and placebo. The intracerebral power density distribution was computed with LORETA from spectrally analyzed data, and subjective effects were measured by means of the Hallucinogen Rating Scale (HRS). Statistically significant differences compared to placebo were observed for LORETA power 60 and 90 min after dosing, together with increases in all six scales of the HRS. *Ayahuasca* decreased power density in the alpha-2, delta, theta and beta-1 frequency bands. Power decreases in the delta, alpha-2 and beta-1 bands were found predominantly over the temporo-parieto-occipital junction, whereas theta power was reduced in the temporomedial cortex and in frontomedial regions. The present results suggest the involvement of unimodal and heteromodal association cortex and limbic structures in the psychological effects elicited by *ayahuasca*.

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0302–282X/04/0501–0089$21.00/0

Accessible online at:
www.karger.com/nps

Manel J. Barbanoj
Àrea d'Investigació Farmacològica, Institut de Recerca
Hospital de la Santa Creu i Sant Pau, St. Antoni Maria Claret, 167
ES–08025 Barcelona (Spain)
Tel. +34 93 291 90 19, Fax +34 93 291 92 86, E-Mail mbarbanoj@hsp.santpau.es

Introduction

The psychoactive plant tea known as *ayahuasca*, a Quechua name meaning vine of the souls or vine of the dead, is a traditional shamanic inebriant used in the Upper Amazon since pre-Columbian times for religious and medicinal purposes [1]. In the second half of the last century, the use of *ayahuasca* reached the urban areas of Amazonian countries, where it is used by mestizo 'ayahuasqueros' for healing and divination. However, modern nonindigenous use of *ayahuasca* mainly takes place within the context of syncretic religious groups, particularly the Brazilian 'Santo Daime' and 'União do Vegetal', which have combined Old World religious beliefs with the sacramental use of the beverage [2]. In recent years, groups of followers of these Brazilian religions have become established in the United States and in several European countries [3].

Botanical research into the plant sources of *ayahuasca* has shown that the main ingredient of the tea is the woody vine *Banisteriopsis caapi* (Malpighiaceae). *Ayahuasca* is obtained by infusing the pounded stems of the vine either alone or more frequently in combination with the leaves of *Psychotria viridis* (Rubiaceae) or *Diplopterys cabrerana* (Malpighiaceae). *B. caapi* contains notable amounts of β-carboline alkaloids, mainly harmine and tetrahydroharmine, and to a lesser extent harmaline and traces of harmol and harmalol [4, 5]. *P. viridis* and *D. cabrerana* also contain indole alkaloids, mainly the potent short-acting psychedelic agent N,N-dimethyltryptamine (DMT) [4]. DMT is structurally related to the neurotransmitter serotonin, and like better-characterized psychedelics such as LSD and mescaline (a phenethylamine), binds to the 5-HT_{2A} receptor sites in the central nervous system (CNS), where it acts as an agonist [6].

The combination of DMT from *P. viridis* with the β-carboline alkaloids from *B. caapi* in a single oral preparation is most remarkable from the pharmacological point of view. It takes advantage of the pharmacodynamic properties of the β-carbolines, which allow access to the system of the otherwise orally inactive tryptamine component. Indeed, DMT is known for its lack of psychoactivity when orally ingested [7], probably due to metabolism by the enzyme monoamine oxidase [8]. On the other hand, the β-carbolines present in *ayahuasca*, particularly harmine and harmaline, are potent natural monoamine oxidase inhibitors [5], apparently preventing the extensive gut and liver first-pass effect on DMT, which is subsequently able to reach the systemic circulation and the CNS unaltered.

In a clinical research setting, *ayahuasca* has been found to induce transient modifications in perception, thought processes and mood that fit a combined stimulatory and psychedelic effect profile, as measured by subjective effect self-assessment instruments [9, 10]. Furthermore, the central effects of *ayahuasca* have also been evidenced by means of objective neurophysiological measures such as topographic pharmaco-electroencephalography (EEG). The effects of *ayahuasca* on the human EEG are characterized by an overall reduction in absolute power in the classical frequency bands (more pronounced in the slow delta and theta bands), and also by an acceleration of the center-of-gravity frequency. These actions on the spontaneous EEG follow a dose-response pattern and closely parallel the time course of subjective effects [11].

The aim of the present study was to assess the differential involvement of cortical brain regions in the acute central effects of *ayahuasca* by means of a recently developed neuroimaging technique: low-resolution electromagnetic tomography (LORETA). Based on the scalp electrical potential distribution obtained by means of classical EEG measures, LORETA provides three-dimensional information regarding the cortical neural generators of brain electrical activity [12]. Furthermore, LORETA computes a unique three-dimensional intracerebral power density distribution for the different EEG frequency bands, allowing their separate analysis. Unlike dipole modeling, LORETA makes no a priori assumptions about the number of sources involved. The only constraint implemented is that of maximal smoothness of the solution, based on the assumption that neighboring neuronal sources are likely to be similarly active (i.e. have similar orientations and strengths). The distribution obtained is thus the smoothest of all possible inverse solutions, as it is considered the most plausible. In a new implementation of LORETA, an additional neuroanatomical constraint restricts the solution space to cortical gray matter volume [13]. The technique has previously been used in the evaluation of acute effects of psychoactive drugs [14, 15].

To our knowledge, regional brain electrical activity has not been evaluated previously by means of LORETA following the administration of *ayahuasca* or other psychedelics with 5-HT_{2A} agonist activity. It is consequently difficult to establish an a priori hypothesis regarding the brain areas involved in the effects of *ayahuasca* on the EEG. However, PET and SPECT investigations of blood flow and glucose metabolism after acute psilocybin (an indoleamine structurally similar to DMT) and mescaline administration have evidenced increased activation in the prefrontal cortex [16–18]. Consequently, we postulated that changes in electrical activity would be identified at least in these regions.

Subjects and Methods

Volunteers

Eighteen healthy volunteers (15 males and 3 females) participated in the study. Eligibility criteria included prior experience with psychedelic drugs on at least five occasions without sequelae derived therefrom, no current or previous history of a neurological or psychiatric disorder, and no family history of an Axis-I psychiatric disorder in first-degree relatives. Volunteers were given a structured psychiatric interview (DSM-III-R) and completed the trait anxiety scale from the State-Trait Anxiety Inventory. Exclusion criteria included alcohol or other substance dependence, and high scores on the trait anxiety scale (over 1 standard deviation above the normative mean). Each participant underwent a complete physical examination that included a medical history, laboratory tests, ECG and urinalysis. Their mean age was 25.7 years (range 19–38 years), mean weight was 66.47 kg (range 50.7–79.5 kg) and mean height was 175.11 cm (range 158–188 cm). In addition to their prior intake of psychedelics, all volunteers had previous experience with cannabis and cocaine. Although prior exposure specifically to *ayahuasca* was not required for participation, two of the volunteers had ingested the drug before inclusion in this study. The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans, and was approved by the hospital's ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of *ayahuasca*, the general psychological effects of psychedelics and their possible adverse effects, as reported in the psychiatric literature. Written informed consent was obtained from all participants.

Drug

The *ayahuasca* employed in the present study was not administered in its original liquid form, but as a lyophilizate. The freeze-dried homogenized material had been obtained from a 9.6-liter batch of *ayahuasca*. The DMT content in the lyophilizate had been determined by HPLC, as described by Callaway et al. [19], and the β-carboline constituents were determined following a modification of the method described in that study. The 9.6-liter batch yielded 611 g of freeze-dried powder, containing 8.33 mg of DMT, 14.13 mg of harmine, 0.96 mg of harmaline and 11.36 mg of tetrahydroharmine per gram. Based on tolerability and subjective effects assessed previously [9], an *ayahuasca* dose containing 0.85 mg DMT/kg body weight was administered to the volunteers. The calculated individual dose for each volunteer was administered by combining 00 gelatin capsules containing 0.5, 0.25 or 0.125 g of freeze-dried *ayahuasca*. Placebo capsules were 00 gelatin capsules containing 0.75 g of lactose. These were administered on the placebo day, and were also combined with active *ayahuasca* capsules when necessary, so that all volunteers took the same number of capsules on each experimental day.

Study Design and Experimental Procedure

EEG recordings were obtained in a double-blind placebo-controlled randomized crossover clinical trial. Two weeks prior to the beginning of the experimental procedure, volunteers were requested to abstain from any medication or illicit drug use until the completion of the study. Volunteers also abstained from alcohol, tobacco and caffeinated drinks 24 h prior to each experimental day. Urine was screened for illicit drug use on each experimental day. Experimental days were at least 1 week apart.

On each experimental day, volunteers remained in the clinical research unit for a period of approximately 10 h. Following arrival in the morning under fasting conditions, EEG electrodes were placed on the scalp, and drug/placebo capsules were administered at approximately 10.00 a.m. with 250 ml of tap water. EEG recordings were obtained at baseline and at regular intervals after treatment administration. For the first 4 h, volunteers remained seated in a reclining chair in a quiet and dimly lit room. The experimenter remained outside the room during the EEG recordings. The last recording was performed at 8 h, and volunteers were discharged approximately 9 h after drug administration.

EEG Acquisition and Processing and LORETA Analysis

Nineteen-lead EEG recordings were obtained by means of scalp electrodes placed according to the international 10/20 system: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1 and O2, referenced to averaged mastoids. Additionally, vertical and horizontal electrooculograms were recorded. The signal was acquired through a Neuroscan SYNAMPS amplifier. Three-minute vigilance-controlled EEG with eyes closed was recorded at baseline, prior to drug administration and at different time points after dosing. During the vigilance-controlled EEG recordings, the experimenter tried to keep the volunteers alert; as soon as drowsiness patterns appeared in the EEG, they were aroused by acoustic stimulation. The EEG signal was band-pass filtered at 0.3–30 Hz, and digitized on-line with a sampling frequency of 100 Hz. EEG recordings were obtained prior to drug administration (–15 min and baseline), and 30, 60, 90, 120, 180, 360 and 480 min after dosing.

A two-step artifact processing procedure was used [20]. It included ocular artifact minimization based on regression analysis in the time domain, as described by Semlitsch et al. [21], and automatic artifact rejection based on a time and frequency domain approach as described by Anderer et al. [22]. Validity of the artifact processing procedure was visually assessed. After recomputation to average reference, spectral analysis was performed for artifact-free 5-second epochs. For each recording, the spectral power of six 5-second epochs of artifact-free, vigilance-controlled EEG was averaged. Data were digitally filtered into seven frequency bands according to Kubicki et al. [23]: delta (1.5–6 Hz), theta (6–8 Hz), alpha-1 (8–10 Hz), alpha-2 (10–12 Hz), beta-1 (12–18 Hz), beta-2 (18–21 Hz) and beta-3 (21–30 Hz).

Subsequently, LORETA was used to estimate the three-dimensional intracerebral current density distribution from the voltage values recorded at the scalp electrodes. The LORETA version employed implements a three-shell spherical head model [24] registered to the human brain atlas of Talairach and Tournoux [25] available as a digitized MRI from the Brain Imaging Centre, Montréal Neurological Institute. The EEG electrode coordinates reported by Towle et al. [26] were used for registration between spherical and realistic head geometry. The LORETA solution space was restricted to the cortical gray matter and hippocampus, based on the Digitized Probability Atlas corresponding to the Talairach atlas and also available from the Brain Imaging Centre, Montréal Neurological Institute. A voxel was included in the solution space if its probability of being gray matter was higher than 33%, and higher than its probability of being either white matter or cerebrospinal fluid. The final solution space consisted of 2,394 voxels with a spatial resolution of 0.343 cm^3 [13]. The EEG lead field was computed numerically with the boundary element method [27]. LORETA images represent the power (i.e. squared magnitude of computed intracerebral current density) in each of the 2,394 voxels. 'LORETA power', synonymous to 'EEG power', refers to the spectral power of current density as estimated by

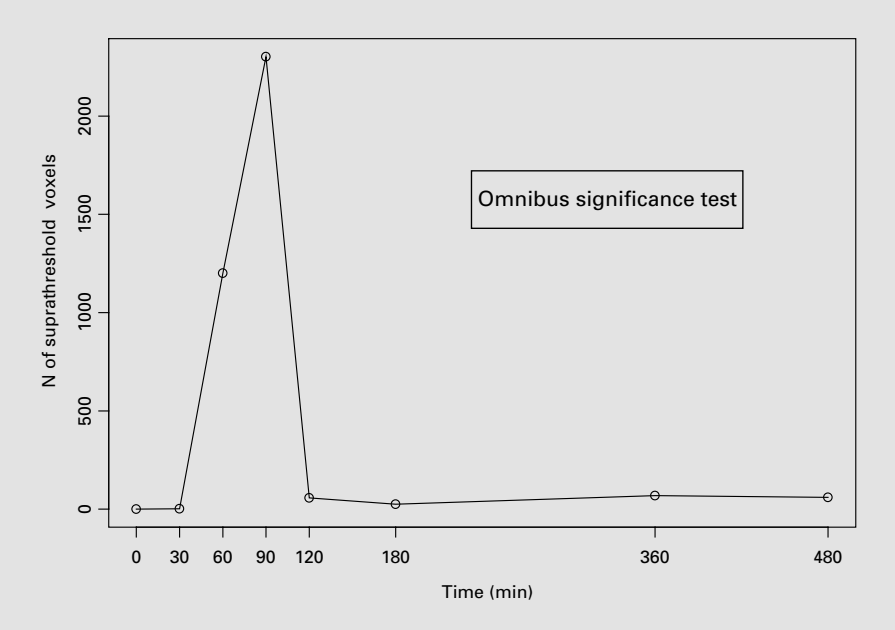


Fig. 1. Omnibus significance test. The total number of suprathreshold voxels at different time points after *ayahuasca* (0.85 mg DMT/kg body weight) administration are shown.

LORETA. Thus, in a first step, current density values were estimated based on the EEG cross-spectral matrix and then squared for each voxel and frequency band [15].

Subjective Effects

Volunteers were requested to answer the Hallucinogen Rating Scale (HRS), a self-report questionnaire measuring psychedelic-induced subjective effects [28]. The HRS includes six subscales: 'somaesthesia', reflecting somatic effects; 'affect', sensitive to emotional and affective responses; 'volition', indicating the volunteer's capacity to willfully interact with his/her 'self' and/or the environment; 'cognition', describing modifications in thought processes or content; 'perception', measuring visual, auditory, gustatory and olfactory experiences, and finally 'intensity', which reflects the strength of the overall experience. In the present study, a Spanish version of the questionnaire was used [29]. The HRS was administered 240 min after administration of *ayahuasca* and placebo.

Statistical Analysis

LORETA Data. In a first step, in order to explore the time course of *ayahuasca* effects, paired-sample t tests were computed for log-transformed LORETA power at each voxel and frequency band for the different time points. To correct for multiple comparisons, a nonparametric single-threshold test was applied on the basis of the theory for randomization and permutation tests developed by Holmes et al. [30]. The omnibus null hypothesis of no activation anywhere in the brain was rejected if at least one t value (i.e. voxel, t_{MAX}) was above the critical threshold (t_{CRIT}) for $p = 0.05$ determined by 5,000 randomizations. The total number of suprathreshold voxels was plotted versus time in order to select the time points with the largest effects. Subsequently, LORETA images were computed for log-transformed normalized data for each separate frequency band at the selected time points and following the same statistical approach. On the basis of the Structure-Probability Maps Atlas [31], the number of significant voxels in each lobe (frontal, parietal, occipital, temporal, limbic and sublobar), gyrus and Brodmann area (BA) of the left and the right hemisphere was computed separately for each suprathreshold region.

Subjective Effects. Scores on the HRS questionnaire after *ayahuasca* administration were compared with those after placebo by means of paired-sample Student's t tests.

Results

LORETA Data

Figure 1 shows the results for the omnibus significance test performed for all voxels and frequency bands at the different time points in order to explore the time course of effects.

A steep rise in the number of suprathreshold voxels was observed 60 min following drug administration, and the pharmacodynamic peak occurred 90 min after dosing. LORETA images were thus computed for the *ayahuasca*-versus placebo-induced changes at these two time points.

The voxel-by-voxel statistical comparison of *ayahuasca*-induced versus placebo-induced effects 60 min after drug administration (0.85 mg DMT/kg body weight dose), followed by Holmes correction, showed statistically significant decreases mainly in the alpha-2 frequency band (459 suprathreshold voxels). As listed in table 1, power density decreases were found in the parietal (135), occipital (79), temporal (170) and limbic (69) lobes in both hemispheres, and at the left frontal (3) and sublobar level

Table 1. *Ayahuasca*- versus placebo-induced decreases in alpha-2 power (10–12 Hz) 60 min after administration

Gyrus	Suprathreshold voxels					
	left hemisphere			right hemisphere		
	N_{sig}	N_{total}	%	N_{sig}	N_{total}	%
Frontal lobe						
Subcallosal gyrus	2	7	29	0	7	0
Precentral gyrus	1	38	3	0	37	0
Parietal lobe						
Postcentral gyrus	6	39	15	6	44	14
Supramarginal gyrus	2	10	20	11	11	100
Superior parietal lobule	16	24	67	4	17	24
Precuneus	48	73	66	18	65	28
Inferior parietal lobule	1	56	2	13	50	26
Angular gyrus	3	4	75	7	7	100
Occipital lobe						
Cuneus	9	35	26	2	30	7
Lingual gyrus	4	38	11	13	31	42
Superior occipital gyrus	3	4	75	5	5	100
Middle occipital gyrus	16	26	62	20	24	83
Inferior occipital gyrus	5	10	50	2	9	22
Temporal lobe						
Superior temporal gyrus	10	85	12	20	95	21
Middle temporal gyrus	30	89	34	30	88	34
Inferior temporal gyrus	10	51	20	4	52	8
Fusiform gyrus	43	58	74	18	53	34
Subgyral	4	12	33	1	9	11
Limbic lobe						
Cingulate	8	8	100	1	8	13
Posterior cingulum	1	15	7	1	20	5
Parahippocampal gyrus	32	33	97	12	31	39
Uncus	14	24	58	0	24	0
Sublobar						
Insula	3	38	8	0	32	0

The number of significant voxels (N_{sig}), the total number of voxels (N_{total}) and the percentage of significant voxels (%) for each gyrus and hemisphere are given (n = 18).

Table 2. *Ayahuasca*- versus placebo-induced decreases in delta power (1.5–6 Hz) 90 min after administration

Gyrus	Suprathreshold voxels					
	left hemisphere			right hemisphere		
	N_{sig}	N_{total}	%	N_{sig}	N_{total}	%
Parietal lobe						
Postcentral gyrus	1	39	3	9	44	20
Supramarginal gyrus	5	10	50	8	11	73
Superior parietal lobule	9	24	38	13	17	76
Precuneus	37	73	51	17	65	26
Inferior parietal lobule	4	56	7	21	50	42
Angular gyrus	4	4	100	7	7	100
Occipital lobe						
Cuneus	10	35	29	1	30	3
Superior occipital gyrus	4	4	100	5	5	100
Middle occipital gyrus	20	26	77	19	24	79
Inferior occipital gyrus	8	10	80	3	9	33
Temporal lobe						
Superior temporal gyrus	19	85	22	15	95	16
Middle temporal gyrus	62	89	70	42	88	48
Inferior temporal gyrus	20	51	39	24	52	46
Fusiform gyrus	41	58	71	40	53	75
Limbic lobe						
Parahippocampal gyrus	0	33	0	3	31	10

The number of significant voxels (N_{sig}), the total number of voxels (N_{total}) and the percentage of significant voxels (%) for each gyrus and hemisphere are given (n = 18).

(3). Suprathreshold voxels were thus found over extensive cortical areas around the temporo-parieto-occipital junction predominantly in the angular gyrus, supramarginal gyrus, precuneus, superior and middle temporal gyri and fusiform gyrus. In the limbic lobe, suprathreshold voxels covered mainly the cingulate and the parahippocampal gyrus. The BAs showing the highest percentage of suprathreshold voxels were BA 7 in the parietal lobe, BA 19 in the occipital lobe, BA 39 and BA 37 in the temporal lobe, and BA 36 and BA 35 in the limbic lobe.

Figure 2 shows LORETA axial brain slices as statistical nonparametric maps corresponding to the suprathreshold regions found for the alpha-2 frequency band, 60 min after drug administration.

In addition to the above results, power decreases were observed for the delta frequency band in a small area covering 15 suprathreshold voxels in the border between the left occipital and temporal lobes in BA 19, BA 37 and BA 21. Finally, decreases in the theta band fell short of statistical significance, with the lowest t value equal to –3.58 (p = 0.0522; cutoff t value = 3.61). This local minimum was located in BA 24 in the medial frontal cortex.

As shown in figure 1, the largest number of suprathreshold voxels, i.e. the pharmacodynamic peak, was observed 90 min after administration of *ayahuasca*. At this time point, statistically significant decreases were found for the delta, theta and beta-1 frequency bands. Alpha-2 power density was also reduced relative to placebo, but contrary to what was observed 60 min after

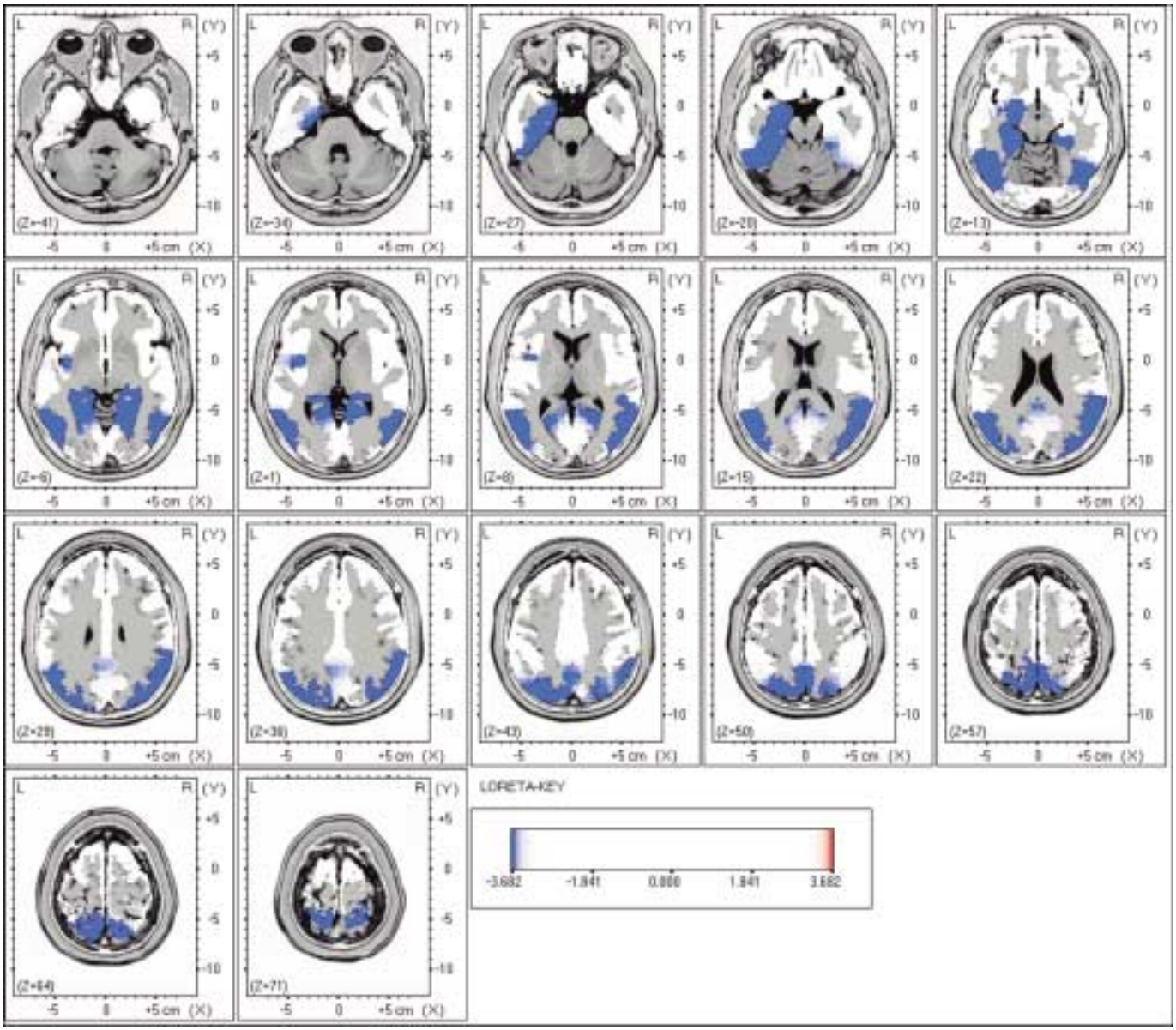


Fig. 2. Effects of *ayahuasca* (0.85 mg DMT/kg body weight) on regional cortical electrical activity 60 min after administration (n = 18). Shown are statistical nonparametric maps based on t values of differences between *ayahuasca*-induced and placebo-induced changes in the alpha-2 (10–12 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($p < 0.05$) as compared to placebo. Axial slices (head seen from above, nose up; L = left; R = right) in steps of 7 mm from most inferior (Z = –41) to the most superior (Z = 71) are shown.

administration, these decreaseswere not statistically significant.

Table 2 lists the anatomical distribution of the power decreases observed for the delta frequency band. Thus, 471 suprathreshold voxels were located in the parietal (135), occipital (70), temporal (263) and limbic (3) lobes in both hemispheres. Suprathreshold voxels were found over extensive cortical areas around the temporo-parieto-occipital junction predominantly in the angular gyrus, superior occipital gyrus, middle temporal gyrus and fusiform gyrus. The BAs showing the highest percentage of suprathreshold voxels were BA 37, BA 19 and BA 39.

Figure 3 shows LORETA axial brain slices as statistical nonparametric maps corresponding to the suprathreshold

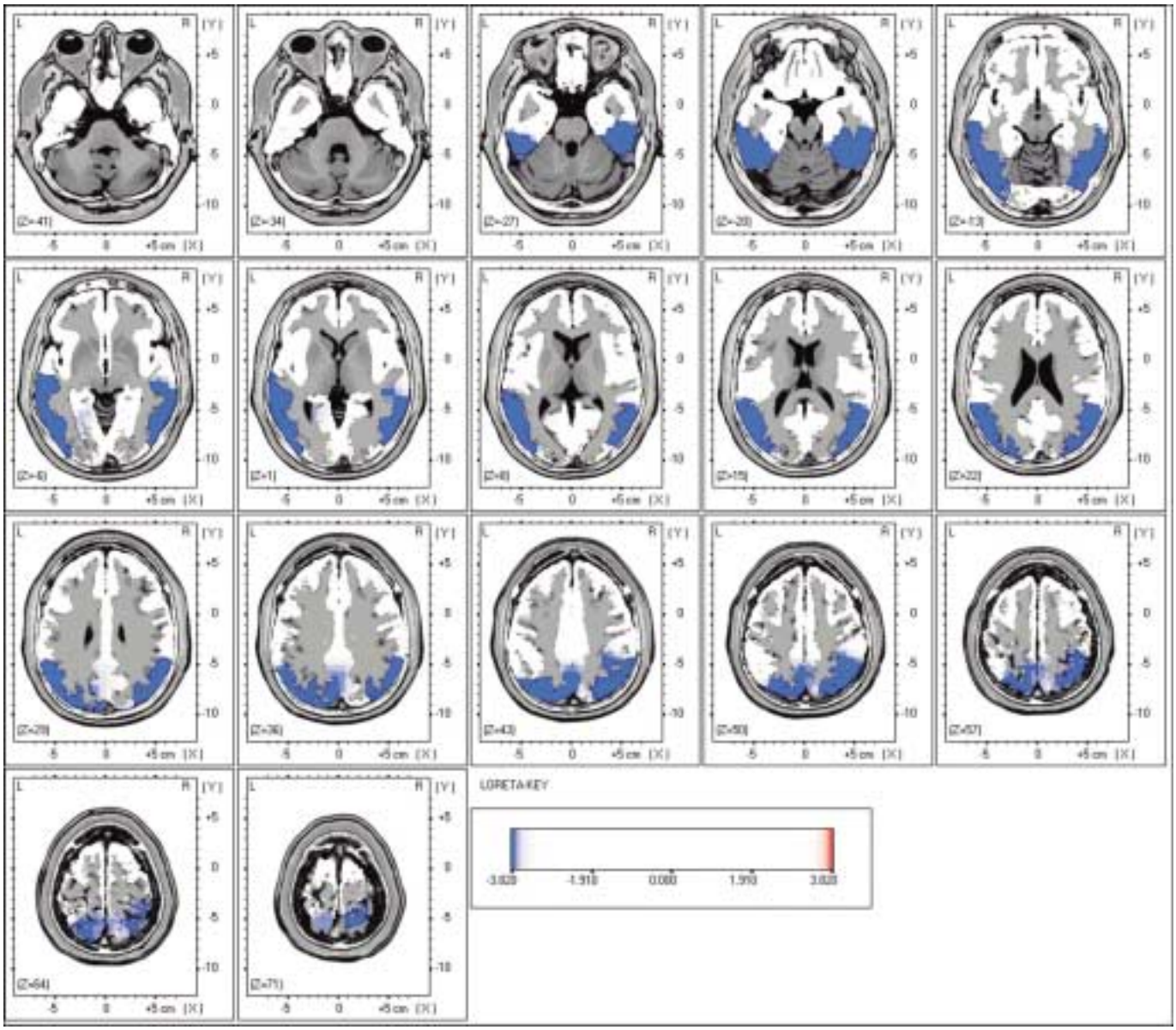


Fig. 3. Effects of *ayahuasca* (0.85 mg DMT/kg body weight) on regional cortical electrical activity 90 min after administration (n = 18). Shown are statistical nonparametric maps based on t values of differences between *ayahuasca*-induced and placebo-induced changes in the delta (1.5–6 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($p < 0.05$) as compared to placebo. Axial slices (head seen from above, nose up; L = left; R = right) in steps of 7 mm from most inferior (Z = –41) to the most superior (Z = 71) are shown.

regions found for the delta frequency band. Note the marked overlap between these brain areas and those found at 60 min for the alpha-2 frequency band.

Areas of power density decrease in the theta frequency band are indicated in table 3. One hundred and twenty-eight voxels showed t values below the statistical threshold. These suprathreshold voxels were found in the frontal lobe (13), in the temporal lobe (58) and in the limbic lobe (57). Suprathreshold voxels were found distributed in three nonconfluent areas, i.e. in the medial and superior frontal gyri (BA 6, 8), in the anterior cingulate (BA 24, 32) and in the left temporomedial cortex comprising mainly the fusiform and parahippocampal gyri and the uncus (BA 36, 35, 28).

Table 3. *Ayahuasca*- versus placebo-induced decreases in theta power (6–8 Hz) 90 min after administration

Gyrus	Suprathreshold voxels					
	left hemisphere			right hemisphere		
	N_{sig}	N_{total}	%	N_{sig}	N_{total}	%
Frontal lobe						
Medial frontal gyrus	2	61	3	2	59	3
Superior frontal gyrus	4	100	4	5	98	5
Temporal lobe						
Middle temporal gyrus	1	89	1	0	88	0
Inferior temporal gyrus	13	51	25	0	52	0
Fusiform gyrus	43	58	74	0	53	0
Subgyral	1	12	8	0	9	0
Limbic lobe						
Anterior cingulum	2	25	8	2	25	8
Cingulate gyrus	8	42	19	7	41	17
Cingulate	6	8	75	0	8	0
Parahippocampal gyrus	18	33	55	0	31	0
Uncus	14	24	58	0	24	0

The number of significant voxels (N_{sig}), the total number of voxels (N_{total}) and the percentage of significant voxels (%) for each gyrus and hemisphere are given (n = 18).

Figure 4 shows LORETA orthogonal slices (axial, sagittal and coronal views) as statistical nonparametric maps corresponding to the three nonconfluent suprathreshold regions found for the theta frequency band, through the voxel of the extreme t value.

Areas of beta-1 power density decrease are shown in table 4. These comprised 139 suprathreshold voxels, located in the parietal lobe (122), occipital lobe (3), temporal lobe (5) and limbic lobe (9). Suprathreshold voxels were found on two nonconfluent areas. The first area comprised voxels in the parietal lobe, extending medially and bilaterally from the posterior cingulate gyrus (BA 30, 31) to the precuneus and superior parietal lobule (BA 7, 19). The second area comprised voxels in the right supramarginal and angular gyri (BA 39).

Figure 5 shows LORETA orthogonal slices (axial, sagittal and coronal views) as statistical nonparametric maps corresponding to the two nonconfluent suprathreshold regions found for the beta-1 frequency band, through the voxel of the extreme t value.

Subjective Effects

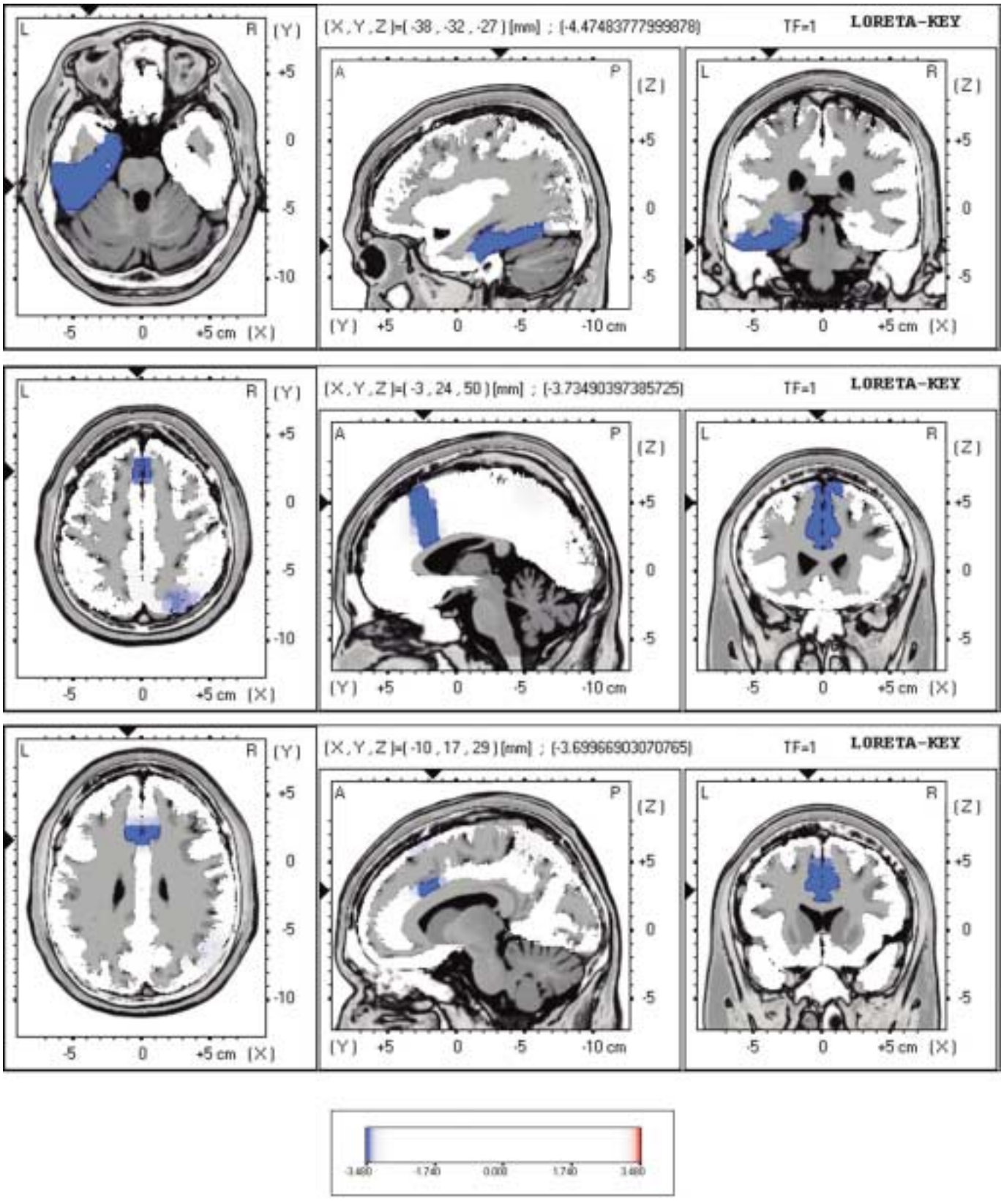
Ayahuasca induced a series of perceptual, mood and cognitive modifications with a characteristic psychedelic pattern, as measured by the HRS questionnaire. Table 5 shows the mean values obtained after placebo and *ayahuasca* for the HRS scores, and the results of the statistical analyses performed.

Drug-induced subjective effects were first noted as early as 15 min, became more marked between 30 and 45 min, showed a steep rise at 60 min, reached a maximum between 90 and 120 min, and decreased thereafter, disappearing entirely at 360 min. All volunteers experienced somatic modifications, which included altered bodily sensations, pins and needles, increased skin sensitivity and physical comfort. Visual and auditory phenomena were also frequently reported but varied greatly in quality and intensity between volunteers, ranging from distortions of objects and sounds to elaborate visions with eyes closed and perception of nonexistent noises. Five subjects reported experiencing auditory and visual synesthesia. In the cognitive sphere, the sense of time was altered, thought speed increased and the ability to focus attention was subjectively perceived to be reduced. Mood modifications were also present, the experience being globally regarded as satisfactory, with most volunteers reporting having experienced happiness, excitation and a 'high'. It is important to note that, at the doses administered, *ayahuasca* did not induce full-blown psychotic symptoms and none of the participants lost awareness of the drug-induced nature of the psychological effects experienced.

Discussion

The analysis of brain electrical activity by means of LORETA identified significant drug-induced changes in the intracerebral power density distribution 60 and 90 min following *ayahuasca* administration. At the peak of

Fig. 4. Effects of *ayahuasca* (0.85 mg DMT/kg body weight) on regional cortical electrical activity 90 min after administration (n = 18). Shown are statistical nonparametric maps based on t values of differences between *ayahuasca*-induced and placebo-induced changes in the theta (6–8 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($p < 0.05$) as compared to placebo. Orthogonal (axial, sagittal, coronal) slices for each of the three nonconfluent suprathreshold regions, i.e. temporomedial (upper row), frontomedial (middle row) and cingulate (lower row), through the voxel of the extreme t value are shown. The Talairach coordinates (X, Y, Z) of the displayed extreme t value are indicated by black triangles on the coordinate axes. L = Left; R = right; A = anterior; P = posterior.


(X,Y,Z)=(-38,-32,-27) [mm] ; (-4.47483777999878)
TF=1
LORETA-KEY
(X,Y,Z)=(-3,24,50) [mm] ; (-3.73490397385725)
TF=1
LORETA-KEY
(X,Y,Z)=(-10,17,29) [mm] ; (-3.69966903070765)
TF=1
LORETA-KEY
-3.480
-1.740
0.000
1.740
3.480

4

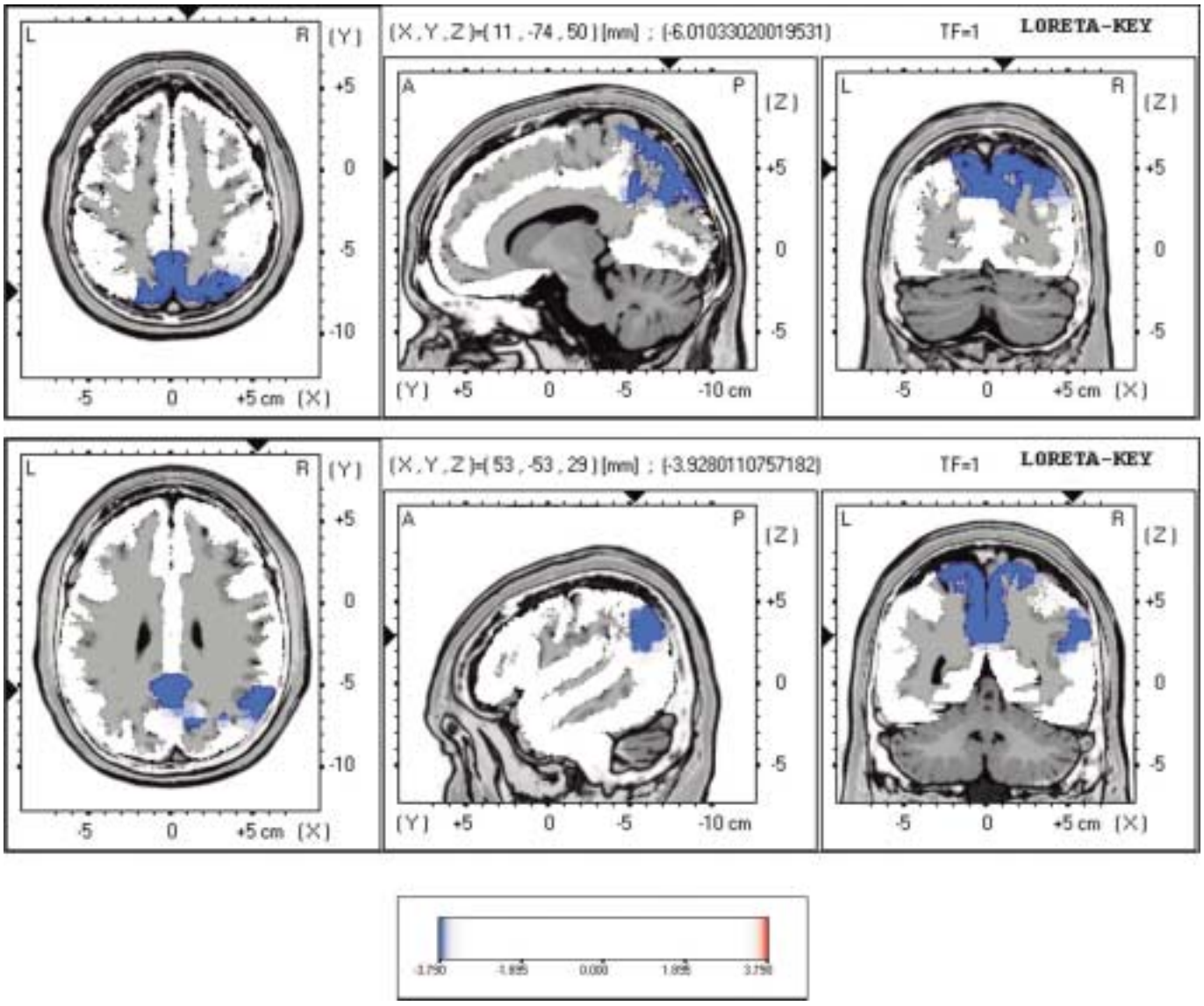


Fig. 5. Effects of *ayahuasca* (0.85 mg DMT/kg body weight) on regional cortical electrical activity 90 min after administration (n = 18). Shown are statistical nonparametric maps based on t values of differences between *ayahuasca*-induced and placebo-induced changes in the beta-1 (12–18 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($p < 0.05$) as compared to placebo. Orthogonal (axial, sagittal, coronal) slices for each of the two nonconfluent suprathreshold regions, i.e. parietomedial (upper row) and parietolateral (lower row), through the voxel of the extreme t value are shown. The Talairach coordinates (X, Y, Z) of the displayed extreme t value are indicated by black triangles on the coordinate axes. L = Left; R = right; A = anterior; P = posterior.

the pharmacodynamic effects, the slow delta rhythm was decreased in posterior brain regions. Additionally, decreases in theta were observed in the medial frontal and medial temporal cortices. Similar effects, although less intense (delta) or bordering on statistical significance (theta), were also observed in analogous brain regions 60 min after dosing. At this time point, however, a widespread power reduction in the alpha-2 band was observed in posterior areas, showing considerable overlap with those demonstrating significant decreases in delta power 90 min after administration. These modifications of the EEG power spectrum were accompanied by a constellation of perceptual, cognitive and mood modifications typical of the psychedelics, as evidenced by significant increases in all subscales of the HRS. The pattern of subjectively reported effects corresponded qualitatively and

Table 4. *Ayahuasca*- versus placebo-induced decreases in beta-1 power (12–18 Hz) 90 min after administration

Gyrus	Suprathreshold voxels					
	left hemisphere			right hemisphere		
	N_{sig}	N_{total}	%	N_{sig}	N_{total}	%
Parietal lobe						
Postcentral gyrus	6	39	15	6	44	14
Supramarginal gyrus	0	10	0	3	11	27
Superior parietal lobule	9	24	38	9	17	53
Precuneus	38	73	52	45	65	69
Inferior parietal lobule	0	56	0	2	50	4
Angular gyrus	0	4	0	4	7	57
Occipital lobe						
Cuneus	0	35	0	3	30	10
Temporal lobe						
Superior temporal gyrus	0	85	0	3	95	3
Middle temporal gyrus	0	89	0	2	88	2
Limbic lobe						
Cingulate gyrus	4	42	10	3	41	7
Posterior cingulum	1	15	7	1	20	5

The number of significant voxels (N_{sig}), the total number of voxels (N_{total}) and the percentage of significant voxels (%) for each gyrus and hemisphere are given (n = 18).

Table 5. Means ± SD of the scores obtained for the HRS questionnaire subscales, and results of the statistical comparisons performed by means of paired-sample Student's t tests (n = 18)

	HRS scores	
	placebo	*ayahuasca*
Somaesthesia	0.07 ± 0.10	0.97 ± 0.40**
Perception	0.09 ± 0.19	1.10 ± 0.67**
Cognition	0.06 ± 0.16	0.96 ± 0.59**
Volition	0.81 ± 0.79	1.35 ± 0.61**
Affect	0.32 ± 0.21	1.02 ± 0.38**
Intensity	0.24 ± 0.45	1.85 ± 0.51**

** $p < 0.01$.

with respect to the time course with previous results obtained in a smaller sample of volunteers [9]. It is of particular interest that these effects involving perceptive and cognitive modifications were regarded by the majority of volunteers as positive and desirable, in contrast with the more psychosis-like profile, including paranoid thoughts and fear of control loss, reported in studies in which other psychedelics, such as psilocybin, have been administered to drug-naive subjects [17]. The fact that the volunteers who enrolled in the present study had prior experience with psychedelics may account for these differences.

The effects of *ayahuasca* on the EEG power spectrum are compatible with its proposed neurochemical mechanism of action. Decreases in slow activity, i.e. delta and theta power, are a general feature of psychostimulants, such as amphetamine and methylphenidate, serotonin releasers such as fenfluramine, and psychedelics displaying 5-HT_2 agonist activity [32–34]. Early pharmaco-EEG research on LSD, a 5-HT_{2A} agonist like DMT – the main psychotropic principle found in *ayahuasca* – had also shown decreases in slow activity after acute drug administration [32]. Delta activity has traditionally been thought to reflect inhibitory activity, and increases in theta have been observed in relaxed and meditative states. Thus, the present results would rather suggest an excitatory or arousing effect for *ayahuasca*. This assumption is further supported by the fact that major tranquilizers with D_2 or mixed D_2/5-HT_2 antagonist activity, such as chlorpromazine and risperidone, are characterized by their delta- and theta-promoting effects [34, 35]. An important difference between *ayahuasca* and psychostimulants is the alpha-2-decreasing properties of the former found in the present study. In addition to slow wave-dampening effects, amphetamine is known to enhance alpha power, a feature not shared by *ayahuasca*. As mentioned above, Itil and Fink [32] found that LSD had inhibitory effects on slow waves, but also reduced alpha. Interestingly, other drugs, such as scopolamine or ketamine, with various mechanisms of action and different overall EEG profiles but able to elicit hallucinatory states, also display an alpha-suppressing effect. Thus, *ayahuasca* would combine alpha-dampening effects, a feature shown by other perception-altering drugs, with slow wave reduction properties, in an overall profile which would bring it closer to drugs such as LSD rather than to pure psychostimulants.

Given the exploratory nature of the present study, it is the authors' view that some hypotheses should be postulated regarding the brain networks and processes targeted by *ayahuasca,* based on the spatial information provided by the LORETA analysis. The changes in intracerebral electrical source distribution showed considerable overlapping between frequency bands, mainly between alpha-2 and delta, although these effects were observed at different time points. Delta and alpha-2 power decreases were located bilaterally over somatosensory, auditory and visual association cortices and over the temporo-parietal heteromodal association cortex [36]. Power decreases in

the beta-1 frequency band were predominantly found in the somatosensory and visual association cortex, and also in the heteromodal association cortex. Areas showing a theta power decrease, however, did not predominate in the association cortex, but in paralimbic structures with relevant roles in emotion and memory processes [36]. Thus, it can be hypothesized that drug-induced bioelectrical changes in the unimodal sensory association cortex may have played a role in the modality-specific modifications in the visual, somatic and auditory perception reported. Additionally, it appears reasonable to assume that effects on transmodal brain areas could account for more complex cognitive modifications which also characterize the subjective experience elicited by *ayahuasca* [9, 10]. In this respect, the temporo-parietal and frontomedial heteromodal association cortex, the cingulate and the temporomedial cortices play relevant roles in the neurobiology of attention, emotion and memory [37–39].

The results obtained in the present study show interesting similarities to, but also differences from, previous SPECT and PET studies involving psychedelics. A recent PET investigation revealed that the most important metabolic increases after psilocybin administration to humans occur predominantly in the temporomedial, frontomedial, frontolateral and anterior cingulate cortices [17]. These areas largely coincide with those showing theta decreases after *ayahuasca* administration. Nevertheless, PET and SPECT studies following psilocybin and mescaline have revealed a hyperfrontality pattern, i.e. increased blood perfusion or glucose metabolism in frontal regions [16, 17]. Metabolic increases in frontomedial regions, and more specifically in the anterior cingulate cortex, appear to be a common feature of psychedelic drug effects, as these have been observed after psilocybin [17, 18] and after subanesthetic doses of ketamine [40]. In the present study, however, only small areas within the frontal lobes were found to show drug-induced changes in the power density distribution. Besides the obvious differences in the drugs being administered, a possible explanation for this divergence could be the different nature of the variables under study (regional brain electrical activity vs. glucose metabolism or blood perfusion). Also, the aforementioned differences in the reported pattern of subjective effects should be taken into consideration.

In conclusion, the effects of *ayahuasca* on the EEG power spectrum involved mainly reductions in the slow delta and theta activity together with decreases in beta-1 and in the alpha-2 frequency band. The assessment of the spatial distribution of intracerebral power density changes singled out the temporo-parieto-occipital junction, and temporomedial and frontomedial areas as target regions of *ayahuasca* in the CNS. These areas comprise the unimodal association cortex in the somatosensory, auditory and visual perception modalities, the heteromodal association cortex, and also key regions within the limbic neural network involved in the integration of multimodal sensory information, and in emotion and memory processes. Future studies specifically addressing drug effects on these aspects of human cognition are needed in order to further our understanding of the complex psychological modifications elicited by *ayahuasca*.

Acknowledgements

This study would not have been possible without the support of the following people, to whom we would like to express our gratitude: Esther Martínez, Félix González and José María Fábregas for their help in the initial stages of our research project; the people of CEFLURIS in Brazil, who agreed to provide the *ayahuasca (Daime)* for evaluation in a clinical trial; James C. Callaway, Department of Pharmaceutical Chemistry, University of Kuopio, Finland, who quantified the DMT in *ayahuasca*; Maria Montero, Hospital de Sant Pau, Barcelona, Spain, who conducted the psychiatric interviews with the volunteers; Roberto Domingo Pascual-Marqui, KEY Institute for Brain-Mind Research, Zurich, Switzerland, who kindly provided the LORETA software; and, finally, Gloria Urbano, Adelaida Morte, Sylvie Cotxet, Llúcia Benito, Susanna Clos and David Martínez for contributing to data collection, and Pablo Ayesta for editing the LORETA figures.

References

1 Schultes RE, Hofmann A: Plantas de los dioses: Orígenes del uso de los alucinógenos. México, Fondo de Cultura Económica, 1982.
2 Dobkin de Rios M: Commentary. On 'Human pharmacology of hoasca': A medical anthropology perspective. J Nerv Ment Dis 1996;184: 95–98.
3 Anonymous: L'Ayahuasca: de l'Amazonie à la jungle urbaine; in: La Géopolitique Mondiale des Drogues 1998/1999. Paris, Observatoire Géopolitique des Drogues, 2000, pp 102–106.
4 Rivier L, Lindgren JE: 'Ayahuasca', the South American hallucinogenic drink: An ethnobotanical and chemical investigation. Econ Bot 1972;26:101–129.
5 McKenna DJ, Towers GHN, Abbott F: Monoamine oxidase inhibitors in South American hallucinogenic plants: Tryptamine and β-carboline constituents of *Ayahuasca.* J Ethnopharmacol 1984;10:195–223.
6 Smith RL, Canton H, Barrett RJ, Sanders-Bush E: Agonist properties of *N,N*-dimethyltryptamine at serotonin 5-HT_{2A} and 5-HT_{2C} receptors. Pharmacol Biochem Behav 1998;61: 323–330.

7 Ott J: Pharmahuasca: Human pharmacology of oral DMT plus harmine. J Psychoactive Drugs 1999;31:171–177.
8 Suzuki O, Katsumata Y, Oya M: Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. Biochem Pharmacol 1981;30:1353–1358.
9 Riba J, Rodríguez-Fornells A, Urbano G, Morte A, Antonijoan R, Montero M, Callaway JC, Barbanoj MJ: Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. Psychopharmacology (Berl) 2001;154:85–95.
10 Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanoj MJ: Human pharmacology of Ayahuasca: Subjective and cardiovascular effects, monoamine metabolite excretion and pharmacokinetics. J Pharmacol Exp Ther 2003;306:73–83.
11 Riba J, Anderer P, Morte A, Urbano G, Jané F, Saletu B, Barbanoj MJ: Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage Ayahuasca in healthy volunteers. Br J Clin Pharmacol 2002;53:613–628.
12 Pascual-Marqui RD, Michel CM, Lehmann D: Low resolution electromagnetic tomography: A new method for localizing electrical activity in the brain. Int J Psychophysiol 1994;18:49–65.
13 Pascual-Marqui RD, Lehmann D, Koenig T, Kochi K, Merlo MCG, Hell D, Koukkou M: Low resolution brain electromagnetic tomography (LORETA) functional imaging in acute, neuroleptic-naive, first-episode, productive schizophrenia. Psychiatry Res 1999;90:169–179.
14 Anderer P, Saletu B, Pascual-Marqui RD: Effects of the 5-HT_{1A} partial agonist buspirone on regional brain electrical activity in man: A functional neuroimaging study using low-resolution electromagnetic tomography (LORETA). Psychiatry Res 2000;100:81–96.
15 Frei E, Gamma A, Pascual-Marqui R, Lehmann D, Hell D, Vollenweider FX: Localization of MDMA-induced brain activity in healthy volunteers using low resolution brain electromagnetic tomography (LORETA). Hum Brain Mapp 2001;14:152–165.
16 Hermle L, Fünfgeld M, Oepen G, Botsch H, Borchardt D, Gouzoulis E, Fehrenbach RA, Spitzer M: Mescaline-induced psychopathological, neuropsychological, and neurometabolic effects in normal subjects: Experimental psychosis as a tool for psychiatric research. Biol Psychiatry 1992;32:976–991.
17 Vollenweider FX, Leenders KL, Scharfetter C, Maguire P, Stadelmann O, Angst J: Positron emission tomography and fluorodeoxyglucose studies of metabolic hyperfrontality and psychopathology in the psilocybin model of psychosis. Neuropsychopharmacology 1997;16:357–372.
18 Gouzoulis-Mayfrank E, Schreckenberger M, Sabri O, Arning C, Thelen B, Spitzer M, Kovar KA, Hermle L, Büll U, Sass H: Neurometabolic effects of psilocybin, 3,4-methylenedioxyethylamphetamine (MDE) and *d*-methamphetamine in healthy volunteers. A double-blind, placebo-controlled PET study with [^{18}F]FDG. Neuropsychopharmacology 1999;20:565–581.
19 Callaway JC, Raymon LP, Hearn WL, McKenna DJ, Grob CS, Brito GC, Mash DC: Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with *ayahuasca*. J Anal Toxicol 1996;20:492–497.
20 Anderer P, Semlitsch HV, Saletu B, Barbanoj MJ: Artifact processing in topographic mapping of electroencephalographic activity in neuropsychopharmacology. Psychiatry Res 1992;45:79–93.
21 Semlitsch HV, Anderer P, Schuster P, Presslich O: A solution for reliable and valid reduction of ocular artifacts, applied to the P300 ERP. Psychophysiology 1986;23:695–703.
22 Anderer P, Saletu B, Kinsperger K, Semlitsch H: Topographic brain mapping of EEG in neuropsychopharmacology. I. Methodological aspects. Methods Find Exp Clin Pharmacol 1987;9:371–384.
23 Kubicki S, Herrmann WM, Fichte K, Freund G: Reflections on the topics: EEG frequency bands and regulation of vigilance. Pharmakopsychiatr Neuropsychopharmakol 1979;12:237–245.
24 Ary JP, Klein SA, Fender DH: Location of sources of evoked scalp potentials: Corrections for skull and scalp thickness. IEEE Trans Biomed Eng 1981;28:447–452.
25 Talairach J, Tournoux P: Co-Planar Stereotaxic Atlas of the Human Brain. Stuttgart, Georg Thieme, 1988.
26 Towle VL, Bolaños J, Suarez D, Tan K, Grzeszczuk R, Levin DN, Cakmur R, Frank SA, Spire JP: The spatial location of EEG electrodes: Locating the best-fitting sphere relative to cortical anatomy. Electroencephalogr Clin Neurophysiol 1993;86:1–6.
27 Pascual-Marqui RD: Review of methods for solving the EEG inverse problem. Int J Bioelectromagn 1999;1:75–86.
28 Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R: Dose-response study of *N*,*N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch Gen Psychiatry 1994;51:98–108.
29 Riba J, Rodríguez-Fornells A, Strassman RJ, Barbanoj MJ: Psychometric assessment of the Hallucinogen Rating Scale. Drug Alcohol Depend 2001;62:215–223.
30 Holmes AP, Blair RC, Watson JDG, Ford I: Nonparametric analysis of statistic images from functional mapping experiments. J Cereb Blood Flow Metab 1996;16:7–22.
31 Lancaster JL, Rainey LH, Summerlin JL, Freitas CS, Fox PT, Evans AC, Toga AW, Mazziotta JC: Automated labeling of the human brain – a preliminary report on the development and evaluation of a forward-transform method. Hum Brain Mapp 1997;5:238–242.
32 Itil T, Fink M: Klinische Untersuchungen und quantitative EEG-Daten bei experimentellen Psychosen. Arzneimittelforschung 1966;16:237–239.
33 Herrmann WM, Schaerer E: Pharmaco-EEG: Computer EEG analysis to describe the projection of drug effects on a functional cerebral level in humans; in Lopes da Silva FH, Storm van Leeuwen W, Rémond A (eds): Handbook of Electroencephalography and Clinical Neurophysiology. Amsterdam, Elsevier, 1986, vol 2: Clinical Application of Computer Analysis of EEG and Other Neurophysiological Signals, pp 385–445.
34 Saletu B, Barbanoj MJ, Anderer P, Sieghart W, Grünberger J: Clinical-pharmacological study with two isomers (*d*-, *l*-) of fenfluramine and its comparison with chlorpromazine and *d*-amphetamine: Blood levels, EEG mapping and safety evaluation. Methods Find Exp Clin Pharmacol 1993;15:291–312.
35 Lee DY, Lee KU, Kwon JS, Jang IJ, Cho MJ, Shin SG, Woo JI: Pharmacokinetic-pharmacodynamic modeling of risperidone effects on electroencephalography in healthy volunteers. Psychopharmacology 1999;144:272–278.
36 Mesulam MM: Behavioral neuroanatomy: Large-scale networks, association cortex, frontal syndromes, the limbic system, and hemispheric specializations; in Mesulam MM (ed): Principles of Behavioral and Cognitive Neurology. New York, Oxford University Press, 2000, pp 1–120.
37 Squire LR, Zola-Morgan S: The medial temporal lobe memory system. Science 1991;253:1380–1386.
38 Devinsky O, Morrell MJ, Vogt BA: Contributions of anterior cingulate cortex to behaviour. Brain 1995;118:279–306.
39 Nyberg L, McIntosh AR, Cabeza R, Habib R, Houle S, Tulving E: General and specific brain regions involved in encoding and retrieval of events: What, where and when. Proc Natl Acad Sci USA 1996;93:11280–11285.
40 Vollenweider FX, Leenders KL, Øye I, Hell D, Angst J: Differential psychopathology and patterns of cerebral glucose utilisation produced by (S)- and (R)-ketamine in healthy volunteers using positron emission tomography (PET). Eur Neuropsychopharmacol 1997;7:25–38.

Psychopharmacology (2004) 173:79–87
DOI 10.1007/s00213-003-1701-x

ORIGINAL INVESTIGATION

E. Frecska · K. D. White · L. E. Luna

Effects of ayahuasca on binocular rivalry with dichoptic stimulus alternation

Received: 28 May 2003 / Accepted: 30 October 2003 / Published online: 8 January 2004

Abstract *Rationale:* During binocular rivalry, two incompatible images are presented to each eye and these monocular stimuli compete for perceptual dominance, with one pattern temporarily suppressed from awareness. One variant of stimulus presentation in binocular rivalry experiments is dichoptic stimulus alternation (DSA), when stimuli are applied to the eyes in rapid reversals. There is preliminary report that in contrast with healthy controls, schizophrenic patients can maintain binocular rivalry even at very high DSA rates. *Objective:* The study was undertaken to investigate whether binocular rivalry survives high rates of DSA induced by the South American hallucinogenic beverage ayahuasca. *Methods:* Ten individuals who were participating in ayahuasca ceremonials were requested to volunteer for binocular rivalry tests (DSA=0, 3.75, 7.5, 15 and 30 Hz) without and after drinking the brew. *Results:* Ingestion of ayahuasca increased mean dominance periods both in standard binocular rivalry conditions (no DSA) and tests with DSA. At higher DSA rates (15 and 30 Hz) the total length of dominance periods was longer on the brew. *Conclusion:* It is discussed that ayahuasca-induced survival of binocular rivalry at high DSA rates may be related to slow visual processing and increased mean dominance periods may result from hallucinogen-induced alteration of gamma oscillations in the visual pathways.

E. Frecska · L. E. Luna
Wasiwaska Research Center,
Florianópolis, SC, Brazil

K. D. White
Department of Psychology,
University of Florida,
Gainesville, Fla., USA

L. E. Luna
Swedish School of Economics,
Helsinki, Finland

E. Frecska (✉)
MHC, Napvirág u. 19, 1025 Budapest II, Hungary
e-mail: efrecska@hotmail.com
Tel.: +36-1-3944388

Keywords Ayahuasca · Binocular rivalry · DMT · Hallucinogens · Human · Visual perception

Introduction

Binocular rivalry belongs to a class of visual illusions in which an ambiguous but unchanging sensory input leads to sudden perceptual switches (Blake 2001). Rivalry occurs when dissimilar images stimulate corresponding retinal areas of the two eyes. For example, if a grating of horizontal lines is presented to the right eye while a grating of vertical lines is presented to the left, the usual experience is of quasi-regular, but unpredictable switches between two perceptions: for 1 s or more, the majority of subjects see only horizontal lines and then for 1 s or more, only vertical lines in an alternating manner (Pettigrew and Miller 1998). Despite the fact that both images are continuously presented during rivalry, the image of one eye disappears from awareness (i.e. suppressed), while the other eye's image is seen (i.e. dominant). Although the phenomenon has been known from the 19th century, lately there has been a resurgence of interest in studying binocular rivalry due to its quantifiable characteristics and new evidence about its neural locus and mechanism.

Several theories have been proposed to account for binocular rivalry (Blake 1989; Fox 1991; Miller et al. 2000; Blake and Logothetis 2002). Such theories generally suggest that because increased thresholds occur during periods of rivalry suppression, the suppression originates from some type of cortical inhibition. Specifically, binocular rivalry was proposed to result from reciprocal inhibition of monocular neurons in the primary visual cortex (Blake 1989). In contrast to the early bottom-up concept, Sheinberg and Logothetis (1997) have demonstrated that activity in higher structures of the visual pathways, such as the inferior temporal cortex and the superior temporal sulcus, correlates better with the visual percept than the activity in the primary visual cortex. Referring to their findings, Dayan (1998) argues that perceptual alternation can be generated by competi-

tion between top-down cortical interpretations for the disparate inputs. Some mapping studies maintain that primary visual cortex is the site of rivalry (Polonsky et al. 2000; Tong 2003), although the possibility has not been excluded that these results could be the consequence of top-down influences from higher levels. The present consensus is that rivalry is a multi-level process that reflects low-level sensory processing as well as high-level cortical activity (Blake and Logothetis 2002).

One variant of stimulus presentation in binocular rivalry experiments is dichoptic stimulus alternation (DSA). In studies using DSA, stimuli are applied to the eyes in rapid alterations instead of keeping the stimulus presented to each eye constant, as in the classical paradigm (Logothetis et al. 1996). DSA at frequencies much higher than the natural frequency of binocular rivalry can unveil underlying deficits in visual neural transitions. This modified method revealed that, in contrast with comparison subjects, schizophrenic patients could maintain slow perceptual alternations even with very high DSA rates (Frecska et al. 2003; Wright et al. 2003). The survival of binocular rivalry at high DSA rates in schizophrenia may reflect impairment in visual information processing and result from loss of presented stimuli when the visual cortex is not capable of following high rates of stimulus change. Slow visual information processing has been repeatedly demonstrated in schizophrenic patients (for review, see McClure 2001). In schizophrenia, visual targets require a longer processing time to "escape" the effects of a subsequent stimulus (Braff and Saccuzzo 1982; Saccuzzo and Braff 1986; Weiner et al. 1990; Rund 1993; Butler et al. 1996). At high DSA rates, there is insufficient time available in schizophrenic patients for extended processing and the resulting under-sampling maintains binocular rivalry, despite the increased frequency (Frecska et al. 2003).

There is evidence that abnormalities in 5-HT_{2A} receptor dependent neurotransmission play a role in some symptoms and perceptual-cognitive deficits in schizophrenia. Some of this evidence comes from the observation that indoleamine hallucinogens can induce 5-HT_{2A} receptor-mediated (Aghajanian and Marek 1999) symptoms that resemble symptoms of schizophrenia (Vollenweider et al. 1998). Dysfunctional 5-HT_{2A} receptors may be involved in abnormal sensory gating mechanisms in schizophrenia (Geyer 1998). Furthermore, the 5-HT_{2A} antagonist property shared by atypical antipsychotic medications may play a role in their ameliorative effects on schizophrenic symptoms not affected by classical D_2 receptor-blocking agents (Meltzer and McGurk 1999; Umbricht et al. 1999; Purdon et al. 2000).

N,N-Dimethyltryptamine (DMT) is the active indoleamine ingredient of ayahuasca which is a hallucinogenic decoction made of psychoactive plants (*Banisteriopsis caapi* and *Psychotria viridis* or *Diplopterys cabrerana*) indigenous to the Amazon and Orinoco river basin of South America. Known under different names such as yagé, natem, mihi, dapa, kamarampi and many others, the brew has been used, probably for millennia, for medico-religious purposes by numerous indigenous groups of the Upper Amazon (Dobkin de Rios 1972; Schultes 1982; Luna 1984, 1986). The ritual use of ayahuasca has spread among Amazonian mestizo population of Colombia, Ecuador, Peru and Brazil. In Brazil, its use has taken a new course as members of synchretic Christian religious organizations, with strong Afro and Amerindian influences, have adopted it as a sacrament under the names of Santo Daime and Hoasca. Although its use is tolerated in countries sharing the Amazon basin, Brazil is the only country where it currently enjoys legalized status. Its active ingredients are the reversible monoamine oxidase inhibitor harmine and harmaline, the serotonin-reuptake inhibitor tetrahydroharmine, which makes the 5-HT_{2A} agonist component DMT bioavailable for oral use, relatively potent and long-acting (Callaway et al. 1999). In this combination, DMT is capable of eliciting a condition characterized by vivid visual imagery, auditory hallucinations, cognitive changes, and depersonalization-derealization type phenomena with profound modification in the sense of self and reality (Szara 1956; Strassman 1996; Riba et al. 2001). The typical onset of action is within 60 min and the peak of effect is between 90 and 120 min after drinking the brew (Riba et al. 2003).

The purpose of present study was to investigate whether binocular rivalry could illuminate common mechanisms in schizophrenia and 5-HT_{2A} mediated hallucinosis induced by ayahuasca. In line with previous findings in schizophrenic patients, we postulate that ayahuasca ingestion will induce a similar condition: the continued presence of binocular rivalry at high DSA rates.

Materials and methods

Subjects

Data were obtained from ten individuals who were participating in ayahuasca ceremonials held in Florianópolis, Brazil between April 1 and 29, 2002 and were volunteering for the binocular rivalry tests. The site in Brazil was chosen for the reason that consumption of ayahuasca is legalized in that country. Enrolled volunteers ingested the decoctum for ritualistic purposes and not for participation in the study. The Principal Investigator (L.E.L.) obtained written informed consent from participants for the binocular rivalry tests prior to the ingestion of the psychoactive brew, conforming to the guidelines set forth by the Ethical Principles and Guidelines for the Protection of Human Subjects of Research (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research 1979). Originally, 15 candidates were considered for participation. One of them was not eligible because she had a dose below the range set in the inclusion criteria. Two individuals did not participate because they preferred not to be disturbed during the sessions, one of them had mixed handedness, and one male was excluded due to cannabis dependence. The final experimental group included eight males and two females with a mean age of 52 (SD=8.8; range=34–65) years and a mean weight of 71.8 (SD=11.4; range=48–82) kg.

Inclusion criteria for experimental subjects were: 1) being experienced in ayahuasca use (having at least five previous sessions); 2) ingesting ayahuasca in a dose not less than 50 ml at the beginning of the experimental session; 3) right handedness, which was evaluated with a modified Edinburgh questionnaire

(Briggs and Nebes 1975); 4) a likelihood ratio (hits/false alarms) at least 3-fold better than random guessing in a signal detection task designed to mimic the perceptual switches of binocular rivalry (see below).

Exclusion criteria for experimental subjects were: 1) personal history of psychiatric or neurological disorder and/or use of psychotropic medication for non-psychiatric condition (e.g. anti-depressant for chronic pain, psychostimulant for weight loss); 2) head injury leading to loss of consciousness for greater than 5 min; 3) alcohol, or illicit drug use in the past 3 weeks; 4) lifetime history of substance dependence as diagnosed by DSM-IV (American Psychiatric Association 1994); 5) body mass index <20 or >25 kg/m^2; 6) having history of cardiac, endocrine illness; 7) nausea with vomiting before the rivalry test was completed; 8) lack or mild degree of ayahuasca experience during the experimental session (see below).

A further sample of 110 undergraduate college students provided control data on binocular rivalry under conditions of DSA that served to verify in a non-age-matched population the very low incidence of perceptual alternation at high rates of DSA in the normal population. Test-retest validation of the quantitative measures of binocular rivalry was performed in 32 subjects.

Ayahuasca session

Participants maintained a salt, sugar and alcohol free diet for 4 weeks during their partaking in the seminar organized by the Wasiwaska Center, had three to four sessions per week, and were enrolled into the rivalry study during week 4. The provided traditional diet did not include fermented or smoked food, aged/dried/cured meat products, and beverages known to have high tyramine content. On the test day, subjects avoided solid food for 12 h starting from 1:30 p.m. At 7:30 p.m. they ingested a self-chosen dose of ayahuasca (obtained from one of the Brazilian synchretic churches) ranging from 50 to 100 (mean=74, SD=21.2) ml. The alkaloid concentrations of the beverage were analyzed later with the method of Callaway et al. (1996) and were as follows: harmine 1.36 mg/ml, tetrahydroharmine 1.05 mg/ml, and DMT 0.73 mg/ml. For the following 4 h, subjects rested supine with lights turned off. Volunteers performed the rivalry test between 90 and 150 min after the ingestion of ayahuasca and had the test done on a separate day in the evening hours without the influence of the decoction (baseline comparison). Six of them had binocular rivalry with ayahuasca as the first procedure and four were scheduled to have the test first without having the brew. Based on participants' previous experiences, the global subjective experience induced by the brew was rated on a 5-point Lickert scale ranging from 0 to 4 (none, mild, moderate, strong, very strong) immediately before starting the binocular rivalry test.

Rivalry procedure

The technique for eliciting binocular rivalry was similar to that already published, with two small targets presented foveally so that the right eye saw, for example, a horizontal grating and the left eye saw a vertical grating, in the same apparent location (Pettigrew and Miller 1998).

Display apparatus

Custom software (written by K.D.W.) generated the rivalry stimuli in frame or field sequential formats, measured responses, and provided user interfaces. In the frame sequential format, odd numbered frames were presented to one eye while even numbered frames were presented to the other eye. The software was triggered by the video vertical synch, which caused the next stimulus to be displayed, then read and stored the status of mouse buttons, and waited for the next vertical synch. Three experimental subjects wore a head-mounted display (Virtual I-O iGlasses) with SVHS resolution, which was synchronized to the frames generated by an IBM Thinkpad computer using 1024×768×256 at 75 Hz non-interlaced graphics (N-mode) by an AverMedia Averkey SVHS scan converter. The iGlasses 3D head-mounted display has separately driven LCD displays (180,000 pixels) for each eye, giving resolutions of 225×266. The other seven experimental subjects wore VRex VR Visualizer LCD shutter glasses, which alternately excluded odd or even fields presented on a 1024×768 resolution 14-inch video monitor. In the latter case, the graphics mode was interlaced at 87 Hz (I-mode). Control subjects wore VRex VR Surfer LCD shutter glasses and viewed 17 inch video monitors on which the 1024×768 display was interlaced at 96 Hz (U-mode).

Stimuli

The stimuli consisted of white binocular fixation guides centered within which was a 1° disk filled with horizontal or vertical grating. These fixation guides consisted of 1) a rectangular frame drawn around the borders of the 12×9° screen, 2) a circle 9° in diameter centered on the screen center, and 3) a plus-sign shaped crosshair at the screen center for which the centermost 3° horizontal and vertical were removed. All stroke widths for the fixation guides were about 1/16° wide. The 1° disk at the screen center was filled with 5 cycles per degree square wave gratings oriented horizontally or vertically. The disk could be presented binocularly (same grating orientation in both eyes) or dichoptically (different grating orientations in the two eyes).

Training in the reporting task

Subjects were trained how to use a two-button computer mouse to indicate what kind of gratings they perceived. In this task they were presented with binocular stimuli (both eyes received the same stimulus, there was no rivalry) while holding the computer mouse in both hands (with left thumb above the left button, right thumb above the right button). These binocular stimuli looked exactly like the experimental stimuli (save that the experimental stimuli used dichoptically presented disks). When the grating was horizontal, subjects were instructed to push, and held down the left button. The left button on the mouse had three black horizontal stripes made of plastic tape in order to be tactually distinctive for the left thumb and to provide tactile cue as reminder. The subjects were advised to move their thumb along and across the stripes to feel this cue for the horizontal report button. They were also told to keep this button held down for the entire time they saw horizontal lines in the disk. The right mouse button had three black plastic tape stripes attached vertically to it.

When subjects saw vertical lines filling the disk, they were supposed to hold down the right button for as long as they saw vertical lines and were reminded to move their thumb along and across the stripes to feel this shape cue. When the disk was filled with superimposed horizontal and vertical lines (a plaid or crosshatch), the subject was told to report this appearance by holding down both mouse buttons. Lastly, in case the disk was filled with a homogeneous field (blank) the subject was instructed to let up both buttons, and, when unsure what to report, to press nothing.

Signal detection analysis of reporting accuracy

This task was run with a computer generated video sequence that simulated rivalry alternation using binocular stimuli. Stimulus durations were sampled at equal probability intervals from a cumulative gamma distribution (Levelt 1965), and the disk content (horizontal, vertical, plaid, blank) randomly assigned that duration, with the proviso that two durations with the same content could not occur in immediate succession. A 90 s sequence of such quasi-randomly selected stimuli changed unpredictably in pattern content

and duration, but in such a way that 25% of the time each pattern appeared. A software program scored the observer's button presses during the video presentation. Responses were scored correct or incorrect by the software depending upon which stimulus pattern preceded pressing of the button(s). These calculations were carried out for time lags between stimulus and response from 0.3 to 3.0 s in 0.1-s steps, comparing button press to stimulus each 1/60 s. Random guessing produces 25% correct (hits) and 75% wrong (false alarm) choices. An observer was not allowed to begin the experiment unless three of the four response types reached a criterion of least 50% hits. The computer video could be repeated if further training was found to be necessary. Repetition did not have to be done more than once with participants of this study. The training and evaluation procedures were generally be completed in about 5 min. Typical performances were 85–95% hits with 5–15% false alarms, by observers without the drug and 70–90% hits with 10–30% false alarms by the same observers under drug influence. Estimated response latency (time lag for maximum percent correct) was 0.65 s without ayahuasca and 0.93 s on ayahuasca. No observer had to be excluded from further participation due to failure to achieve criterion performance (3 times greater likelihood of correct:wrong responses than random chance).

Experimental sequence

We presented standard binocular rivalry for 6 min (in multiple epochs) to obtain the endogenous perceptual alternation rate, and presented dichoptic reversal stimuli at 3.75, 7.5, 15, and 30 reversals per second in four 1.5-min epochs. Order of presentation of standard rivalry and the four reversal rates was counterbalanced within subject. At the end of each epoch, a graph of the responses was displayed. The experimenter described in words the general features shown in the graph in order to verify that these recorded responses were representative of the subject's perceptions. The experimental procedure was completed in 20–30 min.

Data management

For the purpose of identification, all subjects received an alias upon entering the study to aid in maintaining confidentiality. For binocular rivalry, the raw data consisted of the time (in one video frame increments) each button press lasted. These data were imported into spreadsheets and analyzed with macros that extracted the lengths of time when horizontal, vertical, plaid responses or nothing was reported. The following quantitative measures were used to characterize performance for binocular rivalry: 1) "mean dominance period" (MDP)=total duration of horizontal and vertical responses in seconds divided by the number of horizontal and vertical responses; 2) "percentage of dominance periods" (PDP)=% of total time reporting horizontal or vertical responses. Since 0.5 s is close to the mean reaction time during the video simulation sessions, button presses lasting less than 0.5 s were excluded in the analyses, as being too short to be perceptually meaningful. Data were analyzed by Student *t*-test for paired samples and by repeated measures ANOVA (Statistica for Windows v6.0) for multiple variables.

Results

All subjects, regardless of drug condition (with or without ayahuasca), showed binocular rivalry with traditional stimulus presentation (DSA=0 Hz). They reported successive percepts of a horizontal grating or a vertical grating (dominance periods) and sometimes a blend of horizontal and vertical gratings mixed with uncertain responses (non-dominance periods). The typical pattern of perceptual alternation is illustrated for one participant without ayahuasca in Fig. 1. Periods of horizontal dominance appear as blocks below the time line, periods of vertical dominance as blocks above the line, and periods of the blended (plaid) or uncertain percepts as blocks straddling the line.

Analysis of standard rivalry epochs

Our sample of 32 comparison subjects indicates that MDP is stable characteristic within an individual over periods of days or weeks (MDP test-retest r=0.69). Ayahuasca increased MDPs: mean±SD of MDP (no-brew versus on-brew)=2.9±1.5 versus 4.4±0.9, t=–2.29, P<0.05. Our subjects did not respond significantly longer to the video simulation of binocular rivalry (see "Signal detection analysis of reporting accuracy" in Materials and methods) on brew than with no brew (paired t=1.62, NS). Therefore, the longer MDPs during the ayahuasca-induced conditions cannot be explained by response perseveration. The total length of dominance periods in the four standard rivalry epochs was not different on the brew: mean±SD of PDP (no-brew versus on-brew)=86.9%±10.2 versus 79.7%±14.8, t=–1.32, NS. In comparison subjects the PDP had a test-retest value r=0.83.

Analysis of DSA epochs

When conditions of DSA were used in subjects without the brew, patterns of responding unlike those in traditional rivalry were evident. With 3.75 Hz and 7.5 Hz DSA rate, subjects reported horizontal switching with vertical at a very high rate, consistent with the flickering changing orientations presented to either eye and in an effort following the stimulus alternation (Fig. 2). Consequently, after applying the 0.5 s cut-off, less dominance period was reported as compared to the standard epochs, which were performed with constant stimulus presentation to

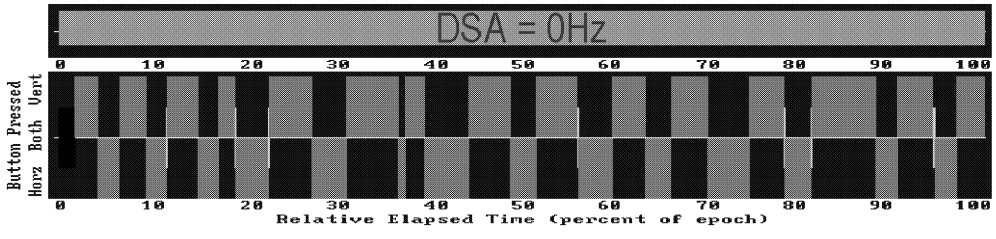


Fig. 1 Typical pattern of perceptual alternation in traditional binocular rivalry without ayahuasca

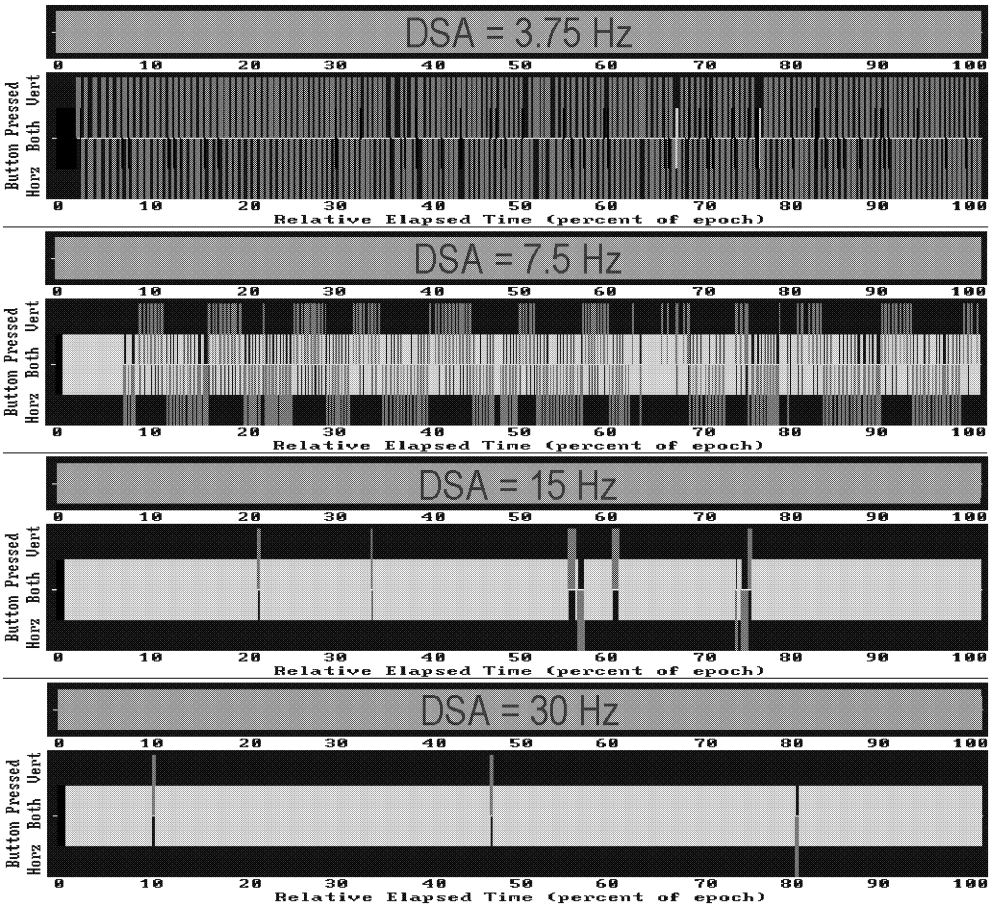


Fig. 2 Typical patterns of perceptual alternation in binocular rivalry at different DSA rates without ayahuasca

each eye. The mean±SD of PDP ($DSA_{3.75\ Hz}$ and $DSA_{7.5\ Hz}$)=24.1±26.3% and 35.1±33.9%. With 15 and 30 DSAs per second, the blended percept (crosshatch) was reported consistently, as though these even more rapidly flickering orientations had fused. Even less dominance periods were noted: mean±SD of PDP ($DSA_{15\ Hz}$ and $DSA_{30\ Hz}$)=1.6±2.3% and 4.2±7.6%. There were individual differences inasmuch as some subjects reported a few periods of horizontal or vertical dominance lasting for a few seconds, with one or more of the DSA rates. The means±SD of MDPs for 3.75 Hz, 7.5 Hz, 15 Hz and 30 Hz DSA rate were 0.9±0.6, 1.4±1.1, 0.9±1.7, and 1.3±2.5, respectively. In summary, the flicker-like response pattern or the blended percept reports were quite typical for subjects in the control condition (no brew). A similar finding was previously reported by Logothetis et al. (1996), although their study used lower DSA rates.

Under the influence of ayahuasca, subjects reverted to show typical patterns of binocular rivalry, which were more striking at the high DSA rates (Fig. 3). At low DSA rates (3.75 Hz and 7.5 Hz), the total length of the dominance periods was the same as in conditions without ayahuasca: mean±SD of PDP ($DSA_{3.75\ Hz}$ and $DSA_{7.5\ Hz}$)=39.5±26.5% and 28.9±30.1% [$F(1,9)=0.34$, NS]. As the DSA rates were increased above 7.5 Hz, the PDPs on the brew diverged from those in the control condition: mean±SD of PDP ($DSA_{15\ Hz}$ and $DSA_{30\ Hz}$) = 28.2±36.1% and 9.8±9.4% [$F(1,9)=6.5$, $P<0.05$]. The average length of binocular rivalry episodes got significantly longer: mean±SD of MDPs for 3.75 Hz, 7.5 Hz, 15 Hz and 30 Hz DSA rate were 5.9±5.5, 2.6±2.7, 6.0±9.8, and 2.4±2.3, respectively [$F(1,9)=9.0$, $P<0.02$]. In brief, subjects on ayahuasca were able to maintain much longer horizontal or vertical dominance periods than without it despite the fact that the stimuli were alternating at rates up to almost 2 orders of magnitude faster than their endogenous rivalry rate.

Discussion

We have found that perceptual switches of binocular rivalry occur despite high rates of DSA in ayahuasca-induced hallucinosis. This finding is similar to what was reported in schizophrenic patients (Frecska et al. 2003).

While this study targeted some common mechanisms in schizophrenia and ayahuasca-induced hallucinosis, it should be noted that according to current views, psychosis and ayahuasca inebriation are not equivalent conditions. Psychosis is neither voluntary nor wished for; it is a disorderly mental state over which the subject has no control. In contrast, ayahuasca, when taken in a traditional-ritual manner, induce a state of being characterized by enhanced internal order. Furthermore, experienced

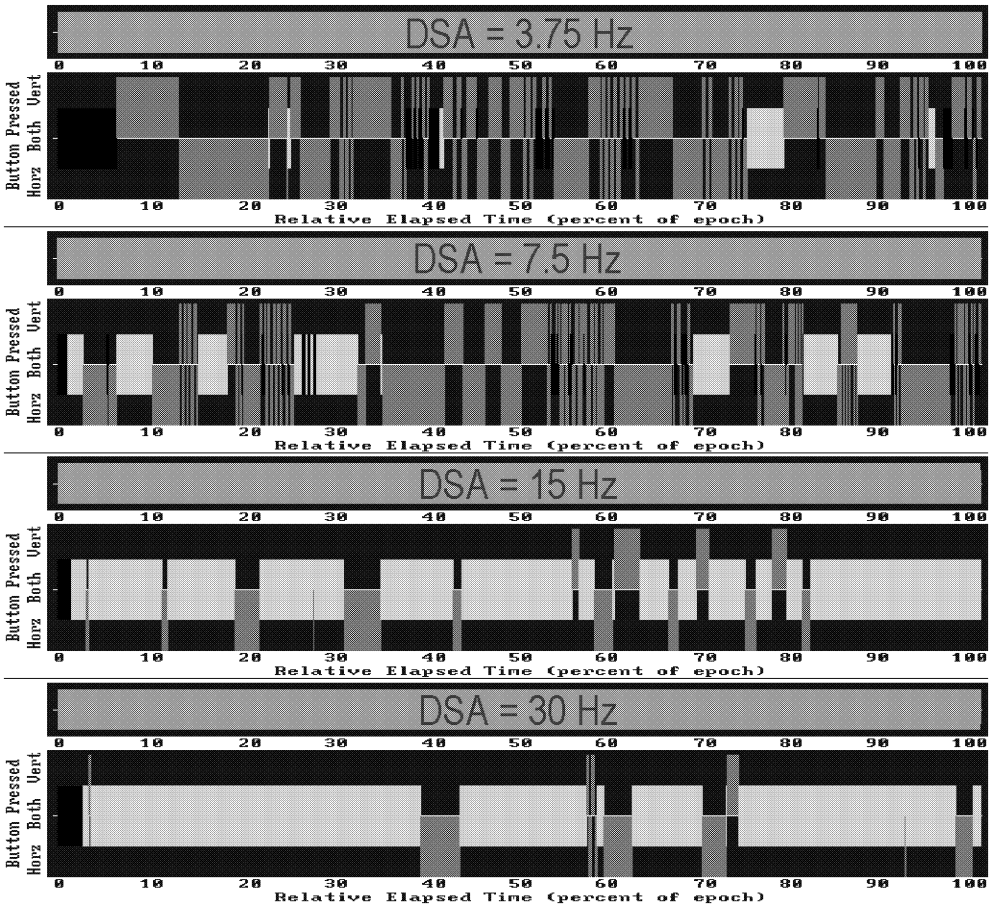


Fig. 3 Typical patterns of perceptual alternation in binocular rivalry at different DSA rates with ayahuasca

ayahuasca drinkers have significant control over what is happening to them under the intoxication (Shanon 2002).

The argument can be made that the response measures we used rely on subjective reports, which might be compromised in some subjects and can be affected by the psychoactive brew. This issue was addressed both by our training procedure and with signal detection analysis, which provided likelihood ratios that the subjects' reports were correct relative to being wrong. All subjects had likelihood ratios significantly greater than the 1:3 ratio for random guessing, as assessed in the pre- and post-experiment "videos". In this way, we emulated binocular rivalry studies in monkeys, who cannot verbally report their perceptual alternations but whose subjective states seem to match those experienced by humans. Moreover, our subjects did not indicate poor performance during video simulations of binocular rivalry while on ayahuasca. No subject's data had to be excluded from analysis due to failure to achieve criterion performance.

A number of visual functions have been reported to be significantly altered by hallucinogenic drugs. These include reduced contrast sensitivity and depth perception (Andre et al. 1994; Leweke et al. 1999), decreased speed of visual processing (Braff et al. 1981), disrupted pre-pulse inhibition (Sipes and Geyer 1997; Jones and Shannon 2000; Javitt and Lindsley 2001; Linn and Javitt 2001; Ouagazzal et al. 2001), poor performance in cognitive tasks involving attention (Jin et al. 1997; Gouzoulis-Mayfrank et al. 2002) and deficit of smooth pursuit eye movements (Ando et al. 1983; Ploner et al. 2002). It has to be noted here that despite a consistently reported decrease of pre-pulse inhibition in animal studies, there is no consensus in the literature on the effect of serotonergic hallucinogens/entactogens on pre-pulse inhibition in humans: with reports on increased (Gouzoulis-Mayfrank et al. 1998; Vollenweider et al. 1999) or lack of effect (Riba et al. 2002). The anomalous visual process that we have described following ayahuasca administration is unlikely to be explained by altered cognitive functions or changes in eye movements. Within each phase of stimulus exposure during DSA at 7.5, 15 or 30 Hz rates, the time is insufficient either for high order information processing or to overcome the latency for initiating smooth pursuit eye movements. Therefore these factors cannot play a determining role in the observed persistence of perceptual alternation. Reduced contrast sensitivity and depth perception have diminishing effects on perceptual rivalry (Cobo-Lewis et al. 2000), and thus provide no explanation.

Considering the limited timeframe, the variation in opinion about the neural mechanism of the relative stability of rivalry in the face of high DSA rates under ayahuasca exposure is then narrowed to early visual processing, so the interpretation of our results will be

presented in that context. We favor the view that an explanation of the persistence of binocular rivalry at high DSA rates requires a neural context that involves the primary visual cortex and visual pathways below. It should be emphasized that this concept is not against the new emerging view that binocular rivalry results from more global processing at higher levels, perhaps coordinated on a hemispheric basis (Pettigrew 2001), with feedback to early visual processing. The model to be presented simply makes an effort to explain how the neural transition of the DSA stimuli escapes steps of regular processing at the "bottom" and becomes subject of perceptual alternation at the "top".

This model, in its simplest form, has already been outlined in the Introduction for schizophrenic patients. To recap and reformulate: slow perceptual processing, which has been implicated in the background of backward masking deficits (Braff et al. 1981), decreased pre-pulse inhibition (Sipes and Geyer 1997; Jones and Shannon 2000; Javitt and Lindsley 2001; Linn and Javitt 2001; Ouagazzal et al. 2001), disturbed auditory event-related potential (Hampson et al. 1989; Javitt et al. 2000; Riba et al. 2002) observed after administration of hallucinogens, and prevented the intoxicated subject "catching up" to the increased DSA rate. At high DSA rates, successive stimuli may exert masking effect on the preceding ones both in schizophrenic patients (Frecska et al. 2003) and subjects influenced by ayahuasca, resulting in relative insensitivity to the higher DSA frequencies and survival of the rivalry. In this way, binocular rivalry with DSA is conceptualized as a continuous series variant of paired stimulus tests. The presented model can explain the persistence of PDP at high DSA rates; however, it cannot give account for the extended MDP induced by ayahuasca.

There is a similarity of the basic perceptual alterations in hallucinogen-induced conditions to those extensively studied in schizophrenic patients and their first degree relatives (see for review, Light and Braff 1999). This parallel observation permits further elaboration of the model and provides an explanation for the extended MDPs in both schizophrenia and drug-induced hallucinosis. In order to address this problem, we will turn to current research on gamma oscillations. The answer will be provided in frame of the gamma synchrony-related "binding theory" of sensory integration.

Patients with schizophrenia experience a wide range of perceptual changes similar to (and even more consistently reported) what we cited above as effect of hallucinogens. Several authors (Green et al. 1999; Lee et al. 2003) suggest that this variety of perceptual changes can be explained in a parsimonious manner. They refer to a type of convergence that can be observed in the literature regarding the underlying mechanisms of the most commonly used perceptual methods. Firstly, pre-pulse inhibition, auditory event-related potentials and visual backward masking all belong to the class of paired-stimulus tests. Secondly, the middle latency auditory event-related potential (P50) appears to be a subset of the auditory gamma band response (Pantev et al. 1993), it is conceptually related to visual masking (Braff et al. 1991) which is also supposed to have gamma-range oscillations as putative neural correlates (Purushothaman et al. 2000). Thirdly, it has been proposed that deficits on P50 may be associated with suppressed cortical oscillations in the gamma range, and Green et al. (1999, 2003) raised the possibility that the visual backward masking deficits of schizophrenics are also linked to cortical gamma oscillations. Oscillations in the gamma range (30–70 Hz) are found over multiple cortical areas and thought to be involved with feature binding (Alais et al. 1998), attentional processes (Singer 1993), motor response preparation (Farmer 1998), and working memory (De Pascalis and Ray 1998). The concept emerges that subtle problems in the gamma range, such as slow frequency undamped waves, prematurely timed early (first burst) or blunted late (second burst) event-related gamma activity might represent the earliest step in a cascade of events leading to deviations in perceptual processing (Green et al. 2003) and other anomalies of integrative brain function (i.e. altered "binding") (Lee et al. 2003).

It has been demonstrated that gamma-frequency synchronization in the visual cortex (V1 and V2) correlates with stimulus selection during binocular rivalry (Fries et al. 2002). According to these authors, both stimulus selection and suppression during dichoptic stimulus presentation is an active process and depends on intact post-retinal gamma-frequency synchronization. It is the relative increase in gamma synchronization of the selected response over the non-selected one that enhances the probability that the given stimulus impacts target neurons at higher processing levels and thereby gains perceptual dominance. One rivaling, but suppressed stimulus can overcome the currently dominant stimulus by power increase in its induced gamma oscillation at V1 and V2. Therefore, binocular rivalry is dependent on alternating increases in gamma synchronization at the visual cortex. We argue that in case of erratic gamma activity, a stimulus can remain dominant for a longer period, since it may take more time for the rivaling stimulus to get beyond the threshold in increased gamma synchronization. There is report on disruption of synchronous interhippocampal gamma oscillations by the hallucinogen mescaline (Doheny and Whittington 1998). We suppose that similar ayahuasca-induced aberration in post-retinal gamma synchronization results in the observed increase in MDPs.

The investigation of the gamma band deficits in hallucinating and/or psychotic individuals is in an early phase yet. Several issues need further clarification. For example, the question can be raised: what kind of visual channels are involved in the discussed phenomenon? Preliminary findings imply that gamma abnormalities are at least partly responsible for visual masking deficits in schizophrenia (Green at al. 2003). The described differences in gamma activity are supposed to originate from the sustained channels (Purushothaman et al. 2000). However, those are subtle compared with the overall

group differences in masking performance. The major component of the early visual deficit in schizophrenia and in drug-induced hallucinosis may derive from other mechanisms, and perhaps due to abnormal processing in the transient channels with uncertain gamma origin. Another series of questions can address the type of gamma pattern change. Most of the studies have focused primarily on reduced gamma power but increased gamma activity was proposed to mediate excessive information processing, i.e. increased "binding" (for review, see Lee et al. 2003), and unsteady frequency is evident behind the sustained visual channel problem in schizophrenia (Green et al. 1999).

In summary, it is premature to specify exactly what kind of underlying neural mechanism can explain the perceptual alterations observed by the use of paired-test procedures and binocular rivalry with DSA. Moreover, according to our knowledge, limited information is available about hallucinogen action on gamma band activity. Our findings are preliminary, our explanation is tentative, but we hope that this study will promote further studies into the field and that the method of binocular rivalry with different stimulus presentations may turn out to be a valuable tool for studying gamma activity-related perceptual functions and dysfunctions. Studies on abnormal gamma activity and its mechanisms may provide an interpretive framework for understanding hallucinogen related breakdown in integrative function.

Acknowledgments The authors thank Eva Wiesenmayer, Richard Bonenfant and Sherman Wissinger for their help in preparation of the manuscript.

References

Aghajanian GK, Marek GJ (1999) Serotonin and hallucinogens. Neuropsychopharmacology 21:16S–23S

Alais D, Blake R, Lee SH (1998) Visual features that vary together over time group together over space. Nat Neurosci 1:160–164

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Press, Washington

Ando K, Johanson CE, Levy DL, Yasillo NJ, Holzman PS, Schuster CR (1983) Effects of phencyclidine, secobarbital and diazepam on eye tracking in rhesus monkeys. Psychopharmacology 81:295–300

Andre JT, Tyrrell RA, Leibowitz HW, Nicholson ME, Wang M (1994) Measuring and predicting the effects of alcohol consumption on contrast sensitivity for stationary and moving gratings. Percept Psychophys 56:261–267

Blake R (1989) A neural theory of binocular rivalry. Psychol Rev 96:145–167

Blake R (2001) A primer on binocular rivalry, including current controversies. Brain Mind 2:5–38

Blake R, Logothetis NK (2002) Visual competition. Nat Rev Neurosci 3:13–21

Braff DL, Saccuzzo DP (1982) Effect of antipsychotic medication on speed of information processing in schizophrenic patients. Am J Psychiatry 139:1127–1130

Braff DL, Silverton L, Saccuzzo DP, Janowsky DS (1981) Impaired speed of visual information processing in marijuana intoxication. Am J Psychiatry 138:613–617

Braff DL, Saccuzzo DP, Geyer MA (1991) Information processing dysfunctions in schizophrenia: studies of visual backward masking, sensorimotor gating, and habituation. In: Steinhauer SR, Gruzelier JH, Zubin J (eds) Neuropsychology, psychophysiology, and information processing (Handbook of Schizophrenia, vol 5). Elsevier Science Publishers, Amsterdam, pp 303–334

Briggs GG, Nebes RD (1975) Patterns of hand preference in a student population. Cortex 11:230–238

Butler PD, Harkavy-Friedman JM, Amador XF, Gorman JM (1996) Backward masking in schizophrenia: relationship to medication status, neuropsychological functioning, and dopamine metabolism. Biol Psychiatry 40:295–298

Callaway JC, Raymon LP, Hearn WL, McKenna DJ, Grob CS, Brito GS, Mash DC (1996) Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. J Anal Toxicol 20:492–497

Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, Andrade EN, Andrade EO, Mash DC (1999) Pharmacokinetics of hoasca alkaloids in healthy humans. J Ethnopharmacol 65:243–256

Cobo-Lewis AB, Gilroy LA, Smallwood TB (2000) Dichoptic plaids may rival, but their motions can integrate. Spat Vis 13:415–429

Dayan P (1998) A hierarchical model of binocular rivalry. Neural Comput 10:1119–1135

De Pascalis V, Ray WJ (1998) Effects of memory load on event-related patterns of 40-Hz EEG during cognitive and motor tasks. Int J Psychophysiol 28:301–315

Dobkin de Rios M (1972) Visionary vine: psychedelic healing in the Peruvian Amazon. Chandler Publishing, San Francisco

Doheny HC, Whittington MA (1998) Synchronous interhippocampal gamma oscillations are disrupted by the hallucinogen mescaline. J Physiol 513P:23P–24P

Farmer SF (1998) Rhythmicity, synchronization and binding in human and primate motor systems. J Physiol 509:3–14

Fox R (1991) Binocular rivalry. In: Regan D (ed) Binocular vision. Macmillan, London

Frecska E, White KD, Leonard CM, Kuldau JM, Bengtson M, Ricciuti N, Luna L, Mahoney B, Pettigrew J (2003) Binocular rivalry in schizophrenia and drug-induced psychosis. Neuropsychopharmacologia Hungarica 5:4–13

Fries P, Schroder JH, Roelfsema PR, Singer W, Engel AK (2002) Oscillatory neuronal synchronization in primary visual cortex as a correlate of stimulus selection. J Neurosci 22:3739–3754

Geyer MA (1998) Behavioral studies of hallucinogenic drugs in animals: implications for schizophrenia research. Pharmacopsychiatry 31:73–79

Gouzoulis-Mayfrank E, Heekeren K, Thelen B, Lindenblatt H, Kovar KA, Sass H, Geyer MA (1998) Effects of the hallucinogen psilocybin on habituation and prepulse inhibition of the startle reflex in humans. Behav Pharmacol 9:561–566

Gouzoulis-Mayfrank E, Thelen B, Maier S, Heekeren K, Kovar KA, Sass H, Spitzer M (2002) Effects of the hallucinogen psilocybin on covert orienting of visual attention in humans. Neuropsychobiology 45:205–212

Green MF, Nuechterlein KH, Breitmeyer B, Mintz J (1999) Backward masking in unmedicated schizophrenic patients in psychotic remission: possible reflection of aberrant cortical oscillation. Am J Psychiatry 156:1367–1373

Green MF, Mintz J, Salveson D, Nuechterlein KH, Breitmeyer B, Light GA, Braff DL (2003) Visual masking as a probe for abnormal gamma range activity in schizophrenia. Biol Psychiatry (in press)

Hampson RE, Foster TC, Deadwyler SA (1989) Effects of delta-9-tetrahydrocannabinol on sensory evoked hippocampal activity in the rat: Principal components analysis and sequential dependency. J Pharmacol Exp Ther 251:870–877

Javitt DC, Lindsley RW (2001) Effects of phencyclidine on prepulse inhibition of acoustic startle response in the macaque. Psychopharmacology 156:165–168

Javitt DC, Jayachandra M, Lindsley RW, Specht CM, Schroeder CE (2000) Schizophrenia-like deficits in auditory P1 and N1

refractoriness induced by the psychomimetic agent phencyclidine (PCP). Clin Neurophysiol 111:833–836
Jin J, Yamamoto T, Watanabe S (1997) The involvement of sigma receptors in the choice reaction performance deficits induced by phencyclidine. Eur J Pharmacol 319:147–152
Jones CK, Shannon HE (2000) Effects of scopolamine in comparison with apomorphine and phencyclidine on prepulse inhibition in rats. Eur J Pharmacol 391:105–112
Lee KH, Williams LM, Breakspear M, Gordon E (2003) Synchronous gamma activity: a review and contribution to an integrative neuroscience model of schizophrenia. Brain Res Brain Res Rev 41:57–78
Levelt WJM (1965) On binocular rivalry. Royal Van Gorcum, Assen
Leweke FM, Schneider U, Thies M, Munte TF, Emrich HM (1999) Effects of synthetic delta9-tetrahydrocannabinol on binocular depth inversion of natural and artificial objects in man. Psychopharmacology 142:230–235
Light GA, Braff DL (1999) Human and animal studies of schizophrenia-related gating deficits. Curr Psychiatr Rep 1:31–40
Linn GS, Javitt DC (2001) Phencyclidine (PCP)-induced deficits of prepulse inhibition in monkeys. Neuroreport 12:117–120
Logothetis NK, Leopold DA, Sheinberg DL (1996) What is rivalling during binocular rivalry? Nature 380:621–624
Luna LE (1984) The healing practices of a Peruvian shaman. J Ethnopharmacol 11:123–133
Luna LE (1986) Vegetalismo. Shamanism among the Mestizo population of the Peruvian Amazon. Almqvist & Wiksell International, Stockholm
McClure RK (2001) The visual backward masking deficit in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 25:301–311
Meltzer HY, McGurk SR (1999) The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. Schizophr Bull 25:233–255
Miller SM, Liu GB, Ngo TT, Hooper G, Riek S, Carson RG, Pettigrew JD (2000) Interhemispheric switching mediates perceptual rivalry. Curr Biol 10:383–392
National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (1979) Ethical principles and guidelines for the protection of human subjects of research. The Belmont Report. US Government Printing Office, Washington
Ouagazzal A, Grottick AJ, Moreau J, Higgins GA (2001) Effect of LSD on prepulse inhibition and spontaneous behavior in the rat. A pharmacological analysis and comparison between two rat strains. Neuropsychopharmacology 25:565–575
Pantev C, Elbert T, Makeig S, Hampson S, Eulitz C, Hoke M (1993) Relationship of transient and steady-state auditory evoked fields. Electroencephalogr Clin Neurophysiol 88:389–396
Pettigrew JD (2001) Searching for the switch: neural bases for perceptual rivalry alternations. Brain Mind 2:85–118
Pettigrew JD, Miller SM (1998) A "sticky" interhemispheric switch in bipolar disorder? Proc R Soc Lond B Biol Sci 265:2141–2148
Ploner CJ, Tschirch A, Ostendorf F, Dick S, Gaymard BM, Rivaud-Pechoux S, Sporkert F, Pragst F, Stadelmann AM (2002) Oculomotor effects of delta-9-tetrahydrocannabinol in humans: implications for the functional neuroanatomy of the brain cannabinoid system. Cereb Cortex 12:1016–1023
Polonsky A, Blake R, Braun J, Heeger DJ (2000) Neuronal activity in human primary visual cortex correlates with perception during binocular rivalry. Nat Neurosci 3:1153–1159
Purdon SE, Jones BD, Stip E, Labelle A, Addington D, David SR, Breier A, Tollefson GD (2000) Neuropsychological change in early phase schizophrenia during 12 months of treatment with olanzapine, risperidone, or haloperidol. The Canadian Collaborative Group for research in schizophrenia. Arch Gen Psychiatry 57:249–258
Purushothaman G, Ogmen H, Bedell HE (2000) Gamma-range oscillations in backward-masking functions and their putative neural correlates. Psychol Rev 107:556–577
Riba J, Rodriguez-Fornells A, Urbano G, Morte A, Antonijoan R, Montero M, Callaway JC, Barbanoj MJ (2001) Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. Psychopharmacology 154:85–95
Riba J, Rodriguez-Fornells A, Barbanoj MJ (2002) Effects of ayahuasca on sensory and sensorimotor gating in humans as measured by P50 suppression and prepulse inhibition of the startle reflex, respectively. Psychopharmacology 165:18–28
Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanoj MJ (2003) Human pharmacology of Ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J Pharmacol Exp Ther 306:73–83
Rund BR (1993) Backward-masking performance in chronic and nonchronic schizophrenics, affectively disturbed patients, and normal control subjects. J Abnorm Psychol 102:74–81
Saccuzzo DP, Braff DL (1986) Information-processing abnormalities: trait- and state-dependent components. Schizophr Bull 12:447–459
Schultes RE (1982) The beta-carboline hallucinogens of South America. J Psychoact Drugs 14:205–220
Shanon B (2002) The Antipodes of the mind: charting the phenomenology of the Ayahuasca experience. Oxford University Press, Oxford
Sheinberg DL, Logothetis NK (1997) The role of temporal cortical areas in perceptual organization. Proc Natl Acad Sci USA 94:3408–3413
Singer W (1993) Synchronization of cortical activity and its putative role in information processing and learning. Annu Rev Physiol 55:349–374
Sipes TE, Geyer MA (1997) DOI disrupts prepulse inhibition of startle in rats via 5-HT_{2A} receptors in the ventral pallidum. Brain Res 761:97–104
Strassman RJ (1996) Human psychopharmacology of N,N-dimethyltryptamine. Behav Brain Res 73:121–124
Szara S (1956) Dimethyltryptamine: its metabolism in man; the relation of its psychotic effect to the serotonin metabolism. Experientia 12:441–442
Tong F (2003) Primary visual cortex and visual awareness. Nat Rev Neurosci 4:219–229
Umbricht D, Javitt D, Novak G, Bates J, Pollack S, Lieberman J, Kane J (1999) Effects of risperidone on auditory event-related potentials in schizophrenia. Int J Neuropsychopharmacol 2:299–304
Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 9:3897–3902
Vollenweider FX, Remensberger S, Hell D, Geyer MA (1999) Opposite effects of 3,4-methylenedioxymethamphetamine (MDMA) on sensorimotor gating in rats versus healthy humans. Psychopharmacology 143:365–372
Weiner RU, Opler LA, Kay SR, Merriam AE, Papouchis N (1990) Visual information processing in positive, mixed, and negative schizophrenic syndromes. J Nerv Ment Dis 178:616–626
Wright P, Nolan NJ, Mahoney BP, White KD, Kuldau JM, Leonard CM (2003) Binocular rivalry is slower in first-degree relatives in schizophrenics. International Congress on Schizophrenia Research, Colorado Springs (poster presentation)

Altered States of Consciousness and Short-Term Psychological After-Effects Induced by the First Time Ritual Use of Ayahuasca in an Urban Context in Brazil†

Paulo Cesar Ribeiro Barbosa, M.Sc.*; Joel Sales Giglio M.D., Ph.D.**
& Paulo Dalgalarrondo M.D., Ph.D.**

Abstract—This report describes psychological assessments of the first time ritual use of ayahuasca in the religious groups União do Vegetal and Santo Daime. Nineteen subjects who tried the beverage in Santo Daime rituals and nine subjects who tried it in União do Vegetal rituals were evaluated one to four days before their first ayahuasca experience in life and one to two weeks after this experience. Semistructured interviews and a structured psychiatric scale were used in the first evaluation to elicit set variables concerning attitudes towards the ayahuasca experience and to elicit mental health status. Mental health status was reassessed in the second evaluation, which also included a semistructured interview concerning the phenomenology of altered states of consciousness (ASCs). Predominantly positive expectancies concerning the ayahuasca experience were the most prominent findings concerning set variables. Visual phenomena, numinousness, peacefulness, insights and a distressing reaction were the most salient ASC experiences. A significant reduction of the intensity of minor psychiatric symptoms occurred in the Santo Daime group after the hallucinogen experience. Subjects in both groups reported behavioral changes towards assertiveness, serenity and vivacity/joy. The set and setting hypothesis, suggestibility processes, as well as the supposed unique effects of ayahuasca are used in discussing these findings.

Keywords—hallucinogens, psychiatric symptoms, religion, ritual, states of consciousness

Ayahuasca tea is a hallucinogenic beverage obtained from decoction of root bark and sometimes the stem cortex of the liana *Banisteriopsis caapi* (which contains harmala alkaloids harmine, harmaline and tetrahydroharmine) and N,N-dimethyltryptamine (DMT)-containing plants, such as the leaves of *Psychotria viridis* bush (Ott 1994). Ethnological data has described the ritual use of the beverage throughout the western Amazon Basin by Amerindian and mestizo populations (Luz 2002; Dobkin de Rios 1989). Its ritualized use among the Brazilian urban population results from the growth of two religious groups: the União do Vegetal (also known as UDV) and the Santo Daime. Both groups are characterized by a syncretism of Christian elements and beliefs in reincarnation. Since ayahuasca is viewed as a tool of spiritual development, ritual use of the beverage is an essential aspect of Santo Daime and UDV (Brissac 1999; Labigalini Jr. & Dunn 1995; MacRae 1992).

†The authors thank Andrea F Semolini and Helymar C Machado of the Comissão de Pesquisa – Estatistica – FCM – UNICAMP, for the statistical support.

*Professor of Science Methods and Mental Health, Departamento de Filosofia e Ciências Humanas, Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia, Brazil.

**Professor of Psychiatry, Departamento de Psicologia Medica e Psiquiatria, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil.

Please address correspondence and reprint requests to Paulo Cesar Ribeiro Barbosa, Av. Moraes Sales, N. 1610 ap. 144, Campinas City, São Paulo State, Brazil, CEP 13010-002; email:pcesarr@yahoo.com.br.

The introduction of ritual use of ayahuasca in urban contexts has raised serious concerns about potential adverse effects to mental health (Casenave 2000).

A well-known hypothesis about the psychological effects of hallucinogens argues that positive or adverse reactions to these substances would be a function of the setting—the physical and social environment in which the drug is consumed—and the set—the subject's emotional state, intentions, expectancies and beliefs concerning the use of the psychoactive substance (Szara 1967). Some studies have suggested that the religious and ritual settings of the UDV and Santo Daime could favor positive outcomes of ayahuasca use because within these settings the hallucinogen tea would be considered a sacrament, and its psychological effects would be ritually structured toward a self-enhancing and spiritually laden experience (Grob et al. 1996; MacRae 1992).

These studies performed assessments of long-term members of UDV or Santo Daime who were acquainted with the states of consciousness induced by the ritual use of ayahuasca as well as with the beliefs and values associated with this use. There is a significant gap in knowledge about a fundamental aspect involved in the religious introduction of ayahuasca in the urban context: knowledge about the first experience with the ritual use of ayahuasca in life, as well as the outcomes of this first experience. This is a moment of crucial importance, since ayahuasca-naïve subjects would neither be acquainted with the states of consciousness expected to be produced by ayahuasca, nor with the ritual settings of the UDV and Santo Daime and their repertoire of beliefs and values.

The present work investigates psychological aspects relevant to set and to mental health in the first experience with the ritual use of ayahuasca in life. Set variables related to religious beliefs, expectancies and intentions concerning the effects of the hallucinogenic tea were assessed before that experience. Mental health variables related to psychiatric symptoms and psychosocial behaviors were evaluated before and reevaluated after the ritual use of ayahuasca. Also, a phenomenological description of the altered states of consciousness (ASC) induced by that use was performed.

BACKGROUND INFORMATION: THE RITUAL SETTINGS OF UDV AND SANTO DAIME

The study was conducted in several Santo Daime and UDV temples located in two large cities in southeastern Brazil: São Paulo and Campinas. The rituals are characterized by the constant preaching of Santo Daime's and UDV's ethos and worldview. There are some significant differences between the rituals of these two religions. In Santo Daime, the preaching is performed by a collective performance of hymns and a synchronized dance called a *bailado*, which are accompanied by vigorous percussion and melodic instruments. All participants are required to sing and dance during the ceremonies, which last four to 12 hours. In UDV, the preaching occurs through questions directed to the preacher by the participants, through popular songs with moral contents played on stereo equipment and also through hymns performed by single participants. During some periods silence predominates. The rituals invariably last four hours and the participants remain seated in a relaxed position most of time.

METHODS

Design

Twenty eight subjects were assessed prospectively from one to four days prior to the first ritual experience with ayahuasca (time 0 or T0) and between seven and 14 days after this experience (time 1 or T1). All subjects had only one ritual experience with ayahuasca between T0 and T1. Nineteen subjects tried ayahuasca in Santo Daime and nine subjects tried it in UDV.

Recruitment

Recruiting naïve subjects to make a group analysis was difficult because ritual opportunities to try ayahuasca for the first time were not frequent, and there were few novices per ritual. Therefore, it was decided to use whatever individuals were available. Novices were invited to participate in the research by elder members of Santo Daime or UDV who were responsible for instruction in the behavior to be adopted during the ritual. Those who accepted the invitation to participate in the research were directed to the researcher.

Parameters

Both structured and qualitative semistructured instruments were used in the evaluation of the subjects. The structured instruments included a sociodemographic questionnaire and a fully standardized psychiatric scale (Clinical Interview Schedule-Revised Edition [CIS-R]) that evaluated the outcome of the intensity of minor psychiatric symptoms between the T0 and T1. Qualitative semistructured interviews elicited expectancies and religious beliefs concerning the ritual use of ayahuasca, the outcomes of behaviors towards various psychosocial facets before and after the experience and the phenomenology of the altered states of consciousness. All semistructured interviews were audiotaped. Qualitative evaluations were used because they fit well with the need for a sensitive preliminary exploration of barely studied phenomena such as the first-time ritual use of ayahuasca in urban contexts. While structured instruments restrict the subjects' information to a previously-determined limited set of responses, qualitative evaluation grants to the subjects more autonomy and spontaneity in eliciting their information, allowing more sensitivity in eliciting potential novelties of untraveled

TABLE 1
Age: Group Differences and Total Sample

	N	Mean	Min	Max	Median
Santo Daime	19	33.0±12.4	18	56	31
UDV	9	41.9±10.8	27	56	42
Total	28	35.7±12.5	18	56	33.5

Note: Mann-Whitney U test; $p = .07$

fields of human experience (Kvale 1999). Therefore, the combination of structured and qualitative semistructured evaluations was made in order to try to meet both the needs of standardized evaluation as well as sensitivity to novel psychological and cultural aspects involved in the first-time ritual use of ayahuasca in an urban context.

Clinical Interview Schedule-Revised Edition (CIS-R). Subjects were administered a structured psychiatric interview (CIS-R) in T0 and T1. The CIS-R is a scale which measures the intensity of minor psychopathological symptoms: somatic symptoms, fatigue, difficulties in concentration, sleep problems, irritability, preoccupation with corporeal functioning, depression, depressive ideation, worries, anxiety, compulsions, and obsessions (Goldberg et al. 1970). A Brazilian version of CIS-R was readily available at the time of the study (Botega et al. 1995).

Sociodemographic profile. Subjects completed a questionnaire on their level of education and occupation.

Inventory of intrinsic religious beliefs profiles. Subjects were asked to tell about their current beliefs in God, spiritual reality, reincarnation and spiritual beings. This was done in T0.

Inventory of expectancies/motivations. Subjects were asked to tell the motives that led them to decide to try ayahuasca, and to tell about their expectancies concerning the experience. This was done in T0.

Phenomenological mapping of the altered states of consciousness induced by ayahuasca. Subjects were asked about seven major dimensions of their ayahuasca experience—mood, thought contents and processes, sense of self, exteroception, interoception, volition/control, and sense of time and space—as well as interpretations of these dimensions of altered states. This semistructured interview was based on previous propositions of phenomenological descriptions of altered states (Walsh 1995; Metzner 1989).

Behavioral changes inventory. This is a semistructured interview designed to elicit possible changes in their attitudes towards relevant psychosocial aspects: family issues, occupational and financial issues, interpersonal issues, self-esteem, stressor events, and subjective experience concerning physical well-being. In T0, subjects were asked about their current attitudes towards these issues; in T1, subjects were asked about possible changes in these attitudes.

Analysis

Data collected by CIS-R were analyzed by the Statistical Analysis System version 8.2 (SAS Institute Inc 1999-2001). All variables were analyzed by considering the total sample and comparing Santo Daime and UDV separately. Categorical variables of sociodemographic data were analyzed according to Fischer's test. The Mann-Whitney U test was used in the analysis of the continual variables of age and minor psychiatric symptoms. The Wilcoxon Signed Rank test was applied to the analysis of the outcome of minor psychiatric symptoms in T0 and T1. All tests adopted $p < .05$ as a level of statistical significance.

Data collected through semistructured interviews underwent qualitative content and phenomenological analyses. Meaningful experiential patterns were identified in the subject's reports and were transformed into major categorical dimensions. In order to provide the reader with an overview of these dimensions they were converted into tables and presented in simple frequencies.

RESULTS

Sociodemographic Profile

Sociodemographic data are presented in Tables 1 and 2. A significantly greater percentage of unmarried subjects were found among Santo Daime subjects ($p = .01$) who also tended to be younger than UDV subjects ($p = .07$). A statistically significant difference was not observed in sex ($p = 1.0$), occupational category (p=0.393) nor in level of education ($p = 0.114$) between the Santo Daime and UDV groups. All the subjects had completed high school.

Intrinsic Religious Beliefs Profile and Motivations/Expectancies, Phenomenological Mapping of Altered States of Consciousness, and Behavioral Changes After Use of Ayahuasca

Subjects' narratives concerning religious beliefs profile and motivations/expectancies before the use of ayahuasca, altered states of consciousness induced by ritual use of ayahuasca and behavioral changes after the use of ayahuasca are summarized in Tables 3, 4 and 5. Due to the dimensional nature of the data shown in these tables the categories are not mutually exclusive, i.e., several subjects'

TABLE 2
Other Sociodemographic Variables: Group Differences and Total Sample

Variable	Categories	Santo Daime N = 19	União do Vegetal N = 9	Total N = 28	*p*
Sex	Male	8 (42.1%)	4 (44.4%)	12 (42.9%)	NS
	Female	11 (57.9%)	5 (55.6%)	16 (57.1%)	
Marital status	Married	5 (26.3%)	8 (88.9%)	13 (46.4%)	.01*
	Separated	4 (21.1%)	0	4 (14.3%)	
	Single	10 (52.6%)	1 (11.1%)	11 (39.3%)	
Education	< Bachelor's	11 (57.9%)	2 (22.2%)	13 (46.4%)	NS
	> Bachelor's	8 (42.1%)	7 (77.8%)	15 (53.6%)	
Occupation	Professionals: education, health business, law and commucations experts	6 (31.6%)	6 (66.7%)	12 (42.9%)	NS
	Service/shop market worker: fortune teller, shop salesperson and demonstrator	4 (21.1%)	1 (11.1%)	5 (17.9%)	
	University students	3 (15.8%)	0	3 (10.7%)	
	Small business owners	1 (5.3%)	1 (11.1'%)	2 (7.1%)	
	Others: technician, clerk, artisan, house-wife, unemployed	5 (26.3%)	1 (11.1%)	6 (21.4%)	

Note: Fisher's test; NS = non-statistically significant
*p<.05

reports contained contents that were assigned to more than one dimension. That is the reason why the sum of the occurrences in the dimensions of Intrinsic Religious Beliefs Profile, Motivations/Expectancies (Table 3) and in Altered States of Consciousness (Tables 4) exceeded the number of subjects in the sample. Also, the totals shown for behavioral changes 7-14 days after the use of ayahuasca (Table 5) did not equal the number of total subjects of the sample because some dimensions were absent from some subjects' reports.

In Table 4, some of the dimensions listed for altered states of consciousness (in particular, Alleged Insights and Distressing Reaction) are highly descriptive because they need to transmit complex meaningful articulations involving subjects' biographies, ritual events and their motivations and expectancies. As these data are intended to transmit new information concerning this little-studied phenomenon, the responses given with low frequency convey new information about the phenomenon and so should be considered relevant information on their own.

Clinical Interview Schedule-Revised Edition (Minor Psychiatric Symptoms)

Data concerning minor psychiatric symptoms elicited by CIS-R are shown in Table 6. A significantly higher prevalence of symptoms was found in the Santo Daime group than in the UDV group in T0 ($p < .05$). A significant reduction of the intensity of minor psychiatric symptoms can be observed in Santo Daime group between T0 and T1 ($p < .01$), which in turn influences the significant difference between T0 and T1 in the overall sample ($p < .01$). In the UDV group, the difference between the two times was not significant, due to its low score in T0 ($p = .88$)

DISCUSSION

Since a convenience sample was used and inasmuch as the number of subjects was small, and since a substantial part of the data were elicited and analyzed by means of qualitative procedures, a cautious attitude is required when generalizing the findings. Nevertheless, this is the first investigation which attempts to study variables related to set, altered states of consciousness and mental health status prior and after the first experience with the ritual use of ayahuasca. The results need to be considered a preliminary exploration towards this untravelled field. Certainly future studies must be compared to these preliminary findings in order to evaluate their significance.

The educational level of the subjects is well above the Brazilian and even above the more developed São Paulo State standards. While all subjects had completed high school level, only 16.3% of Brazilian and 18.4% of São

TABLE 3

Dimensions of Intrinsic Religious Beliefs Profile and Motivations/Expectancies Before the First Ayahuasca Experience: Group Differences and Total Sample

Dimensions	Santo Daime N = 19	UDV N = 9	Total N = 28
Intrinsic Religious Beliefs Profile			
Beliefs in reincarnation: Kardecist conceptions of spiritual and moral evolution throughout successive reincarnations (from Allan Kardec, the nineteenth century founder of this kind of spiritism).	13 (68.4%)	5 (55.6%)	18 (64.3%)
Metaphysical religiosity: Beliefs concerning supernatural beings, parallel dimensions, cosmic energy and eastern influences (e.g., the idea of chakras), and practices such as yoga and meditation.	12 (63.2%)	4 (44.4%)	16 (57.1%)
Unstructured religious beliefs: Beliefs in the existence of a spiritual reality with no structured ideas about this reality.	5 (26.3%)	2 (22.2%)	7 (25%)
Agnosticism: No belief in spiritual reality at all.	0	1 (11.1%)	1 (3.6%)
Motivations/Expectancies			
Self-knowledge: Search for self-knowledge.	9 (47.4%)	3 (33.3%)	12 (42.9%)
Spiritual latencies: Search for awakening of supposed hidden spiritual attributes (e.g., "the superior self").	7 (36.8%)	1 (11.1%)	8 (28.6%)
Curiosity: Desire to know about the effects of ayahuasca.	6 (31.6%)	1 (11.1%)	7 (25%)
Healing: Search for healing of psychosocial problems (e.g., family/marital, work, interpersonal and self-esteem problems).	6 (31.6%)	0	6 (21.4%)
Equilibrium: Search for improvement in general well-being and behavior.	5 (26.3%)	0	5 (17.9%)

Paulo State populations above 25 years of age have completed it (IBGE 2000). While 53.6% of subjects had a bachelors degree, only 6.4% of Brazilian and 9.4% of São Paulo State populations above 25 years of age possess one (IBGE 2000). The high proportion of professionals in the occupational category of subjects reflects their high educational level. High educational level and specialized occupation are major indices of social status pertaining to middle classes as opposed to the working classes of Brazilian society (IPEA 2000). Therefore, the subjects' sociodemographic profile is a sign that, despite the Amazonian origins of Santo Daime and UDV among working classes (Brissac 1999; MacRae 1992), the religious use of ayahuasca in southeastern Brazilian large cities seems to be a predominantly middle and educated social class phenomenon.

All but one category (curiosity) identified by the inventory of expectancies/motivations clearly reflect sensitivity to the supposed properties of the beverage as a medium of spiritual or psychological development and improved health. This affinity with UDV's and Santo Daime's conceptions corroborates a hypothesis that the search for an ayahuasca experience reflects a cultural change of desires of a part of the Brazilian urban middle class, which seeks in spiritual and mystical experiences an alternative way of life to the growing materialistic and utilitarian values recently developed in Brazilian society (Soares 1990). This is coherent with the prevalence of metaphysical religiosity elicited by the intrinsic religious beliefs inventory, which revealed a prior attitude of searching for practices and beliefs related to alterations of consciousness (Glick 1988).

TABLE 4
Dimensions of Altered States of Consciousness: Group Differences and Total Sample

Dimensions	Santo Daime N = 19	UDV N = 9	Total N = 28
Visual phenomena: extraordinary visual experiences, which included kaleidoscopic lights, geometric forms, tunnels, animals, humans and supernatural beings.	12 (63.2%)	6 (66.7%)	18 (64.3%)
Peace: a prominent sense of inner calm, silence and harmony.	7 (36.8%)	8 (88.9%)	15 (53.6%)
Numinousness: a mixture of terror and fascination, which results from the sense of a superior and powerful presence.	10 (52.6%)	2 (22.2%)	12 (42.9%)
Alleged insights: elucidating thoughts about biographic, existential and behavioral aspects; some insights were attached to great fascination and profundity and to a deep meaning of ritual events; frequently they were perceived as received "from outside".	9 (47.4%)	2 (22.2%)	11 (39.3%)
Alterations in self-body image: alterations such as fusions with the environment and separation between the conscious self and the body.	7 (36.8%)	2 (22.2%)	9 (32.1%)
Distressing reaction: an overwhelming affliction due to an experience of "being imposed" an ideation of a "prophecy" of an imminent personal tragedy; the prophecy was attributed to a supposed "superior self," which was a previously expected spiritual latency; idiosyncratically considered as part of the superior self, the ritual events were interpreted as an irrefutable confirmation of the prophecy.	1 (5.3%)	0	1 (3.6%)

TABLE 5
Dimensions of Behavioral Changes 7-14 Days After the Use of Ayahuasca: Group Differences and Total Sample

Dimensions	Santo Daime N = 19	UDV N = 9	Total N = 28
Serenity: more serenity towards previous stressor psychosocial aspects.	6 (31.6%)	2 (22.2%)	8 (28.6%)
Assertiveness: more assertiveness towards previous psychosocial aspects.	4 (21.1%)	1 (11.1%)	5 (17.9%)
Vivacity/joy: more energy and happiness in daily life.	2 (10.5%)	2 (22.2%)	4 (14.3%)
Relaxation/Satisfaction: unusual satisfaction and relaxation the day after the experience.	2 (10.5%)	2 (22.2%)	4 (14.3%)
Worry: the subject who underwent the distressing reaction reported a lot of preoccupation.	1 (5.3%)	0	1 (3.6%)

TABLE 6
The CIS-R Scale: Group Differences and Outcome of Minor Psychiatric Symptoms in T0 and T1

N	Santo Daime N = 19		UDV N = 9		Total N = 28		p^b
	Mean	Median	Mean	Median	Mean	Median	
T0	11±8.8	13	2.6 ±2.5	2	8.3 ±8.3	5.5	.01*
T1	5.2 ±4.3	4	2.3 ±3.9	1	4.3±4.3	3	.03*
p^a	.007**		NS		.009**		

[a]Wilcoxon Signed Rank test: outcome of minor psychiatric symptoms in T0 and T1; NS = non-statistically significant
**$p<.01$
[b] Mann-Whitney U test: group differences
*$p<.05$

Since Christian principles and the idea of spiritual and moral evolution throughout successive reincarnations are present both in Santo Daime/UDV and in Kardecist-inspired ideas of reincarnation (Goulart 2002; Andrade 1995), the presence of the latter in the subjects' religiosity seems also to reveal the subjects' affinity with Santo Daime's and UDV's systems of religious beliefs. These findings suggest that, although the religious use of psychoactive substances is not a trait of western societies (Galanter 1989), some specific cultural changes in religious practices and beliefs in contemporary Brazilian urban contexts (e.g., the spread of the New Age movement and beliefs in reincarnation) contributed to the positive and optimistic attitudes of most subjects towards the ayahuasca ritual experience.

The score on minor psychiatric symptoms among the Santo Daime group previous to the ayahuasca experience is higher than the CIS-R score found by Botega and colleagues (1995) among patients in a general hospital setting in Brazil (mean = 7.6; median = 6; SD = 7.5). Significantly, the category concerning the search for healing elicited by the motivations/expectancies inventory was found only among Santo Daime subjects.

The experiential dimensions elicited by phenomenological mapping confirm psychedelic studies performed four decades ago, which qualified as invariant hallucinogenic effects phenomena like alterations in the sense of self, insightful and aesthetic visual experiences (Unger 1963) and which proposed mystical experiences as a consequence of hallucinogen consumption in religious set and settings (Pahnke & Richards 1971). Therefore, the pleasant feelings and sensations and personal and religious insights elicited by the phenomenological mapping seem to have been influenced by the subjects' positive expectancies and motivations and by the Santo Daime/UDV ritual settings. These settings would structure the altered states towards "positive" experiences through actualizations of altruistic and optimistic contents of their doctrinaire repertories (MacRae 1992).

However, other findings suggest a more complex relationship between the ASCs and the set/setting, rather than the unidirectional determination of altered states by the set/setting described above. The phenomena of attachment of deep meaning to ritual events and the idiosyncratic interpretations of these events, described respectively in the categories of alleged insights and distressing reaction, are examples of how ayahuasca-induced alterations in consciousness may influence the processing of ritual information, and seem to reflect two transcultural characteristics of ASC: suggestibility and impaired judgment (Ludwig 1966).

The experience of receiving cognitions and feelings from "outside," identified in the distressing reactions and in the "positive" dimensions of ASCs has been identified as a transcultural trait of ayahuasca states of consciousness, independent of the set and setting (Shanon 1999). Therefore, although the content of the ASCs may be explained by the set/setting hypothesis, the way this content is actualized in the subjects' experiences must take into account the radical cognitive and affective alterations induced by ayahuasca intoxication.

Retrospective studies confirm the occurrence of personal insights in the first experience with ayahuasca (Grob et al. 1996). Since the mid twentieth century, mental health professionals have proposed the use of hallucinogens as a psychotherapeutic adjunct because these substances would facilitate association and memory processes (Strassman 1995; Grinspoon & Bakalar 1979). According to the perspective of UDV and Santo Daime members, insights experienced during the ASCs are crucial to self-knowledge and spiritual evolution (Couto 1989). The alleged property of ayahuasca as an insight and self-knowledge inducer seems to be quite charming. However, data concerning the attribution of fascination and profundity to the insights are also signs of the suggestibility and impaired judgment discussed above. This observation demands a more careful interpretation of the alleged insights: Wouldn't subjects be attributing a greater importance to their thoughts than their real influence in the subjects' lives?

An intriguing finding in the phenomenological mapping was the occurrence of peaceful states as a prominent

trait of the ASCs. Although experiences of "peace" and "inner silence" have been described as a possibility of psychedelic experiences (Pahnke & Richards 1971; Barron, Jarvik & Bunnel 1964), the rate of 53.6% contrasts with predominant characterizations of hallucinogenic drugs as emotional liability, anxiety and excitement inducers (Fischer 1971; Szara 1967). It does appear that the mild sedative properties attributed to ayahuasca's β-carbolines act as modulators of the typical hallucinogenic properties attributed to DMT (Ott 1994), softening it and configuring the unique psychological effects of ayahuasca. Difference in the rate at which subjects expressed feelings of peace between Santo Daime (36.8%) and UDV (88.9%) suggests other problems concerning set and setting variables. It would appear that the significantly lower score on CIS-R of UDV subjects previous to the ayahuasca experience reflects more emotional stability than is seen in the Santo Daime subjects, which in turn would determine more proneness to stable experiences. Perhaps the seated and relaxed position of UDV rituals, the absence of percussion instruments and periods of silence facilitate the peaceful states, while the vigorous collective singing and dancing to percussion instruments of Santo Daime rituals would promote more typical hallucinogenic potentialities, such as numinous excitement.

The remarkable reduction of minor psychiatric symptoms evaluated by CIS-R and calmer, more assertive and vivacious behaviors elicited by the behavioral changes inventory suggest interpretations based on psycholytic and psychedelic therapy, which advocated that positive transformations in attitudes may result from insights induced by hallucinogenic drugs used in psychotherapeutic settings (Bravo & Grob 1989; Savage, Terril & Jackson 1962). More optimistic perspectives about ayahuasca's behavioral effects favor these models (Peláez 2002). Nevertheless, it would be prudent to raise two more parsimonious hypotheses concerning more superficial psychological phenomena which may also contribute to the interpretation of the data: the first concerns the rupture with daily life, and the second concerns suggestibility processes.

Extraordinary experiences may work, at least temporarily, as antidotes to boredom caused by the monotonous repetition of daily life, destitute of events which mobilize enthusiasm. This hypothesis fits the data concerning motivations/expectancies, which reflects the desire to transcend everyday life. Another "salutary" effect of this rupture would be an abrupt interruption of distressing psychosocial aspects. During ASCs, these problems would be replaced by extraordinary and/or peaceful experiences, which would be extended to the following days. Hence, the improvement of emotional states would be a consequence of the repose or emotional catharsis which is substituted for the monotony and stress related to the subjects' daily lives.

The important role of suggestibility in ASC was mentioned above. Reports on motivations/expectancies and on experiences during and after ASCs suggest a synergistic combination of three suggestibility processes which may determine the emotional and behavioral improvement: (1) existential and spiritual searches elicited by the motivations/expectancies inventory would reflect subjects' wishes for changes in their relationships with the world and themselves; this seems to suggest to them to behave or at least to report behavioral changes according to those wishes; (2) emotional and perceptual excitement experienced in "exotic" ritual environments would satisfy the desire to transcend everyday life, working as pretext to concretize previously yearned-for behavioral changes; and (3) suggestibility to acceptance of morally and optimistically laden ritual contents during ASC would also be extended to following days, influencing the elicited reports on experiential changes.

A fundamental step towards explanatory models of the psychological effects of ritual use of ayahuasca is a follow-up investigation that is presently being conducting by the authors. Long-term maintenance or disappearance of emotional and behavioral improvement will throw some light upon the question of how profound or superficial these effects are. Also, such study will shed some light on possible adverse or positive mental health outcomes of this kind of experience. In subsequent studies the novel information and hypotheses involving data obtained from qualitative procedures should be standardized through structured instruments in order to corroborate, reformulate of refute them in a more rigorous way.

REFERENCES

Andrade, A.P. 1995. *O Fenômeno do Chá e a Religiosidade Cabocla: Um Estudo Centrado na União do Vegetal* (dissertation). São Bernardo do Campo (SP), Brazil: Instituto Metodista de Ensino Superior.

Barron, F.; Jarvik, M.E. & Bunnel Jr., S. 1964. Hallucinogenic drugs. *Scientific American* 210 (4): 29-37.

Botega, N.J.; Pereira, W.A.B.; Bio, M.R.; Garcia Jr., C. & Zomignani, M. A. 1995. Psychiatric morbidity among medical inpatients: a standardized assessment (GHQ-12 and CIS-R) using lay interviewers in a Brazilian hospital. *Social Psychiatry and Psychiatric Epidemiology* 30 (3): 127-31.

Bravo, G. & Grob, C.S. 1989. Shamans, sacraments and psychiatrists. *Journal of Psychoactive Drugs* 21 (1): 123-28.

Brissac, S.C.T. 1999. *A Estrela do Norte Iluminando até o Sul:Uma Etnografia da União do Vegetal em um Contexto Urbano* (dissertation). Rio de Janeiro (RJ), Brazil: Museu Nacional.

Casenave, S.O.S. 2000. Banisteriopsis caapi: ação alucinógena e ritual. *Revista de Psiquiatria Clínica* 27 (1): 32-35.

Couto, F.R. 1989. *Sinais Dos Tempos: Santos e Xamãs* (dissertation). Brasília (DF), Brazil: UnB.

Dobkin de Rios, M. 1989. A modern-day shamanistic healer in the Peruvian Amazon: Pharmacopoeia and trance. *Journal of Psychoactive Drugs* 21 (1): 91-99.

Fischer, R. 1971. A cartography of the ecstatic and meditative states. *Science* 174 (12): 897-904.

Galanter, M. 1989. *Cults: Faith Healing and Coercion*. New York: Oxford University Press.

Glick, D.C. 1988. Symbolic, ritual and social dynamics of spiritual healing. *Social Science and Medicine* 27 (11): 1197-206.

Goldberg, D.; Cooper, B.; Eastwood, M.R.; Kedward, H.B. & Shepherd, M. 1970. A standardized psychiatric interview for use in community surveys. *British Journal of Preventive and Social Medicine* 24 (1): 18-23.

Goulart, S.L. 2002. O contexto do surgimento do culto da Santo Daime: Formação da comunidade e do calendário ritual. In: B.C. Labate & W.S. Araújo (Eds.) *O Uso Ritual da Ayahuasca*. Campinas, Brazil: Mercado de Letras.

Grinspoon, L. & Bakalar, J.B. 1979. *Psychedelic Drugs Reconsidered*. New York: Basic Books.

Grob, C.S.; Mackenna, D.J.; Callaway, J.C.; Brito, G.S.; Neves, E.S.; Oberlaender, G.; Saide, O.L.; Labigalini, E.; Tacla, C.; Miranda, C.; Strassman, R.J. & Boone, K.B. 1996. Human psychopharmacology of hoasca: A plant hallucinogen used in ritual context in Brazil. *Journal of Nervous and Mental Disease* 184 (2): 86-94.

Instituto Brasileiro de Geografia e Estatística (IBGE). 2000. *Censo Demográfico 2000: Resultados da Amostra (CR-ROM)*. Rio de Janeiro (RJ), Brazil. IBGE.

Instituto de Pesquisa Econômica Aplicada (IPEA). 2000. *Atlas do Desenvolvimento Humano no Brasil*. Available at www.ipea.gov.br.

Kvale, S. 1996. *Interviews: An Introduction to Qualitative Research Interviewing*. Thousand Oaks, California: Sage Publications.

Labigalini Jr., E. & Dunn, J. 1995. The Union of Vegetable: The ritualized use of hoasca tea. *Psychiatric Bulletin* 19 (5): 313-14.

Ludwig, A.M. 1966. Altered states of consciousness: *Archives of General Psychiatry* 15 (3): 225-34.

Luz, P. 2002. O uso ameríndio do caapi. In: B.C. Labate & W.S. Araújo (Eds.) *O Uso Ritual da Ayahuasca*. Campinas (SP), Brazil: Mercado de Letras.

MacRae, E. 1992. *Guiado Pela Lua: Xamanismo e Uso Ritual da Ayahuasca no Culto do Santo Daime*. São Paulo (SP), Brazil: Brasiliense.

Metzner, R. 1989. States of consciousness and transpersonal psychology. In: R. Valle & S. Halling (Eds.) *Existential-Phenomenological Perspectives in Psychology*. New York: Plenum Press.

Ott, J. 1994. *Ayahuasca Analogues*. Kennewick: Natural Products Co.

Pahnke, W.N. & Richards, W.A. 1971. Implications of LSD and experimental mysticism. In: C.T. Tart (Ed.) *Altered States of Consciousness*. New York: Anchor Books.

Peláez, M.C. 2002. Santo Daime, transcendência e cura. Interpretações sobre as possibilidades terapêuticas da bebida ritual. In: B.C. Labate & W.S. Araújo (Eds.) *O Uso Ritual da Ayahuasca*. Campinas (SP), Brazil: Mercado de Letras.

SAS Institute Inc. 1999-2001. *Statistical Analysis System Version 8.2*. Cary, North Carolina: SAS Institute.

Savage, C.; Terril, J. & Jackson D.D. 1962. LSD, transcendence and the new beginning. *Journal of Nervous and Mental Disease* 135 (5):425-39.

Shanon, B. 1999. Ayahuasca, mind and consciousness. *Noetic Journal* 2 (3): 305-15.

Soares, L.E. 1990. O Santo Daime no contexto da nova consciência religiosa. In: L. Landin (Ed.). *Sinais dos Tempos: Diversidade Religiosa*. Rio de Janeiro (RJ), Brazil: Instituto de Estudos da Religião.

Strassman, R.J. 1995. Hallucinogenic drugs in psychiatric research and treatment: Perspectives and prospects. *Journal of Nervous and Mental Disease* 183 (3): 127-38.

Szara, S. 1967. The hallucinogenic drugs: Curse or blessing? *American Journal of Psychiatry* 123 (12): 1513-18.

Unger, S.M. 1963. Mescaline, psilocybin and personality change: *Psychiatry* 26 (2): 111-25.

Walsh, R. 1995. Phenomenological mapping: A method for describing and comparing states of consciousness. *Journal of Transpersonal Psychology* 27 (1): 25-56.

Available online at www.sciencedirect.com
ScienceDirect
Journal of Ethnopharmacology 112 (2007) 507–513
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Effects of ayahuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members

R.G. Santos [a,*], J. Landeira-Fernandez [b], R.J. Strassman [c], V. Motta [a], A.P.M. Cruz [a]

[a] *Departamento de Processos Psicológicos Básicos, Instituto de Psicologia, Universidade de Brasília, Asa Norte, Brasília-DF 70910-900, Brazil*
[b] *Department of Psychiatry, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA*
[c] *Departamento de Psicologia, Pontifícia Universidade Católica do Rio de Janeiro, PUC-RJ, Brazil*

Received 21 December 2006; received in revised form 16 April 2007; accepted 18 April 2007
Available online 25 April 2007

Abstract

The use of the hallucinogenic brew ayahuasca, obtained from infusing the shredded stalk of the malpighiaceous plant *Banisteriopsis caapi* with the leaves of other plants such as *Psychotria viridis*, is growing in urban centers of Europe, South and North America in the last several decades. Despite this diffusion, little is known about its effects on emotional states. The present study investigated the effects of ayahuasca on psychometric measures of anxiety, panic-like and hopelessness in members of the Santo Daime, an ayahuasca-using religion. Standard questionnaires were used to evaluate state-anxiety (STAI-state), trait-anxiety (STAI-trait), panic-like (ASI-R) and hopelessness (BHS) in participants that ingested ayahuasca for at least 10 consecutive years. The study was done in the Santo Daime church, where the questionnaires were administered 1 h after the ingestion of the brew, in a double-blind, placebo-controlled procedure. While under the acute effects of ayahuasca, participants scored lower on the scales for panic and hopelessness related states. Ayahuasca ingestion did not modify state- or trait-anxiety. The results are discussed in terms of the possible use of ayahuasca in alleviating signs of hopelessness and panic-like related symptoms.

Keywords: Ayahuasca; *Banisteriopsis caapi*; *Psychotria viridis*; Emotional states; BHS; ASI-R

1. Introduction

Ayahuasca is a Quechua term derived from the juxtaposition of the terms: *Aya*–"*soul*" "*dead spirit*"; *Waska*–"*rope*", "*vine*", and thus is loosely translatable "vine of the souls" or "vine of the dead". Ayahuasca refers to the vine used as the principle ingredient of a psychoactive beverage used by more than 70 different indigenous groups spread along Brazil, Colombia, Peru, Venezuela, Bolivia and Ecuador (Goulart, 2005; Luna, 2005). The word *ayahuasca* is used to describe the spiritual force in the beverage or the beverage itself, which is made with several species of the *Banisteriopsis* vine (e.g., *Banisteriopsis caapi*, *Banisteriopsis muricata*) (Malpighiaceae), usually in combination with other plants, such as *Psychotria viridis* or *Diplopterys cabrerana* (Ott, 1994). Ayahuasca also may refer to preparations containing only the vine, as made by the Maku Indians, for example (Davis, 1997).

Since the beginning of the 20th century ayahuasca has been used by syncretic religious cults created in the Amazonian Brazilian states of Acre and Rondônia. These groups, of which Santo Daime, Barquinha and União do Vegetal (UDV) are the main ones, use ayahuasca as a healing tool and as a vehicle to gain access to the divine realm. These churches are highly syncretic, containing influences from popular Catholicism, European esoteric and spiritual beliefs, African cosmologies, and indigenous botanical knowledge. Their use of ayahuasca as sacrament resembles that of the Christian Eucharist. In the Santo Daime and UDV rites, members drink ayahuasca usually twice a month, and in Barquinha it is not unusual to consume the brew four times per week (Araújo, 1999; Labate and Araújo, 2004).

Abbreviations: DMT, *N*,*N*-dimethyltryptamine; THH, tetrahydroharmine; 5-HT, 5-hydroxytryptamine; MAO, monoamine oxidase; SSRIs, selective serotonin reuptake inhibitors; SPECT, single photon emission computerized tomography; GC/MS, gas chromatography/mass spectrometry; STAI, state–trait-anxiety inventory; A-state, state-anxiety; A-trait, trait-anxiety; ASI-R, revised anxiety sensitivity index; BHS, Beck hopelessness scale; UDV, União do Vegetal

* Corresponding author. Current address: Centre d'Investigació de Medicaments, Institut de Recerca, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau (HSCSP), St. Antoni Maria Claret, 167, Barcelona 08025, Spain. Tel.: +34 93 2919019; fax: +34 93 2919286.
E-mail address: banisteria@gmail.com (R.G. Santos).

0378-8741/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2007.04.012

Several studies (e.g., McKenna and Towers, 1981; McKenna et al., 1984; Callaway, 1988; McKenna et al., 1990, 1998; Callaway et al., 1999) have indicated that the main components of ayahuasca, the *N*,*N*-dimethyltryptamine (DMT) and some beta-carbolines, are structurally similar to serotonin (5-hydroxytryptamine or 5-HT), a neurotransmitter present in the nervous system of most animal species. Correspondently, further observations found these ayahuasca components to display high affinity for serotonin receptors, especially the 5-HT_2 receptor subtype (Smith et al., 1998; Grella et al., 2003).

The beta-carbolines in ayahuasca include harmine, harmaline and tetrahydroharmine (THH). DMT is an ultra-short-acting hallucinogenic tryptamine (Callaway et al., 1999) present in several plants used as admixtures to the *Banisteriopsis caapi* vine in ayahuasca preparations. DMT also is present in tissues of mammals, marine animals and amphibians. In human beings it also is endogenous, being found in blood, urine and cerebrospinal fluid (Strassman, 2001). Despite being a potent psychoactive chemical, DMT is inactive following oral administration at doses up to 1000 mg, probably due to degradation by gastrointestinal and liver MAO (monoamine oxidase) (McKenna et al., 1984). However, when DMT is combined with inhibitors of the MAO enzymes, as are the beta-carbolines present in ayahuasca, it becomes able to reach the systemic circulation and the central nervous system, thus producing its effects (McKenna et al., 1984).

The beta-carbolines present in ayahuasca are potent natural, selective, reversible, competitive inhibitors of peripheral MAO, and are more active against MAO-A than MAO-B. They also have a relatively low affinity for liver MAO compared to brain MAO (McKenna et al., 1984, 1998). There is some evidence, however, that THH, the second most abundant beta-carboline in the beverage, acts as a selective inhibitor of serotonin reuptake as well as an MAO inhibitor (McKenna et al., 1998; Frecska et al., 2004). The inhibition of both systems – MAO and serotonin reuptake – by ayahuasca's beta-carbolines may result in elevated levels of brain serotonin (McKenna et al., 1998; Luna, 2005).

Despite the fact that the use of ayahuasca is a relatively widespread practice in countries like Brazil, Peru and Colombia, and that this practice is spreading to the United Sates, Europe and other parts of the world, there are few studies that have examined the brew with rigorous methodologies. One of these investigations is the biomedical study conducted by Grob et al. (1996), who investigated 15 long-term users of the brew regarding its acute and long-term psychological effects as well assessing peripheral serotonergic function (Callaway et al., 1994; Grob et al., 1996).

Grob et al.'s study reported that the ayahuasca-using members of the UDV church among whom the volunteers came were more reflective, confident, gregarious and optimistic compared to the control group, which had never used ayahuasca and was age-, gender-, education-, and socio-economically matched.

However, with respect to our research objectives, the most interesting findings were those that showed that before membership in the religion, 11 of the participants were diagnosed as having previously been afflicted with alcohol abuse disorders, 2 with major depressive disorders, 4 with drug abuse (cocaine and amphetamines), 11 with tobacco addiction, and 3 with phobic anxiety disorders. Five of the participants with a history of alcoholism also had histories of violent behavior associated with binge drinking. All of these psychiatric diagnoses remitted following entry into the religion. All participants reported that their use of ayahuasca within the religious context led to improved mental and physical health and significant improvements in interpersonal, work, and family interactions (Grob et al., 1996; McKenna et al., 1998).

Assessing serotonergic function the authors found a significant up-regulation in the density of the serotonin transporter in blood platelets of the ayahuasca drinkers compared to the control group (Callaway et al., 1994). Up- or down-regulation of peripheral platelet receptors is considered to reflect similar biochemical events occurring in the brain. None of the participants showed evidence of any neurological or psychiatric deficit.

Callaway hypothesized that the causative agent of this platelet effect was tetrahydroharmine (THH). In a self-experiment, he underwent single photon emission computerized tomography (SPECT) scans of his own brain 5-HT uptake receptors before and after a 6-week course of daily dosing with THH. He found that the density of central 5-HT receptors in the prefrontal cortex had increased. During recovery over the next several weeks their density gradually returned to previous levels.

According to McKenna et al. (1998) a deficit of serotonin reuptake sites in frontal cortex has been found to correlate with aggressive behavior in alcoholics. If THH were able to specifically reverse this deficit, it may have applications in the treatment of this behavior. As mentioned previously, the majority of the participants in the Grob et al. study had a previous history of alcoholism, some displaying violent behavior. Although the behavioral transformation could be due to the supportive social and psychological environment of a religious group, the finding of this long-term change in precisely the serotonin system that is deficient in violent alcoholics argues that biochemical factors also may play a role (McKenna et al., 1998).

It is well established that anxiety, panic and depressive-related symptoms are significantly attenuated by 5-HT agonists, such as tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs), as well as by MAO-A inhibitors that also exert an indirect agonist action on serotonergic neurotransmission (for recent reviews, see Wikinski, 2004; Nash and Nutt, 2005; Starcevic, 2006). The fact that ayahuasca preparations contain alkaloids that inhibit both the serotonin reuptake and MAO-A suggests that the brew might attenuate emotional states regulated by the serotoninergic system. The present study investigated this possibility by assessing anxiety, panic and depression psychometric items in a double-blind protocol in long-term members of the ayahuasca-using Santo Daime cult.

2. Methodology

2.1. Subjects

Nine healthy volunteers (6 males and 3 females; between 35 and 56 years of age) of the Santo Daime cult community

Céu do Planalto participated in the study. Eight volunteers had graduated high school, while one had finished college.

Eligibility criteria included: (1) voluntary participation as documented through the signing of written informed consent; (2) long-term use of the brew, as defined by a minimum of ten years use at a frequency of once every 2 weeks, within the context of the Santo Daime cult.

Exclusion criteria included: (1) prior history of hypertension, diabetes or cardiac pathology; (2) current treatment with any of the following drugs: antipsychotics, anxiolytics, antidepressants, mood stabilizers, or appetite suppressants related to amphetamine; (3) pregnant or breastfeeding women.

No alcoholic beverages or other drugs were consumed 24 h before the psychometric evaluation, but five of the volunteers had consumed ayahuasca before data collection, at 22, 20, 18, 16 and 6 h (data not treated differently). Volunteers refrained from tobacco and caffeine-rich beverages 1 h before the data collection.

The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans, and was approved by the university's ethics committee at the University of Brasilia, Brazil (#059/2005).

2.2. Ayahuasca

We obtained a 2 l standard sample of the brew prepared by members of the Santo Daime community consisting of the stalks of *Banisteriopsis caapi* Spruce ex Grisebach (Malpighiaceae) combined with the washed leaves of *Psychotria viridis* Ruíz et Pavón (Rubiaceae), boiled and concentrated for several hours. All the ayahuasca used in the study was from this first sample.

To each liter of ayahuasca were added 70 g of artificial grape juice (®*Fresh*, Kraft Foods Brazil S.A.), 3 ml of cherry essence (®*Saborfort*, Mix Industry of Food Products LTDA., Brazil) and 3 ml of saccharin- and cyclamate-based artificial sweetener (®*Finn*, Boehringer Ingelheim Brazil). This procedure was designed to disguise the distinctive flavor, odor and color of ayahuasca in order to blind the volunteers regarding whether they received ayahuasca or vehicle-control.

The vehicle-control solution consisted of the same admixtures, while mineral water was used instead of ayahuasca. This solution was used only in the pre-treatment session.

In order to give the control solution the flavor of ayahuasca, to minimize the probability of solution identification by the participants of the study, 60 ml/l of ayahuasca were added to the vehicle-control solution used in the experimental sessions. As the doses used during the study were established in 50 ml, each individual dose of this ayahuasca-flavored solution contained approximately 3 ml of ayahuasca.

Solutions were administered in a double-blind manner.

2.3. Chemical analysis

A 500 ml sample of the ayahuasca used in the present study was assayed for the concentration of relevant alkaloids. This analysis was performed using gas chromatography/mass spectrometry (GC/MS) with the following instruments: chromatograph Agilent Technologies 6890N, mass selective detector (operating at 70 eV) Agilent Technologies 5973-Inert (500-40 *m/z*), automatic injector Agilent Technologies 7683B Series equipped with a DB-5ms column (0.2 mm × 25 m × 0.33 μm). Initial temperature was 200 °C, while the final was 300 °C. The ramp rate was 50 °C/min initiated 0 min after the injection of the sample. Injector temperature was 280 °C. About 0.2 μl of ayahuasca were injected.

2.4. Psychometric instruments

As opposed to the relative lack of physiological measures of motivation, psychometric research has developed several scales to evaluate the explicit motivational system. We used three scales to evaluate anxiety: one to evaluate state, or current, anxiety (anxiety-state), one to evaluate more persistent traits of anxiety (anxiety-trait), and one to evaluate panic.

State- and trait-anxiety were measured by the state–trait-anxiety inventory (STAI), previously translated and validated for a Brazilian sample. The STAI quantifies state-anxiety (A-state), which fluctuates in intensity throughout time. State-anxiety is to be distinguished from, trait-anxiety (A-trait), characterized by more persistent nature of one's reactivity to the environment.

In order to evaluate panic-like related states, the anxiety sensitivity index (ASI-R) was used, a Brazilian version that is still in the validation phase. The concept of anxiety sensitivity relates to the *fear* of feeling anxious; in other words, the belief that the somatic anxiety symptoms may have disastrous consequences. A person with high scores in anxiety sensitivity, for example, will more likely interpret somatic anxiety symptoms such as palpitations, dizziness, nausea and sweating as indicative of a highly pathological process, compared to a person that is less sensitive to these symptoms. Several studies have showed that this anxiety sensitivity index is closely related to the diagnosis of panic disorder.

In order to evaluate hopelessness related symptoms the Beck hopelessness scale (BHS) was used. This instrument is composed of statements regarding thoughts and beliefs about the future. These items measure three aspects of hopelessness: pessimism about the future, loss of motivation, and negative expectations. This hopelessness construct operates in many mental disorders and is highly correlated with measures of clinical depression and suicidal ideation.

2.5. Study design and experimental procedure

Initially, a general description of the study was made to the members of the Santo Daime cult, including information about the objectives of the research and methods to be used.

Volunteers were enrolled through the signing of a written informed consent. Baseline, pre-treatment, evaluations were then performed to assess state-anxiety, trait-anxiety, state panic, and depression, using a ritual developed exclusively for this study. During this 1 h ceremony the study participants remain seated chanting religious hymns, and the inactive vehicle-control solution is administered in a non-blind manner. We designed this procedure, which is essentially a Santo Daime ceremony

without active ayahuasca, in order that the participants could experience the taste, odor and color of the vehicle-control solution.

The inactive vehicle solution was administered to all participants at the beginning of the ritual. One hour after the consumption of this solution the questionnaires were distributed to each participant in a randomized fashion and participants were then instructed as how to fill out the rating scales. Since ayahuasca can produce effects in different times from person to person, this randomized method guarantees more veracity to the results when the participants are under the effects of ayahuasca in the experimental sessions, because each questionnaire will be answered in distinct moments. We chose this 1 h point after the consumption of the solution because the most intense effects of ayahuasca occur between 60 and 120 min (Riba et al., 2001).

The following week the first experimental session took place, using the same methodology and structure of the pre-treatment session. However, in this session, five participants took the full-ayahuasca solution and four took the ayahuasca-flavored solution in a double-blinded manner. Questionnaires were distributed similarly as in the pre-treatment session, and one of the authors briefly reminded subjects of the correct procedure to fill out the questionnaires.

A week later, the second experimental session took place in which those who had received the full-ayahuasca solution took the ayahuasca-flavored solution, and those who had consumed the ayahuasca-flavored solution took the full-ayahuasca solution.

The solutions were in opaque plastic cups in order to further disguise the appearance of each solution. Each volunteer was assigned a number by which he or she would know which cup to take from a table upon which the cups were placed.

2.6. Statistical analysis

The results were initially evaluated by a *t*-test to verify an order ingestion effect upon any of the outcome measures. Then, *t*-test was also employed to examine any treatment (vehicle × ayahuasca) effect. We required a significance level of $P < 0.05$ to be considered statistically significant.

3. Results

3.1. Chemical analysis

The analysis showed the presence of the beta-carbolines harmine, tetrahydroharmine (THH), harmaline, harmol, and *N*,*N*-dimethyltryptamine (DMT) (Fig. 1). These findings are consistent with the data from previous investigations that found harmine, tetrahydroharmine, harmaline, and *N*,*N*-dimethyltryptamine as the mains constituents of ayahuasca (McKenna et al., 1984; Callaway et al., 1999). Our identification of harmol confirms previous reports of the presence of trace concentrations of other beta-carbolines in ayahuasca (McKenna et al., 1998; Riba et al., 2003).

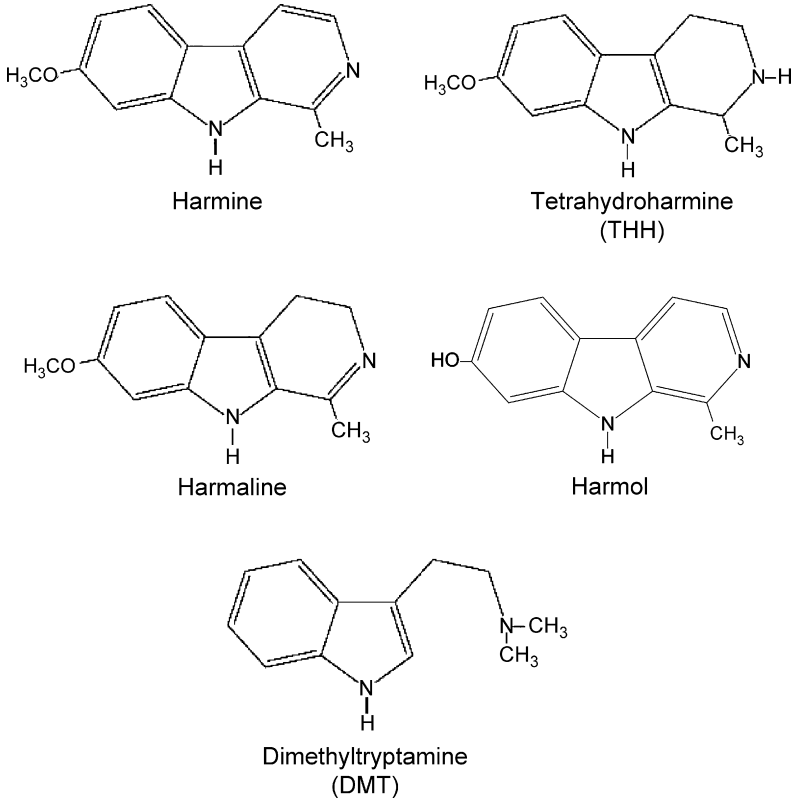


Fig. 1. Chemical analysis by gas chromatography/mass spectrometry (GC/MS) of the ayahuasca preparation used in this study.

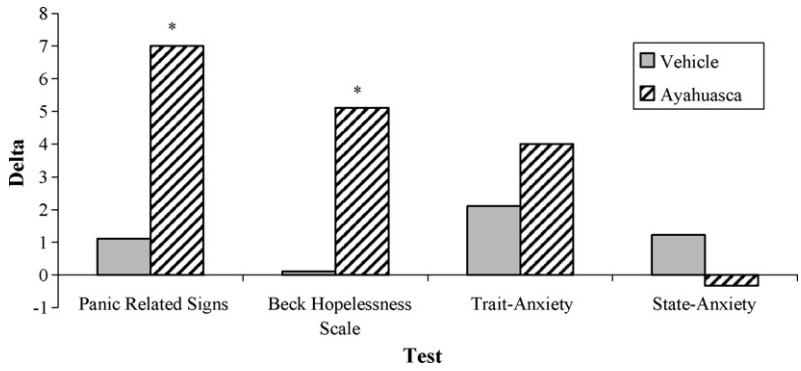


Fig. 2. Mean of changing scores from baseline to experimental sessions across the four different test. Participants ($n = 9$) ingested the vehicle-control solution (ayahuasca-flavored solution) or the full-ayahuasca solution. Asterisk (*) indicates a statistically difference between vehicle and ayahuasca treatments with a significance level of $P < 0.05$.

3.2. Psychometric measures

t-Test revealed that there was no significant effect of order of ingestion upon any outcome measures [panic-like related signs: vehicle ($t(7) = 0.24$; $P = 0.8$), ayahuasca ($t(7) = 0.46$; $P = 0.7$); Beck hopelessness scale: vehicle ($t(7) = 0.04$; $P = 0.9$), ayahuasca ($t(7) = 1.31$; $P = 0.1$); trait-anxiety: vehicle ($t(7) = 0.91$; $P = 0.3$), ayahuasca ($t(7) = 0.47$; $P = 0.7$); state-anxiety: vehicle ($t(7) = 1.24$; $P = 0.1$), ayahuasca ($t(7) = 0.98$; $P = 0.3$)]. Therefore data from the first and the second experimental sessions were pooled together. Moreover, statistical analyses were performed in change values, in which each score test from baseline was subtracted from those resulting from vehicle and from those resulting from ayahuasca and then compare these change scores to each other. A positive value indicates a reduction in the score whereas a negative value indicates an increase in the score from baseline to the experimental session.

Fig. 2 presents the mean change across the four measures. *t*-Test revealed a statistical significant change in panic-like related signs ($t(8) = 7.84$; $P < 0.001$) and Beck hopelessness scale: vehicle ($t(8) = 6.20$; $P < 0.001$), but no significant changes in trait-anxiety ($t(8) = 1.01$; $P = 0.2$) or state-anxiety vehicle ($t(8) = 0.64$; $P = 0.5$).

4. Discussion and conclusions

The present study investigated the effects of ayahuasca in anxiety, hopelessness and panic-like measures through a double-blind protocol in Santo Daime members. Acute ingestions of the brew significantly attenuated hopelessness and panic-like parameters as detected by BHS and ASI-R psychometric scales, respectively.

In accordance with previously reported studies (McKenna et al., 1984; Callaway et al., 1999), our sample of ayahuasca revealed the presence of the beta-carbolines harmine, THH and harmaline, and the tryptamine *N*,*N*-dimethyltryptamine (DMT). These chemical constituents are structurally similar to serotonin (McKenna et al., 1998; Callaway et al., 1999), exhibit great affinity for 5-$HT_{2A/2C}$ receptor subtypes (Smith et al., 1998; Grella et al., 2003) and exert an indirect agonist action in the serotonergic system. For example, THH selectively inhibits serotonin reuptake, while also inhibiting MAO-A (McKenna et al., 1998; Frecska et al., 2004). Harmine and harmaline reversibly inhibit the MAO-A and thus raise central levels of noradrenalin and serotonin. Also, it has been further indicated that these beta-carbolines preferentially inhibit MAO-A, the form of enzyme in which serotonin, and presumably other tryptamines, including DMT, are the preferred substrates in the brain (McKenna et al., 1984, 1998).

Taking into account this indirect agonist action of ayahusca at the monaminergic system, including the noradrenergic but preferentially the serotonergic system, we might speculate that the reduction of BHS and ASI-R psychometric parameters observed in the present study were mediated at least in part by this mechanism. This suggestion is supported by the fact that the majority of antidepressant/antipanic drugs also enhance noradrenergic and serotonergic functions, either inhibiting noradrenalin and serotonin reuptake, such as the tricycle antidepressants, or selectively inhibiting serotonin reuptake, such as the widely used selective serotonin reuptake inhibitors.

It also is possible that DMT, a 5-$HT_{2A/2C}$ agonist that exerts an effect similar of serotonin itself (Smith et al., 1998), could attenuate panic-like parameters since 5-HT_2 receptor activation in the dorsal periaqueductal grey has been associated to an alleviation of panic symptoms (Deakin and Graeff, 1991; Graeff et al., 1996). However, it is important to note that none of our volunteers had panic disorders or pathological depression, so clinical implication of these findings should be analyzed with caution.

In contrast to significant effect of ayahuasca in the BHS and ASI-R psychometric scales, the brew did not affect state- and trait-anxiety as assessed by STAI. To this respect, we must consider that our volunteers, all quite experienced in the use of ayahuasca, and all long-term members of the Santo Daime church, may have begun this study with low levels of anxiety. Thus, there was little room for change in a relatively low-anxiety group, and any putative anxiolytic effect of ayahuasca would not be apparent when pre-treatment levels were so low to begin with. Further studies might compare anxiolytic effects in ayahuasca-naïve volunteers. Besides, the complexity of ayahuasca's pharmacology, acting simultaneously upon other

different neurotransmitter systems, selectively affecting different subtypes of serotoninergic receptors, located throughout the brain, could also explain the absence of effects of ayahuasca on state- and trait-anxiety. Some receptors may have anxiogenic effects, while others could be anxiolytic, neutralizing the action of one against the others.

Considering that the volunteers, as members of the Santo Daime cult, believe that participation in the rituals, using ayahuasca, is helpful, the psychological changes observed in this study should be interpreted with caution. The acute psychological effects of ayahuasca can include euphoria, visions, new insights and even mystical experiences, all with potential beneficial effects, especially with people that have organized their live around the religious ceremonies with the brew.

Nevertheless, even if someone is not a member of a religious group that use ayahuasca, those profound psychological effects, mediated somehow by the pharmacology of ayahuasca, might by their own merit affect the psychological changes described. This line of reasoning, although considering the extrapharmacological variables, suggests that the brew, as solely a pharmacological agent, can produce beneficial effects on mood and anxiety.

Although this study investigated acute effects of the brew in those using it for at least 10 consecutive years, it is worth speculating that these positive effects may be applicable to the larger population. Many of the commonly prescribed and effective anxiolytic, anti-panic and antidepressant drugs have the same mechanisms of action as those of ayahuasca. In addition, the psychological effects of ayahuasca may have their own set of beneficial properties.

The possible therapeutic use of substances like ayahuasca also must take into account extra-pharmacological variables, usually referred to as *set* and *setting*. *Set* contains the motivation, expectation, and preparation of the individual, as well as his or her biology and personality. The *setting* is the environment, social and interpersonal, within which the person's experience takes place; it subsumes, as well, the personalities of the people administering the compound of interest. From a quality control and experimental consistency point of view, one also must consider the purity and consistency of the drug itself, and dose effects.

Careful consideration of these variables and their optimal management will be necessary to maximize the therapeutic potential and minimize adverse sequelae associated with hallucinogenic substances (Strassman, 1984; Grof, 2001).

Acknowledgements

This work was made possible by the support from Fernando de la Rocque Couto and other members of the Centro Eclético da Fluente Luz Universal Alfredo Gregório de Melo (CEFLAG), who provided the ayahuasca and participated in the study. We also wish to thank Vitor Augusto Motta Moreira for his help in the research project, Adriano Maldaner, who did the chemical analyses of our ayahuasca sample, and Jordi Riba for his critical comments on the manuscript. Research supported grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) to A.P.M. Cruz and J. Landeira-Fernandez. R.G. Santos was recipient of a master fellowship from CNPq.

References

Araújo, W.S., 1999. Navegando sobre as ondas do Daime: história, cosmologia e ritual da Barquinha. Ed. da Unicamp, São Paulo.

Callaway, J.C., 1988. A proposed mechanism for the visions of dream sleep. Medical Hypotheses 36, 119–124.

Callaway, J.C., Airaksinen, M.M., McKenna, D.J., Brito, G., Grob, C.S., 1994. Platelet serotonin uptake sites increased in drinkers of *ayahuasca*. Psychopharmacology 116, 385–387.

Callaway, J.C., McKenna, D.J., Grob, C.S., Brito, G.S., Raymon, L.P., Poland, R.E., Andrade, E.N., Andrade, E.O., Mash, D.C., 1999. Pharmacokinetics of Hoasca alkaloids in healthy humans. Journal of Ethnopharmacology 65, 243–256.

Davis, W., 1997. One River: Explorations and Discoveries in the Amazon Rain Forest. Simon and Schuster Inc., Touchstone, New York.

Deakin, J. F.W., Graeff, F.G., 1991. 5-HT and mechanisms of defense. Journal of Psychopharmacology 5, 305–315.

Frecska, E., White, K.D., Luna, L.E., 2004. Effects of ayahuasca on binocular rivalry with dichoptic stimulus alternation. Psychopharmacology 173, 79–87.

Goulart, S.L., 2005. Contrastes e continuidades em uma tradição religiosa amazônica: os casos do Santo Daime, da Barquinha e UDV. In: Labate, B.C., Goulart, S.L. (Orgs.), O uso ritual das plantas de poder. Mercado de Letras, Campinas, pp. 355–396.

Graeff, F.G., Guimarães, F.S., de Andrade, T.G., Deakin, J.F.W., 1996. Role of 5-HT in stress, anxiety and depression. Pharmacology Biochemistry and Behavior 54, 129–141.

Grella, B., Teitler, M., Smith, C., Herrick-Davis, K., Glennona, R.A., 2003. Binding of beta-carbolines at 5-HT_2 serotonin receptors. Bioorganic and Medicinal Chemistry Letters 13, 4421–4425.

Grob, C.S., McKenna, D.J., Callaway, J.C., Brito, G.S., Neves, E.S., Oberlaender, G., Saide, O.L., Labigalini, E., Tacla, C., Miranda, C.T., Strassman, R.J., Boone, K.B., 1996. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brasil. Journal of Nervous and Mental Disease 184, 86–94.

Grof, S., 2001. LSD psychotherapy. Multidisciplinary Association for Psychedelic Studies (MAPS). Sarasota, Florida.

Labate, B.C., Araújo, W.S. (Orgs.), 2004. O uso ritual da ayahuasca. Mercado de Letras, Campinas.

Luna, L.E., 2005. Narrativas da alteridade: a ayahuasca e o motivo de transformação em animal. In: Labate ,B.C., Goulart, S.L. (Orgs.), O uso ritual das plantas de poder. Mercado de Letras, Campinas, pp. 333–354.

McKenna, D.J., Towers, G.H.N., 1981. Ultra-violet mediated cytotoxic activity of beta-carboline alkaloids. Phytochemistry 20, 1001–1004.

McKenna, D.J., Towers, G.H.N., Abbott, F., 1984. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of Ayahuasca. Journal of Ethnopharmacology 10, 195–223.

McKenna, D.J., Repke, D.B., Lo, L., Peroutka, S.J., 1990. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. Neuropharmacology 29, 193–198.

McKenna, D.J., Callaway, J.C., Grob, C.S., 1998. The scientific investigation of Ayahuasca: a review of past and current research. The Heffter Review of Psychedelic Research 1, 65–77.

Nash, J.R., Nutt, D.J., 2005. Psychopharmacoterapy of Anxiety. Handbook of Experimental Pharmacology, Vol. 169, pp. 401–469.

Ott, J., 1994. Ayahuasca Analogues: Pangaean Entheogens. Natural Books Co., Kennewick, WA.

Riba, J., Rodrígues-Fornells, A., Urbano, G., Morte, A., Antonijoan, R., Monteiro, M., Callaway, J.C., Barbanoj, M.J., 2001. Subjective effects and tolerability of the South American psychoactive beverage *Ayahuasca* in healthy volunteers. Psychopharmacology (Berl) 154, 85–95.

Riba, J., Valle, M., Urbano, G., Yritia, M., Morte, A., Barbanoj, M.J., 2003. Human pharmacology of ayahuasca: subjective and cardiovascular effects,

monoamine metabolite excretion, and pharmacokinetics. Journal of Pharmacology and Experimental Therapeutics 306, 73–83.

Smith, R.L., Canton, H., Barret, R.J., Sanders-Bush, E., 1998. Agonist properties of *N*,*N*-dimethyltryptamine at 5-HT_{2A} and 5-HT_{2C} serotonin receptors. Pharmacology Biochemistry and Behavior 61, 323–330.

Starcevic, V., 2006. Anxiety states: a review of conceptual and treatment issues. Current Opinion in Psychiatry 19, 79–83.

Strassman, R.J., 1984. Adverse reactions to psychedelic drugs: a review of the literature. The Journal of Nervous and Mental Disease 172, 577–595.

Strassman, R.J., 2001. DMT: The Spirit Molecule. Park Street Press, Rochester, Vermont.

Wikinski, S., 2004. Depression and anxiety: from clinic to pharmacological treatment. Vertex 15, 208–212.

Psychopharmacology (2008) 196:315–326
DOI 10.1007/s00213-007-0963-0

ORIGINAL INVESTIGATION

Daytime *Ayahuasca* administration modulates REM and slow-wave sleep in healthy volunteers

Manel J. Barbanoj · Jordi Riba · S. Clos · S. Giménez · E. Grasa · S. Romero

Received: 2 May 2007 / Accepted: 19 September 2007 / Published online: 21 November 2007

Abstract
Objectives Ayahuasca is a traditional South American psychoactive beverage and the central sacrament of Brazilian-based religious groups, with followers in Europe and the United States. The tea contains the psychedelic indole *N*, *N*-dimethyltryptamine (DMT) and β-carboline alkaloids with monoamine oxidase-inhibiting properties that render DMT orally active. DMT interacts with serotonergic neurotransmission acting as a partial agonist at 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor sites. Given the role played by serotonin in the regulation of the sleep/wake cycle, we investigated the effects of daytime *ayahuasca* consumption in sleep parameters.
Measurements and results Subjective sleep quality, polysomnography (PSG), and spectral analysis were assessed in a group of 22 healthy male volunteers after the administration of a placebo, an *ayahuasca* dose equivalent to 1 mg DMT kg^{-1} body weight, and 20 mg *d*-amphetamine, a proaminergic drug, as a positive control. Results show that *ayahuasca* did not induce any subjectively perceived deterioration of sleep quality or PSG-measured disruptions of sleep initiation or maintenance, in contrast with *d*-amphetamine, which delayed sleep initiation, disrupted sleep maintenance, induced a predominance of 'light' vs 'deep' sleep and significantly impaired subjective sleep quality. PSG analysis also showed that similarly to *d*-amphetamine, *ayahuasca* inhibits rapid eye movement (REM) sleep, decreasing its duration, both in absolute values and as a percentage of total sleep time, and shows a trend increase in its onset latency. Spectral analysis showed that *d*-amphetamine and *ayahuasca* increased power in the high frequency range, mainly during stage 2. Remarkably, whereas slow-wave sleep (SWS) power in the first night cycle, an indicator of sleep pressure, was decreased by *d*-amphetamine, *ayahuasca* enhanced power in this frequency band.
Conclusions Results show that daytime serotonergic psychedelic drug administration leads to measurable changes in PSG and sleep power spectrum and suggest an interaction between these drugs and brain circuits modulating REM and SWS.

Keywords *Ayahuasca* · *d*-amphetamine · Polysomnograpy · Spectral analysis · Subjective evaluations · Healthy volunteers

Part of the results were presented in abstract form at the XIV Reunión Anual de la Asociación Ibérica de Patología del Sueño (AIPS), Almería, Spain, May 26–28, 2005 (Vigilia-Sueño 2005; 17: 49).

M. J. Barbanoj · J. Riba · S. Clos · S. Giménez · E. Grasa · S. Romero
Centre d'Investigació del Medicament, Institut de Recerca, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

M. J. Barbanoj
Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Barcelona, Spain

S. Romero
Centre de Recerca en Enginyeria Biomèdica, Departament ESAII, Universitat Politècnica de Catalunya, Barcelona, Spain

M. J. Barbanoj (✉)
Centre d'Investigació del Medicament, Hospital de la Santa Creu i Sant Pau, Sant Antoni M. Claret, 167, 08025 Barcelona, Spain
e-mail: mbarbanoj@santpau.es

Introduction

Ayahuasca is a psychotropic plant concoction that contains the psychedelic indole *N*,*N*-dimethyltryptamine (DMT) and has mind-modifying properties that have played a central role in shaping the world view of the indigenous peoples of the Amazon (Schultes and Hofmann 1987). The tea is commonly obtained by infusing the stems of the *Banisteriopsis caapi* liana together with the leaves of the *Psychotria viridis* bush, and it is used in traditional medicine and magico-religious practices (Schultes and Hofmann 1987). In recent times, several synchretic religions known generically as '*ayahuasca* churches' have appeared in Brazil, blending Christian beliefs with the sacramental use of *ayahuasca*. These congregations have contributed significantly to the introduction of *ayahuasca* use to the United States and Europe. The US Supreme Court recently ruled in favor of allowing one of these churches, the *União do Vegetal*, the religious use of *ayahuasca* among its members, a status analogous to that held by the Native American Church for the use of peyote (*Lophophora williamsii*).

Research into *ayahuasca* has shown that the tea combines the orally labile DMT from *P. viridis* with monoamine oxidase (MAO)-inhibiting β-carboline alkaloids (Buckholtz and Boggan 1977b) from *B. caapi* in a single preparation. Remarkably, these β-carbolines block the metabolic breakdown of DMT by the visceral MAO, allowing its access to systemic circulation (Riba et al. 2003). In the central nervous system, *ayahuasca* interacts mainly with serotonergic neurotransmission, with DMT acting as a partial agonist at the 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor sites (Deliganis et al. 1991).

In previous studies conducted by our group to characterise the pharmacology of *ayahuasca*, this tea showed a psychedelic and stimulatory profile compatible with 5-HT_{2A} agonism and pro-aminergic effects. Self-report questionnaires demonstrated somatic, perceptual and cognitive modifications, together with increases in positive mood and activation (Riba et al. 2003). Electroencephalography measures during wakefulness indicated reductions in slow (delta and theta) and alpha-2 activity (Riba et al. 2002) and measures of regional cerebral blood flow showed increased perfusion in paralimbic and frontal brain regions (Riba et al. 2006).

Sleep disturbances have been described for recreational drugs acting on serotonergic neurotransmission, such as 3,4 methylenedioxymethamphetamine (Morgan 2000). Serotonin plays a prominent role in the regulation of sleep–wake cycles. Both the firing of serotonergic neurons in the raphe nuclei and serotonin release increase during waking, decrease in non-REM sleep and are absent during the REM stage (McGinty and Harper 1976). This regulation mechanism is very complex and involves the interplay of several serotonin receptor subtypes, with 5-HT_{1A} agonism suppressing REM sleep (Driver et al. 1995), 5-HT_{2A}, 5-HT_{2C} antagonism enhancing slow-wave sleep (SWS) (Landolt et al. 1999) and 5-HT_{2C} agonism decreasing SWS (Katsuda et al. 1993).

In the present study, we investigated possible sleep disturbances associated with acute daytime *ayahuasca* administration. The sleep effects of daytime *ayahuasca* administration were compared vs placebo and vs *d*-amphetamine as a positive control. *d*-Amphetamine shares with *ayahuasca* stimulatory properties and an inhibitory effect on the waking EEG slow activity (delta and theta) but it lacks the psychedelic effects associated with serotonergic 5-HT_{1A} and 5-$HT_{2A/2C}$ agonism. Based on the arousing effects of *ayahuasca*, we postulated that a deterioration of sleep initiation, maintenance and subjective quality would be observed, similar to that previously reported for *d*-amphetamine (Saletu et al. 1989). However, given the pharmacological mechanism responsible for the psychedelic effects of DMT, i.e., 5-HT_{2A} agonism, we also postulated that *ayahuasca* would modulate REM and slow-wave sleep.

Materials and methods

Volunteers

Twenty-two young healthy male volunteers were recruited. Mean age was 27.1 years (range 20–38), mean weight was 67.6 kg (range 60–85) and mean height was 1.77 m (range 1.60–1.90). Eligibility criteria included prior use of psychedelics on at least ten occasions without sequelae derived thereof. The most frequently consumed psychedelics were LSD and psilocybian mushrooms. All volunteers underwent a structured psychiatric interview (DMS-IV). Exclusion criteria included current or past history of Axis-I disorders and alcohol or other substance dependence. A medical examination and laboratory tests were performed before study initiation. All participants were screened for sleep disturbances (Pittsburg sleep quality index < 5). Pre-study examinations also included drug screening and serological testing (for hepatitis B and C and HIV). The alcohol, coffee and cigarette consumption reported in all volunteers was insufficient to lead to withdrawal symptoms during the 48-h period around the experimental session where consumption was not allowed (≤39 g absolute alcohol/day, ≤100 mg caffeine/day, ≤5 cigarettes/day). No strenuous physical exercise or naps were allowed in the 24 h before each experimental session or in the ensuing 24 h. Participants were requested not to take any medications or illicit drugs during the study and were asked to keep regular sleep–wake habits during the fortnight before the study and

during the study proper. Urine was tested for illicit drug use on each experimental session.

The study was approved by the local Ethics Committee and the Spanish Ministry of Health, and was conducted following the principles stated in the Declaration of Helsinki and the guidelines of Good Clinical Practice. All volunteers gave their written informed consent to participate.

Drugs

The administered drugs were a placebo, 20 mg *d*-amphetamine and a freeze-dried encapsulated formulation of *ayahuasca* equivalent to 1 mg DMT/kg body weight. One gram of lyophilizate contained: 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg tetrahydroharmine (THH) per gram. The calculated individual dose of *ayahuasca* for each volunteer was administered by combining gelatine capsules (00 size) containing 0.5, 0.25 or 0.125 g of lyophilizate. Volunteers received the same number of capsules on each experimental session.

Study design

The study was conducted according to a randomized, double-blind, placebo-controlled, cross-over design and involved participation in three experimental sessions at least 1 week apart, plus an adaptation night in the sleep laboratory. The adaptation night was used to familiarize the participants with the laboratory and the recording procedures and was held within the 2 weeks before the first experimental session. Data from this adaptation night were not included in the statistical analyses. On each of the three experimental sessions, volunteers arrived in the laboratory at 7:00 A.M. and had a light breakfast before 10:00 A.M. At 12:00 noon, they received the capsules containing one of the three treatments and they then remained in the laboratory until 12:00 the next day. Volunteers were constantly supervised and they were not allowed to nap. Sleep recordings were conducted from 11:00 P.M. of the experimental session until 7:00 A.M. of the next day. Data from these three nights were subsequently submitted to statistical analysis. Additionally, to measure pattern and intensity of the acute subjective effects induced by *ayahuasca* during the day, participants responded self-report questionnaires before and after 4 h of drug administration (see below).

Subjective effect measures

Self-rated subjective effects were measured by administering Spanish versions of the Hallucinogen Rating Scale or HRS (Riba et al. 2001) and the Addiction Research Center Inventory or ARCI (Lamas et al. 1994). The HRS measures psychedelic-induced subjective effects and includes six scales: *somaesthesia*, reflecting somatic effects; *affect*, sensitive to emotional and affective responses; *volition*, indicating the volunteer's capacity to willfully interact with his/her 'self' and/or the environment; *cognition*, describing modifications in thought processes or content; *perception*, measuring visual, auditory, gustatory and olfactory experiences; and finally *intensity*, which reflects the strength of the overall experience. The range of scores for all HRS scales is 0 to 4. The ARCI consists of five scales or groups: MBG, morphine-benzedrine group, measuring euphoria and positive mood; PCAG, pentobarbital-chlorpromazine-alcohol group, measuring sedation; LSD, lysergic acid diethylamide scale, measuring somatic-dysphoric effects; BG, the benzedrine group, measuring intellectual energy and efficiency, and the A scale, an empirically derived scale measuring amphetamine-like effects. The range of scores is 0 to 16 for MBG, −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG and 0 to 11 for A. Volunteers answered the ARCI immediately before drug administration, and 4 h after drug intake, whereas the HRS was only answered at 4 h post-administration.

Before statistical analysis, ARCI scores were transformed to differences from pre-administration values. The transformed ARCI scores and raw HRS scores were analyzed by means of multivariate and univariate tests as indicated below.

Recordings and sleep stage classification

Sleep recordings were performed in individual, sound-attenuated, temperature-regulated rooms and volunteers were supervised by qualified technical staff. Volunteers had dinner by 8.00 P.M. and started sleep procedures. The total time in bed was fixed at 8 h. The lights were turned off around 11.00 P.M. and turned on around 7 A.M. the next morning. In addition, the volunteers completed a self-rating scale on the subjective quality of sleep and awakening no later than 15 min after having woken up each morning (Saletu et al. 1987).

Data were acquired by means of either (1) the Coherence 32E-Deltamed system, or (2) the SleepLab-Aequitron Medical Recordings system; the same system was used for each volunteer. Recordings consisted of six EEG-channels (Fp1, Fp2, C3, C4, O1, O2, vs the average of both mastoids (A1-A2) according to the 10/20 International System), two electro-oculographic leads (EOG) (right and left, registered between both external canthi, with a capacity to detect ocular movements in both directions: horizontal and vertical), and one chin electromyographic channel (EMG), consisting of two electrodes placed on the submentonian muscles, which monitored the muscular tone.

Three channels were included to monitor the respiratory function: one for airflow signal (by means of a thermistor placed in the path of airflow from nose and mouth) and two channels to record rib cage and abdominal motion (by calibrated transducers). Finally, two channels (linked electrodes on the left and right anterior tibialis) were used to register limb movements. EEG and EOG channels were filtered to a bandwidth of 0.1–75 Hz with a sensitivity of 10 μV/mm. EMG was filtered to a bandwidth of 10–75 Hz with a sensitivity of 5 μV/mm. A 50-Hz notch filter was used to attenuate electrical noise. The electrodes were gold-plated. Channels were calibrated before each recording and the electrode impedance was kept below 10 kΩ.

The sleep recordings were visually scored in a 30-s epoch resolution according to the traditional standard R&K criteria (Rechtschaffen and Kales 1968) using the View and Rate (Cdatentechnik GbR, © 1995–1999 3.02 version) program. Analysis was performed by two independent sleep scorers. Discrepancies were solved by a third expert from the same laboratory. Each scorer was blind to the other raters' analysis and treatment received by the participant.

Sleep variables were derived by visual scoring using standard criteria. Total sleep time (TST) is the amount of actual sleep time in the total sleep period (TSP); equal to TSP less wakefulness (stage 0). TSP is the total time available for sleep during an attempt to sleep and comprises NREM and REM sleep, as well as wakefulness; in addition to TST, TSP includes stage 0. The number of awakenings refers to the arousals to wakefulness during TSP. The sleep efficiency index (SEI) is the proportion of sleep in the recorded period, and is calculated by dividing TST by the total time in bed (TIB) and multiplied by 100. Sleep stages 1, 2, slow-wave sleep (3+4) (SWS) and REM are expressed in minutes and in percentages of the TST. Movement time is identified when more than a half of the scoring epoch is obscured because of movement but the preceding and subsequent epochs are of sleep. Movement time is expressed in minutes and in percentage of the TST. Latency to stages 1, 2 and 3 defines the period of time measured from lights out to the appearance of sleep stages 1, 2 and 3, respectively. REM latency is defined as clock time from the first epoch of stage 2 (followed by ≥8 min sleep in the next 10 min) to the first REM period of at least 3 min. In addition, the number of NREM and REM periods as well as the average duration of NREM and REM periods and of sleep cycles was computed. Consecutive NREM-REM cycles were defined according to modifications of the criteria proposed by Feinberg and Floyd (1979). NREM episodes were defined as starting with stage 2, containing at least 15 min of stages 2, 3 and 4, and being followed by REM episodes of at least 5-min duration. No minimal criterion for the REM duration was applied for the completion of the first cycle.

EEG power spectra

Despite its widespread use in visual scoring, the conventional R&K criteria (Rechtschaffen and Kales 1968) provides insufficient information about the continuity of sleep stages. All-night spectral analysis is a sensitive method for documenting pharmacological effects on sleep EEG (Borbély et al. 1985). It not only detects shifts between the various sleep stages during the night, but also takes into account the qualitative alterations of certain stages (Schlösser et al. 1998). To conduct spectral analysis, the EEG signal was high-pass (0.3 Hz) and low-pass (35 Hz) filtered before being converted from analogue to digital and the sampling frequency was 256 Hz. Power spectra of 5-s artefact-free epochs, weighted by a Hanning window, were computed using the Fast Fourier Transform and matched with the sleep scores. Epochs containing artefacts caused either by saturation or muscle activity were automatically identified and eliminated. Mean (±SEM) artefact-free recordings, in seconds, computed for the different experimental nights were: 5.245±50.3 after placebo, 5.335±30.9 after *ayahuasca*, and 5.024±88.2, after *d*-amphetamine, being 91.4, 93.1 and 87.7% of the corresponding TIB, respectively. Power density values (C4A1 derivation) were averaged into 0.4 Hz (0.2–6.0 Hz) and 0.8 Hz (6.2–26.0 Hz) bins. The spectra were calculated separately for non-REM sleep (NREMS stages: 1, 2, 3 and 4), stage 2 (S2), slow wave activity (SWS: stages 3 and 4) and REM sleep (REMS).

Dynamics of slow-wave activity, spindle frequency activity during whole night and delta EOG activity during REM sleep

Slow-wave activity (SWA) is defined as the power in the delta band (0.5–4.0 Hz) and spindle frequency activity (SFA) as the power in the sigma band (11.0–15.0 Hz) for the C4A1 derivation during whole night sleep. Delta EOG activity (DEA) is defined as the power in the delta band (0.5–4.0 Hz) for the EOG channels during REM sleep. These variables were computed from 5-s artifact-free epochs. DEA was used as a representative of phasic REM activity intensity. A moving average estimation of 5-min duration was computed to smooth the signal throughout the night.

To compensate for the individual differences in the occurrence and duration of the NREM–REM cycles, a method derived from that of Aeschbach and Borbély was used (Aeschbach and Borbély 1993). For SWA and SFA, each NREM period of unequal length was subdivided into 24 equal parts, and each REM period into four equal parts. They were then averaged across subjects.

For DEA, each REM period of unequal length was subdivided into 24 equal parts and averaged across subjects. Changes in the three dynamic activities were evaluated on the raw data by calculating areas under the curve (AUC) in each cycle.

Sleep and awakening quality self-rating scale

A Spanish version of the 'Self-assessment Scale for Sleep and Awakening Quality (SSA)' (Saletu et al. 1987) was used. The SSA consists of 20 items grouped in three categories: (1) SSA-1 (seven items), which evaluates subjective sleep quality; (2) SSA-2 (eight items), evaluating subjective awakening quality; and (3) SSA-3 (five items), evaluating the presence of somatic complaints. Responses are coded according to an ordinal scale with four possibilities (not at all, slightly, moderately and extremely). In the coding process, the values 1, 2, 3 or 4 are assigned in such a way that a higher score means a worse subjective quality, with the theoretical score ranging from: 20–80 for the global scale (SSA), 7–28 for SSA-1, 8–32 for SSA-2 and 5–20 for SSA-3. Furthermore, the questionnaire presents five additional open questions related to different moments of the night, from which subjective sleep latency (SSL) and subjective sleep efficiency (SSE) are calculated.

Statistical analysis

To decrease the risk of type I error, PSG variables were grouped in three different clusters comprising: (1) sleep initiation and maintenance variables; (2) sleep architecture variables; and (3) NREM–REM period variables. SSA variables were grouped in a fourth cluster. Each cluster and a subjective effect measures cluster obtained from the self-report questionnaires (HRS and ARCI combined) were subjected to a multivariate analysis of variance (MANOVA). Within each cluster, the MANOVA yielded the result of a general linear model with one within-subject factor (treatment: three levels) for each variable. Greenhouse-Geisser ε correction was used. Pairwise comparisons were conducted by means of repeated measures *t* tests corrected for multiple comparisons (Sidak).

To assess the effects on EEG power spectra at the different sleep stages and on the AUCs of its dynamics, repeated measures *t* tests were applied to each frequency bin or AUC, respectively, comparing placebo with each active treatment.

Differences were considered significant when the probability of type I error was less than 0.05 (two-sided).

Results

Final study population

Because of technical problems during the acquisition phase (electrode failure at some time point during the sleep recording), the data from four subjects had to be excluded from the analysis, leaving a final sample of 18 with a complete set of PSG recordings. No significant differences in demographic characteristics were observed between the initial and the final sample. All 18 participants completed the trial and were compliant with the study protocol, and all active treatments were well tolerated.

Subjective effect measures

Significant results were observed for subjective effects as measured by self-report questionnaires (Pillai's trace: 1.45; $F=5.98$, $df=22, 50$; $p<0.001$). Results for the individual subscales are shown in Table 1.

PSG variables

Sleep initiation and maintenance

Significant results were observed for sleep initiation and maintenance variables (Pillai's trace: 1.14; $F=3.10$, $df=20, 52$; $p=0.001$). Significant results were observed for all sleep latencies. *d*-Amphetamine induced significant increases in latencies to stage 1, stage 2, and REM compared to placebo and to *ayahuasca*. Additionally, it showed a trend to increase stage 3 latency. In contrast, *ayahuasca* only showed a tendency to increase REM latency compared to placebo (Table 2).

Sleep maintenance variables also showed significant results. *d*-Amphetamine induced significant decreases in TSP, TST and SEI and significant increases in stage 0, total wake time and awakenings/TSP as compared to either placebo or *ayahuasca*. *Ayahuasca* did not differ from placebo in any of these variables (Table 2).

Sleep architecture

Significant results were observed for sleep architecture variables (Pillai's trace: 1.28; $F=4.60$, $df=20, 52$; $p<0.001$). Duration of all sleep stages showed significant effects except for SWS in percentage of TST and movement time (in minutes and percentage of TST). Compared to placebo and *ayahuasca*, *d*-amphetamine induced significant decreases in REM as measured in minutes or as percentage of TST. Significant decreases in SWS and significant increases in stage 1 sleep were also seen after *d*-amphetamine but only when the former was

Table 1 Acute subjective effects induced by placebo, *ayahuasca* 1 mg DMT/kg and *d*-amphetamine 20 mg

	Placebo	*Ayahuasca*	*d*-Amphetamine	GLM (*p* values)	Adjusted for multiple paired comparisons: Sidak		
					PLA:AYA	PLA:AMP	AYA:AMP
HRS							
Somaesthesia	0.03 (0.07)	1.36 (0.73)	0.56 (0.44)	<0.001	***	***	***
Affect	0.33 (0.19)	1.43 (0.60)	0.72 (0.33)	<0.001	***	***	***
Perception	0.01 (0.04)	1.61 (0.89)	0.12 (0.17)	<0.001	***		***
Cognition	0.02 (0.07)	1.45 (1.07)	0.34 (0.35)	<0.001	***	**	***
Volition	0.78 (0.63)	1.77 (0.69)	0.88 (0.39)	<0.001	***		**
Intensity	0.06 (0.16)	2.25 (0.97)	1.10 (0.79)	<0.001	***	***	***
ARCI							
A	0.11 (1.18)	3.06 (1.59)	2.22 (1.93)	<0.001	***	**	
BG	0.33 (1.19)	−0.17 (1.95)	2.83 (2.77)	<0.001		**	**
MBG	0.06 (1.86)	3.11 (3.68)	3.33 (2.74)	0.008	*	**	
PCAG	−0.89 (3.83)	0.06 (3.28)	−3.72 (3.32)	0.007			*
LSD	0.06 (1.39)	4.61 (2.59)	2.50 (1.62)	<0.001	***	**	*

Means (SD) of the scores obtained for the HRS and ARCI questionnaires subscales (n=18), and results of the statistical analysis performed.
PLA placebo, *AYA ayahuasca*, *AMP d*-amphetamine, *A* amphetamine, *BG* benzedrine group, *MBG* morphine–benzedrine group, *PCAG* pentobarbital–chlorpromazine–alcohol group, *LSD* lysergic acid diethylamide scale
*p<0.05
**p<0.01
***p<0.001

measured in minutes and the latter in percentage. In addition, *d*-amphetamine produced significant effects on stage 2. An increase was found when measured in minutes, but when expressed as percentage, this difference was only observed in relation to placebo. *Ayahuasca*'s significant effects as compared to placebo consisted in decreases in REM (in minutes and percentage of TST). A tendency to increase stage 2 (in minutes and percentage of TST) was also observed (Table 3).

NREM–REM periods

Significant results were observed for NREM–REM period variables (Pillai's trace: 0.89; F=4.97, df=10, 62; p<0.001). The number of NREM and REM periods as well as the average duration of NREM periods and sleep cycles showed significant differences between treatments. Both active compounds, *d*-amphetamine and *ayahuasca*, decreased the number of periods and increased the duration

Table 2 Sleep initiation and maintenance after daytime administration of *ayahuasca* 1 mg DMT/kg and *d*-amphetamine 20 mg

	Placebo	*Ayahuasca*	*d*-Amphetamine	GLM (*p* values)	Adjusted for multiple paired comparisons: Sidak		
					PLA:AYA	PLA:AMP	AYA:AMP
Latency to stage 1 (minutes)	19.39±5.01	13.78±2.64	91.42±16.51	<0.001		***	***
Latency to stage 2 (minutes)	21.58±5.30	16.97±3.38	109.29±19.82	<0.001		***	***
Latency to stage 3 (minutes)	21.86±4.00	17.11±2.00	62.89±18.93	0.035			*
REM latency (minutes)	92.44±8.65	113.72±10.55	210.27±18.91	<0.001	*	***	**
Total sleep period (minutes)	458.72±5.35	463.56±2.95	361.06±22.22	<0.001		***	***
Total sleep time (minutes)	444.67±6.48	450.14±3.99	297.97±24.05	<0.001		***	***
Sleep efficiency (%)	92.52±1.37	93.87±0.83	61.94±4.98	<0.001		***	***
Wake (minutes)	19.67±5.05	14.42±2.79	116.58±21.79	<0.001		***	***
Stage 0 (minutes)	13.72±3.30	13.03±2.70	62.94±13.01	<0.001		**	***
Awakenings/TSP	3.01±0.73	2.81±0.59	18.48±4.12	0.001		**	**

Mean±SEM (n=18)
PLA Placebo, *AYA ayahuasca*, *AMP d*-amphetamine
*p<0.10
**p<0.01
***p<0.001

Table 3 Sleep architecture after daytime administration of *ayahuasca* 1 mg DMT/kg and *d*-amphetamine 20 mg

	Placebo	*Ayahuasca*	*d*-Amphetamine	GLM (*p* values)	Adjusted for multiple paired comparisons: Sidak		
					PLA:AYA	PLA:AMP	AYA:AMP
Stage 1 (minutes)	15.44±2.48	14.44±2.61	21.53±3.54	0.024			*
Stage 1 (%)	3.48±0.57	3.25±0.61	8.08±1.33	<0.001		****	****
Stage 2 (minutes)	239.58±9.22	261.83±8.37	180.39±17.60	<0.001	*	***	****
Stage 2 (%)	53.71±1.64	58.09±1.68	59.77±2.39	0.015	*	**	
SWS (minutes)	86.03±3.88	83.94±6.06	58.50±5.51	<0.001		***	****
SWS (%)	19.40±0.93	18.67±1.37	20.50±1.88	0.572			
REM (minutes)	98.28±4.57	82.42±4.14	33.42±5.48	<0.001	***	****	****
REM (%)	22.19±1.08	18.32±0.93	10.21±1.63	<0.001	***	****	****
Movement time (minutes)	5.81±0.94	4.61±0.68	4.14±0.82	0.061			
Movement time (%)	1.32±0.21	1.02±0.15	1.44±0.28	0.095			

All percentages are expressed relative to total sleep time (TST). Mean±SEM (n=18).
PLA Placebo, *AYA ayahuasca*, *AMP d*-amphetamine
*p<0.10
**p<0.05
***p<0.01
****p<0.001

of NREM periods and sleep cycles as compared to placebo. However, *d*-amphetamine's effects were significantly larger than those obtained after *ayahuasca* (Table 4).

Spectral analysis

EEG power spectra

The mean all-night power spectra are presented in Fig. 1. The values for nights after an active treatment are expressed as a percentage of the placebo night.

The NREM power spectrum showed differences as a function of the active compound. Compared to placebo, values were significantly higher after *d*-amphetamine in the high-frequency range (frequencies higher than 15 Hz), while after *ayahuasca*, significantly higher values were limited to the 15–20 Hz frequency band. The spectrum in stage 2 was very similar to that of NREM, but the SWA spectrum showed no significant differences between placebo and the active compounds. In REM, power density was significantly reduced in comparison to placebo after *d*-amphetamine in the 2.4–2.8 and 5.6–8.4 Hz frequency ranges.

Table 4 NREM-REM periods after daytime administration of *ayahuasca* 1 mg DMT/kg and *d*-amphetamine 20 mg

	Placebo	*Ayahuasca*	*d*-Amphetamine	GLM (*p* values)	Adjusted for multiple paired comparisons: Sidak		
					PLA:AYA	PLA:AMP	AYA:AMP
Number NREM periods	4.06±0.17	3.61±0.14	1.72±0.18	<0.001	*	***	***
Number REM periods	4.06±0.17	3.61±0.14	1.56±0.23	<0.001	*	***	***
Average duration NREM periods (minutes)	82.20±4.13	95.34±3.84	146.51±13.18	<0.001	**	***	**
Average duration REM periods (minutes)	26.46±1.39	29.64±3.44	19.94±3.30	0.051			
Average duration sleep cycles (minutes)	110.92±4.28	123.12±4.10	210.23±18.92	<0.001	*	***	***

Mean±SEM (n=18)
PLA Placebo, *AYA ayahuasca*, *AMP d*-amphetamine
*p<0.05
**p<0.01
***p<0.001

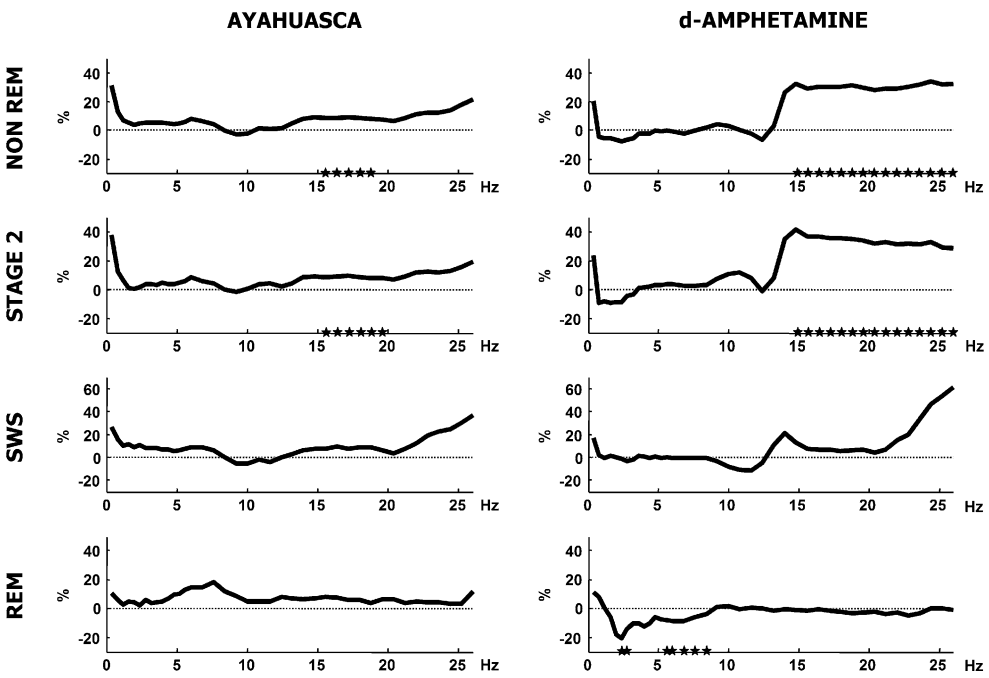


Fig. 1 EEG power density (C4A1-derivation) in non-REM sleep (stages 1, 2, 3 and 4) stage 2 (S2), slow-wave sleep (stages 3 and 4) and REM sleep after daytime administration of *ayahuasca* 1 mg DMT/kg and *d*-amphetamine 20 mg. For each frequency bin (n=18), means are expressed as a percentage of the corresponding value after placebo (*horizontal dashed lines* at 0%). *Asterisks at the bottom of the panels* indicate frequency bins which differ significantly from placebo (p<0.05, t test for repeated measures)

Dynamics of slow-wave activity

On all nights, either after placebo or active drugs, SWA was higher in NREM episodes and lower in REM episodes, and a declining trend was observed over consecutive NREM episodes. The dynamics of SWA throughout the night after all experimental interventions is presented in Fig. 2. A significant reduction in SWA after *d*-amphetamine relative to placebo was obtained in the first cycle (t=2.41, df=14, p=0.030). On the contrary, a trend to increase SWA between *ayahuasca* and placebo in the first cycle was also evidenced (t=1.82, df=17, p=0.086).

As it has been shown that the delta power in a cycle is dependent on the number of cycles in that night (Preud'homme et al. 2000), it has been proposed that to avoid a potential bias, cycle-by-cycle comparisons should be conducted on nights with the same number of cycles. As the number of sleep cycles was significantly affected by the active treatments, a reanalysis was performed incorporating only those volunteers who showed the same number of cycles after all three treatments. No such analysis could be performed after *d*-amphetamine as all subjects had a lower number of cycles in relation to placebo. However, eight subjects presented the same number of cycles after *ayahuasca* and placebo. In this subgroup of volunteers, SWA showed a significant increase in activity after *ayahuasca* as compared to placebo. This increase was clearly circumscribed to the first cycle (t=2.78, df=7, p=0.027).

Dynamics of spindle frequency activity

The typical pattern of SFA with low values in REM episodes, and a higher level, U-shaped pattern (with lowest values coinciding with highest values of SWA) in NREM episodes, was observed after all three treatments. No significant changes were observed in the amount of SFA between any active treatment and placebo (Fig. 2).

Dynamics of slow activity in EOG channels (DEA) during REM sleep

Slow activity in EOG channels during REM sleep did not show any significant changes when comparing the night after placebo with the nights after active compounds.

Subjective sleep and awakening quality

Significant results were observed for subjective sleep and awakening quality variables (Pillai's trace: 0.81; F=4.21, df=10, 62; p<0.001). No significant treatment effects were observed for somatic complaints. However, after *d*-amphetamine, there was a significant impairment of the global score and the subjective sleep quality score in comparison to placebo and *ayahuasca*. Significant effects were also obtained in subjective sleep latency and subjective sleep efficiency. *d*-Amphetamine induced an increase of the former and a decrease of the latter in comparison to placebo and *ayahuasca* (Table 5).

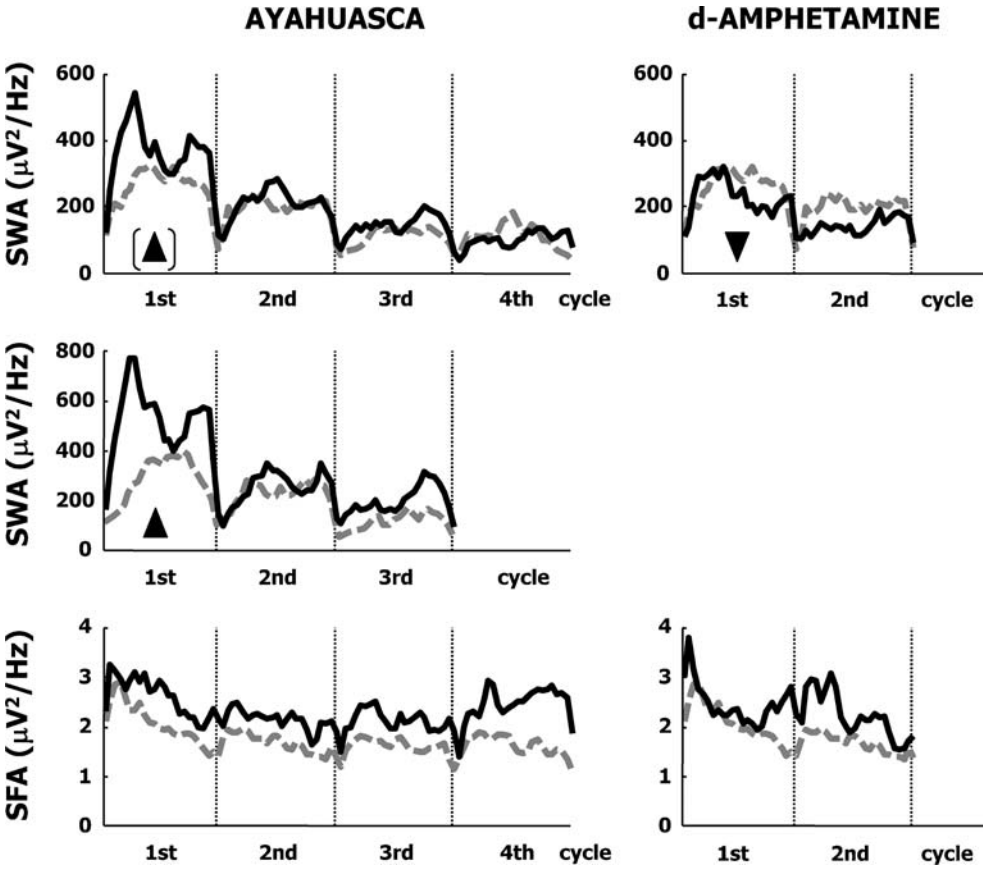


Fig. 2 Time course of EEG slow-wave activity (SWA, 0.5–4.0 Hz range; C4-A1-derivation) and spindle frequency activity (SFA, 11.0–15.0 Hz range; C4-A1-derivation) plotted after daytime administration of *ayahuasca* 1 mg DMT/kg and *d*-amphetamine 20 mg (*continuous curves*) against placebo (*dashed curves*). For each subject, individual NREM episodes were subdivided into 20 equal time bins. Data were averaged across subjects (n=18, for *ayahuasca* and n=14 for *d*-amphetamine) and plotted against the mean timing of NREM sleep. *Dashed vertical lines* delimit NREM episodes. *Triangles* indicate significant AUC increases or decreases in relation to placebo for a given NREM episode. *Ayahuasca* showed a trend increase in the first NREM episode (*t* test for repeated measures: p=0.086; *upper left panel*), which became significant (p=0.027) when the analysis was performed with only those volunteers who showed the same number of cycles after *ayahuasca* and placebo (n=8; *middle panel*)

Table 5 Subjective sleep and awakening quality after daytime administration of *ayahuasca* 1 mg DMT/kg and *d*-amphetamine 20 mg

	Placebo	*Ayahuasca*	*d*-Amphetamine	GLM (p values)	Adjusted for multiple paired comparisons: Sidak		
					PLA:AYA	PLA:AMP	AYA:AMP
SSA-T	34.67±1.22	33.72±1.02	41.44±1.60	<0.001		**	*
SSA-1	13.89±0.64	14.28±0.73	19.67±0.85	<0.001		**	**
SSA-2	15.28±0.76	13.94±0.50	16.11±0.85	0.047			
SSA-3	5.50±0.20	5.50±0.23	5.67±0.24	0.715			
SSL (minutes)	31.56±5.80	37.33±7.52	115.22±18.19	<0.001		*	*
SSE (minutes)	85.64±3.02	85.43±2.52	59.25±5.64	<0.001		**	**

Mean±SEM (n=18)

SSA Self-assessment scale for sleep and awakening quality: *T*=total score, *1* subjective sleep quality, *2* subjective awakening quality, *3* somatic complains; *SSL* subjective sleep latency, *SSE* subjective sleep efficiency, *PLA* placebo, *AYA ayahuasca*, *AMP d*-amphetamine

*p<0.01

**p<0.001

Discussion

Daytime drug administration in the present study caused significant psychotropic effects as measured by self-report questionnaires. *d*-Amphetamine showed a pattern typical of the psychostimulants with high scores in the ARCI-A, ARCI-BG and ARCI-MBG subscales (Martin et al. 1971). Similarly, *ayahuasca* showed significant effects in the ARCI-A and ARCI-MBG subscales, which measure amphetamine-like stimulatory effects and euphoria, respectively, but not in the ARCI-BG which measures intellectual efficiency. Both drugs induced somatic–dysphoric effects as measured by the ARCI-LSD scale. The most relevant differences between the two drugs were the significant increases in the HRS-perception and HRS-volition subscales observed for *ayahuasca* only and which reflect modifications in perception and increased impairment, respectively. The overall pattern of subjective effects induced by *ayahuasca* replicates results found by our group in a previous study (Riba et al. 2003).

Regarding sleep measures, *d*-amphetamine caused a clear deterioration of subjective sleep quality and objective sleep measures, delaying sleep initiation, disrupting sleep maintenance, increasing light sleep, and decreasing the duration of REM sleep and the number of non-REM and REM cycles. These effects are in line with the well-documented effects of amphetamine on sleep. This compound and its derivatives suppress REM sleep, and as the doses rise, vigilance is increased and sleep continuity is disturbed (Saletu et al. 1989).

Ayahuasca, on the other hand, did not induce a deterioration of sleep, and no significant effects on sleep initiation or maintenance variables were evidenced. However, like *d*-amphetamine, *ayahuasca* increased stage 2, decreased REM stage duration and showed a trend to increase REM latency. Furthermore, we observed decreases in the number of non-REM and REM periods and increases in the average duration of non-REM periods and sleep cycles, but these were of less magnitude than after *d*-amphetamine. Also in contrast with *d*-amphetamine, no subjectively measured deterioration was observed compared to placebo.

As evidenced by results in the present and prior (Riba et al. 2003) studies, psychedelics have the capacity to induce increases in activation that can be measured by self-report questionnaires and by EEG (Riba et al. 2002). We had thus postulated that both *ayahuasca* and *d*-amphetamine would impair sleep initiation and maintenance variables and would suppress REM. The mentioned trend to increase REM latency and the significant decrease in duration were in fact the only common effects observed by means of PSG. In this respect, it is worth mentioning that Gouzoulis et al. (1992) reported the complete suppression of REM after the nighttime administration of MDE, a compound with a chemical structure related to both amphetamines and psychedelic phenylethylamines.

To our knowledge, the sleep effects of pharmacologically closer compounds, i.e., the classical serotonergic psychedelics, have been studied in very few reports, most of which were published in the 1960s. Muzio et al. (1966) administered LSD in doses ranging from 0.08 to 73 μg/kg to 12 volunteers on a total of 36 nights and compared the data obtained with that from 69 control nights in the same subjects. The drug was administered orally just before sleep or after 1 h of sleep. These authors found LSD increased the duration of the first or second REM period. They also observed that when an abnormal excess of REM sleep had been induced early in the night there was a below-normal amount of REM sleep during the second half of the night (a kind of reverse 'rebound' within the same night). This acute facilitation of the REM stage in humans was also reported by Green (1965) and by Torda (1968). Such findings clearly differ from those obtained in the present study. However, in the mentioned studies, LSD-induced REM increases were always observed after the immediate administration of the compound, whereas in our study *ayahuasca* was administered at 12:00 noon. Given the complex chemical nature of *ayahuasca*, other alkaloids besides DMT may have played a role in the effects observed on REM. While harmine, an abundant and pharmacologically potent β-carboline, appears to undergo an intense first-pass metabolization, substantial levels of THH can be measured in plasma after oral *ayahuasca* (Riba et al. 2003). THH is a weaker MAO inhibitor than harmine but a stronger serotonin reuptake inhibitor (Buckholtz and Boggan 1977a, b). Reversible MAO inhibitors such as moclobemide (Blois and Gaillard 1990) and selective serotonin reuptake inhibitors (SSRI), such as paroxetine (Hicks et al. 2002), have all been mainly characterized by their ability to decrease REM sleep and to increase stage 2. It is interesting to note that the sleep effects after SSRIs have been reported to be more evident after morning than after evening drug administration (Barbanoj et al. 2005).

Despite the presence of MAOIs and SSRIs in *ayahuasca*, its acute pharmacological effects in humans are those of the classical serotonergic psychedelics acting at the 5-HT_{1A} and 5-$HT_{2A/C}$ sites. The role of these receptors in sleep physiology has been the subject of many studies over the last 40 years. The electrical activity of raphe neurons and the release of 5-HT are increased during waking and decreased during sleep (McGinty and Harper 1976). The available evidence indicates a role for 5-HT_{1A} receptors on REM sleep regulation and for 5-$HT_{2A/C}$ receptors in SWS regulation. Thus, selective activation of somatodendritic 5-HT_{1A} receptors in the dorsal raphe induces an increase of REM sleep, although activation of the postsynaptic 5-HT_{1A}

receptors at the level of cholinergic neurons located in tegmentum nuclei decreases REM sleep occurrence (reviewed in Monti and Monti (2000)). 5-HT_{1A} agonists have been shown to suppress REM sleep (Driver et al. 1995; Gillin et al. 1994). Regarding SWS, drugs antagonising 5-HT_{2A} or 5-HT_{2C} demonstrate an enhancing effect on SWS (Landolt et al. 1999; Sharpley et al. 1994), whereas 5-HT_{2C} agonists appear to lower SWS (Katsuda et al. 1993).

Spectral analysis showed that *d*-amphetamine leads to increases in power in the high-frequency range (higher than 15 Hz), an effect mainly observed during stage 2. In addition, the amount of SWA in the first night cycle was also reduced. To our knowledge, there are no published data on *d*-amphetamine effects on night EEG power spectra. However, the above effects could be expected if we take into account the alerting pattern associated with the morning intake of the *d*-amphetamine. The first effect would be related to the vigilance promoting effects associated to increases in power in the higher frequencies (Coull 1998). The second effect would be related to attenuation of sleep propensity associated with wakefulness (Johns 2002). Similar EEG power spectra changes have been reported after caffeine 200 mg intake in the morning (Landolt et al. 1995).

Ayahuasca also showed increases in power in the high frequencies, although these were limited to the 15–20 frequency range. In contrast with *d*-amphetamine, an increase in slow-wave power was observed in the first night cycle. This finding was unexpected, given the SWS decreasing effects that have been associated with 5-HT_2 agonism (Katsuda et al. 1993). From a neurochemical perspective, these results could be explained by the agonist properties of DMT at the 5-HT_{1A} sites (Seifritz et al. 1996) or through a functional desensitization of the 5-HT_2 (Saucier et al. 1998). Activation of 5-HT_{1A} receptors seems to result in a decrease of neural activity at 5-HT_2 sites. This might be because of either activation of presynaptic autoreceptors within the dorsal raphe nucleus leading to a decrease of activity at postsynaptic projection sites (Sprouse and Aghajanian 1987) and/or activation of postsynaptic 5-HT_{1A} receptors which would exert a modulatory inhibition of 5-HT_2 receptors (Araneda and Andrade 1991). An alternative explanation is that after the acute effects of *ayahuasca*, 'sleep pressure' is increased. The increases observed in SWS are typical of certain situations. After sleep deprivation, SWS activity increases are observed limited to the first night cycle (Borbély et al. 1981). Similarly, according to a recent meta-analysis (Driver and Taylor 2000), physical exercise has also been found to induce increases in SWS, reductions in REM and increases in REM latency. The changes observed in SWS could reflect a reaction to the physical and mental stress induced by the drug, as tiredness is frequent after *ayahuasca* and cortisol levels are augmented in the course of the experience (Callaway et al. 1999).

In summary, the present results did not evidence a deterioration of sleep quality after daytime consumption of *ayahuasca*. Sleep architecture showed *ayahuasca* to inhibit REM and spectral analysis demonstrated increases in slow-wave activity in the first night cycle. Results suggest an interaction between serotonergic psychedelics and brain circuits modulating REM and SWS.

Acknowledgements The authors thank the staff at the Centre d'Investigació de Medicaments de l'Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, in particular Adelaida Morte, Llúcia Benito, David Martínez and Liria da Graça for their technical assistance during data collection and Angeles Funes for copy-editing the manuscript. The present research complies with Spanish law. This study was supported by a grant from the Spanish Ministry of Education and Science (SAF 2002-02746) and by the Spanish Ministry of Health, Instituto de Salud Carlos III, RETICS RD06/0011 (REM-TAP Network).

References

Aeschbach D, Borbély AA (1993) All-night dynamics of the human sleep EEG. J Sleep Res 2:70–81

Araneda R, Andrade R (1991) 5-Hydroxytryptamine2 and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex. Neuroscience 40: 399–412

Barbanoj MJ, Clos S, Romero S, Morte A, Gimenez S, Lorenzo JL, Luque A, Dal-Re R (2005) Sleep laboratory study on single and repeated dose effects of paroxetine, alprazolam and their combination in healthy young volunteers. Neuropsychobiology 51:134–147

Blois R, Gaillard JM (1990) Effects of moclobemide on sleep in healthy human subjects. Acta Psychiatr Scand Suppl 360:73–75

Borbély AA, Baumann F, Brandeis D, Strauch I, Lehmann D (1981) Sleep deprivation: effect on sleep stages and EEG power density in man. Electroencephalogr Clin Neurophysiol 51:483–495

Borbély AA, Mattmann P, Loepfe M, Strauch I, Lehmann D (1985) Effect of benzodiazepine hypnotics on all-night sleep EEG spectra. Hum Neurobiol 4:189–194

Buckholtz NS, Boggan WO (1977a) Inhibition by beta-carbolines of monoamine uptake into a synaptosomal preparation: structure-activity relationships. Life Sci 20:2093–2099

Buckholtz NS, Boggan WO (1977b) Monoamine oxidase inhibition in brain and liver produced by beta-carbolines: structure-activity relationships and substrate specificity. Biochem Pharmacol 26:1991–1996

Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, Andrade EN, Andrade EO, Mash DC (1999) Pharmacokinetics of Hoasca alkaloids in healthy humans. J Ethnopharmacol 65:243–256

Coull JT (1998) Neural correlates of attention and arousal: insights from electrophysiology, functional neuroimaging and psychopharmacology. Prog Neurobiol 55:343–361

Deliganis AV, Pierce PA, Peroutka SJ (1991) Differential interactions of dimethyltryptamine (DMT) with 5-HT1A and 5-HT2 receptors. Biochem Pharmacol 41:1739–1744

Driver HS, Taylor SR (2000) Exercise and sleep. Sleep Med Rev 4:387–402

Driver HS, Flanigan MJ, Bentley AJ, Luus HG, Shapiro CM, Mitchell D (1995) The influence of ipsapirone, a 5-HT1A agonist, on sleep patterns of healthy subjects. Psychopharmacology (Berl) 117:186–192

Feinberg I, Floyd TC (1979) Systematic trends across the night in human sleep cycles. Psychophysiology 16:283–291

Gillin JC, Jernajczyk W, Valladares-Neto DC, Golshan S, Lardon M, Stahl SM (1994) Inhibition of REM sleep by ipsapirone, a 5HT1A agonist, in normal volunteers. Psychopharmacology (Berl) 116:433–436

Gouzoulis E, Steiger A, Ensslin M, Kovar A, Hermle L (1992) Sleep EEG effects of 3,4-methylenedioxyethamphetamine (MDE; "eve") in healthy volunteers. Biol Psychiatry 32:1108–1117

Green WJ (1965) The effect of LSD on the sleep-dream cycle. An exploratory study. J Nerv Ment Dis 140:417–426

Hicks JA, Argyropoulos SV, Rich AS, Nash JR, Bell CJ, Edwards C, Nutt DJ, Wilson SJ (2002) Randomised controlled study of sleep after nefazodone or paroxetine treatment in out-patients with depression. Br J Psychiatry 180:528–535

Johns MW (2002) Sleep propensity varies with behaviour and the situation in which it is measured: the concept of somnificity. J Sleep Res 11:61–67

Katsuda Y, Walsh AE, Ware CJ, Cowen PJ, Sharpley AL (1993) meta-Chlorophenylpiperazine decreases slow-wave sleep in humans. Biol Psychiatry 33:49–51

Lamas X, Farre M, Llorente M, Cami J (1994) Spanish version of the 49-item short form of the Addiction Research Center Inventory (ARCI). Drug Alcohol Depend 35:203–209

Landolt HP, Werth E, Borbely AA, Dijk DJ (1995) Caffeine intake (200 mg) in the morning affects human sleep and EEG power spectra at night. Brain Res 675:67–74

Landolt HP, Meier V, Burgess HJ, Finelli LA, Cattelin F, Achermann P, Borbely AA (1999) Serotonin-2 receptors and human sleep: effect of a selective antagonist on EEG power spectra. Neuropsychopharmacology 21:455–466

Martin WR, Sloan JW, Sapira JD, Jasinski DR (1971) Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. Clin Pharmacol Ther 12:245–258

McGinty DJ, Harper RM (1976) Dorsal raphe neurons: depression of firing during sleep in cats. Brain Res 101:569–575

Monti JM, Monti D (2000) Role of dorsal raphe nucleus serotonin 5-HT1A receptor in the regulation of REM sleep. Life Sci 66:1999–2012

Morgan MJ (2000) Ecstasy (MDMA): a review of its possible persistent psychological effects. Psychopharmacology (Berl) 152:230–248

Muzio JN, Roffwarg HP, Kaufman E (1966) Alterations in the nocturnal sleep cycle resulting from LSD. Electroencephalogr Clin Neurophysiol 21:313–324

Preud'homme XA, Lanquart JP, Mendlewicz J, Linkowski P (2000) Characteristics of spontaneous sleep with varying NREMS Episodes in healthy men: implication for delta activity homeostasis. Sleep 23:193–203

Rechtschaffen A, Kales A (1968) Techniques and scoring system for sleep stages in human subjects. US Government Printing Office, Washington, DC, USA

Riba J, Rodriguez-Fornells A, Strassman RJ, Barbanoj MJ (2001) Psychometric assessment of the Hallucinogen Rating Scale. Drug Alcohol Depend 62:215–223

Riba J, Anderer P, Morte A, Urbano G, Jane F, Saletu B, Barbanoj MJ (2002) Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage ayahuasca in healthy volunteers. Br J Clin Pharmacol 53:613–628

Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanoj MJ (2003) Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J Pharmacol Exp Ther 306:73–83

Riba J, Romero S, Grasa E, Mena E, Carrio I, Barbanoj MJ (2006) Increased frontal and paralimbic activation following ayahuasca, the pan-Amazonian inebriant. Psychopharmacology (Berl) 186:93–98

Saletu B, Wesseley P, Grünberger J, Schultes M (1987) Erste klinische Erfahrungen mit einem neuen Schlafanstossenden Benzodiazepin Cinolazepam mittels eines Selbstbeurteilungsbögens für Schlaf- und Aufwachqualität (SSA). Neuropsychiatrie 1:169–176

Saletu B, Frey R, Krupka M, Anderer P, Grunberger J, Barbanoj MJ (1989) Differential effects of a new central adrenergic agonist-modafinil-and D-amphetamine on sleep and early morning behaviour in young healthy volunteers. Int J Clin Pharmacol Res 9:183–195

Saucier C, Morris SJ, Albert PR (1998) Endogenous serotonin-2A and -2C receptors in Balb/c-3T3 cells revealed in serotonin-free medium: desensitization and down-regulation by serotonin. Biochem Pharmacol 56:1347–1357

Schlösser R, Roschke J, Rossbach W, Benkert O (1998) Conventional and spectral power analysis of all-night sleep EEG after subchronic treatment with paroxetine in healthy male volunteers. Eur Neuropsychopharmacol 8:273–278

Schultes RE, Hofmann A (1987) Plants of the gods: origins of hallucinogenic use. van der Marck Editions, New York

Seifritz E, Moore P, Trachsel L, Bhatti T, Stahl SM, Gillin JC (1996) The 5-HT1A agonist ipsapirone enhances EEG slow wave activity in human sleep and produces a power spectrum similar to 5-HT2 blockade. Neurosci Lett 209:41–44

Sharpley AL, Elliott JM, Attenburrow MJ, Cowen PJ (1994) Slow wave sleep in humans: role of 5-HT2A and 5-HT2C receptors. Neuropharmacology 33:467–471

Sprouse JS, Aghajanian GK (1987) Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. Synapse 1:3–9

Torda C (1968) Contribution to serotonin theory of dreaming (LSD infusion). N Y State J Med 68:1135–1138

Psychopharmacology
DOI 10.1007/s00213-011-2434-x

ORIGINAL INVESTIGATION

Pharmacology of ayahuasca administered in two repeated doses

Rafael G. dos Santos · Eva Grasa · Marta Valle · Maria Rosa Ballester · José Carlos Bouso · Josep F. Nomdedéu · Rosa Homs · Manel J. Barbanoj · Jordi Riba

Received: 30 May 2011 / Accepted: 27 July 2011

Abstract
Rationale Ayahuasca is an Amazonian tea containing the natural psychedelic 5-$HT_{2A/2C/1A}$ agonist *N*,*N*-dimethyltryptamine (DMT). It is used in ceremonial contexts for its visionary properties. The human pharmacology of ayahuasca has been well characterized following its administration in single doses.
Objectives To evaluate the human pharmacology of ayahuasca in repeated doses and assess the potential occurrence of acute tolerance or sensitization.
Methods In a double-blind, crossover, placebo-controlled clinical trial, nine experienced psychedelic drug users received PO the two following treatment combinations at least 1 week apart: (a) a lactose placebo and then, 4 h later, an ayahuasca dose; and (b) two ayahuasca doses 4 h apart. All ayahuasca doses were freeze-dried Amazonian-sourced tea encapsulated to a standardized 0.75 mg DMT/kg bodyweight. Subjective, neurophysiological, cardiovascular, autonomic, neuroendocrine, and cell immunity measures were obtained before and at regular time intervals until 12 h after first dose administration.

Manel J. Barbanoj is deceased.

R. G. dos Santos · J. C. Bouso · J. Riba
Human Experimental Neuropsychopharmacology, IIB Sant Pau,
Sant Antoni María Claret 167,
08025 Barcelona, Spain

R. G. dos Santos · M. R. Ballester · J. C. Bouso · M. J. Barbanoj · J. Riba
Centre d'Investigació de Medicaments,
Hospital de la Santa Creu i Sant Pau,
Sant Antoni Maria Claret 167,
08025 Barcelona, Spain

R. G. dos Santos · M. Valle · M. R. Ballester · M. J. Barbanoj · J. Riba
Departament de Farmacologia i Terapèutica,
Universitat Autònoma de Barcelona,
Barcelona, Spain

E. Grasa · M. Valle · M. R. Ballester · M. J. Barbanoj · J. Riba
Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM,
Barcelona, Spain

M. Valle
Pharmacokinetic and Pharmacodynamic Modelling and Simulation, IIB Sant Pau,
Sant Antoni Maria Claret 167,
08025 Barcelona, Spain

J. F. Nomdedéu
Servei Laboratori d'Hematologia,
Hospital de la Santa Creu i Sant Pau,
Sant Antoni María Claret 167,
08025 Barcelona, Spain

R. Homs
Servei de Bioquímica Clínica,
Hospital de la Santa Creu i Sant Pau,
Sant Antoni María Claret 167,
08025 Barcelona, Spain

J. Riba (✉)
Human Experimental Neuropsychopharmacology,
Institut de Recerca, Hospital de la Santa Creu i Sant Pau,
St. Antoni Maria Claret 167,
Barcelona 08025, Spain
e-mail: jriba@santpau.cat

Published online: 13 August 2011

Results DMT plasma concentrations, scores in subjective and neurophysiological variables, and serum prolactin and cortisol were significantly higher after two consecutive doses. When effects were standardized by plasma DMT concentrations, no differences were observed for subjective, neurophysiological, autonomic, or immunological effects. However, we observed a trend to reduced systolic blood pressure and heart rate, and a significant decrease for growth hormone (GH) after the second ayahuasca dose.
Conclusions Whereas there was no clear-cut tolerance or sensitization in the psychological sphere or most physiological variables, a trend to lower cardiovascular activation was observed, together with significant tolerance to GH secretion.

Keywords Ayahuasca · Psychedelics · Repeated dose administration · Tolerance

Introduction

Ayahuasca is a psychoactive tea that was originally used for its visionary properties in shamanic, religious, and medicinal contexts in the Amazon but is now used worldwide in ceremonial and lay contexts (Tupper 2008). The tea is prepared from *Banisteriopsis caapi*, its key botanical ingredient, plus several other plants, typically *Psychotria viridis* (see, for a review, Riba 2003). Chemical analyses have shown that the main components of ayahuasca are alkaloids with β-carboline structure (harmine, harmaline, and tetrahydroharmine (THH)) from *B. caapi* plus *N,N*-dimethyltryptamine (DMT; Yritia et al. 2002; Riba 2003) from *P. viridis*. The monoamine-oxidase-inhibiting properties of the β-carbolines block the metabolic degradation of DMT, an orally labile psychedelic 5-$HT_{2A/2C/1A}$ receptor agonist (Smith et al. 1998; Riba 2003), and allow its access to systemic circulation after ayahuasca ingestion (McKenna et al. 1984; Riba 2003). In recent years, ayahuasca has been the object of various biomedical studies that have assessed its pharmacological profile in humans. Its effects when administered in single doses are well characterized. In a clinical research setting, it has been found to induce transient perceptual, cognitive, and affective modifications typical of the psychedelics, plus physiological effects that include elevations in diastolic blood pressure, cortisol and prolactin and lymphocyte redistribution, and electroencephalographic changes (Riba et al. 2002; 2003; Santos et al., in press). Ayahuasca is relatively well tolerated in healthy volunteers. The most commonly reported unpleasant effects are nausea and physical discomfort (Riba et al. 2001a).

In the context of the ceremonial use of ayahuasca, it is a common practice to drink several doses in each session, but the pharmacology of the drug in repeated doses has not yet been studied in a clinical trial. Laboratory information regarding possible increased toxicity after repeated administration is thus lacking. Furthermore, studying the pharmacology of ayahuasca administered in repeated doses is of interest from a basic science perspective. DMT appears to be different from other serotonergic psychedelics such as LSD, mescaline, and psilocybin, in terms of its tolerance-inducing capacity. While tolerance development to LSD was described over 50 years ago (Isbell et al. 1956), tolerance to DMT has not been conclusively demonstrated either in animals (Cole and Pieper 1973; Gillin et al. 1973; Kovacic and Domino 1976) or in humans (Gillin et al. 1976; Strassman et al. 1996).

In this present work, we studied the pharmacology of two consecutive doses of ayahuasca on subjective, physiological, and neurophysiological variables and assessed for potential acute tolerance or sensitization.

Materials and methods

Volunteers

A total of 17 volunteers (all male) with experience in psychedelic drug use were recruited. Eligibility criteria required prior use of psychedelics on at least ten occasions without sequelae derived thereof, i.e., psychedelic-related disorders as described in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*; American Psychiatric Association, 1994). A physician conducted a physical examination, ECG, and standard laboratory tests in all volunteers, confirming their good health. Prior to physical examination, volunteers were interviewed by a clinical psychologist (Spanish version of the Structured Interview for *DSM-IV* [*SCID*]; First et al. 1999). We excluded any volunteers who had a present or past history of Axis-I disorders (including alcohol or other substance dependence) and any who had parents or siblings with a present or past history of psychotic disorders. The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans and was approved by the hospital ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of ayahuasca and the general psychological effects of psychedelics and their possible adverse effects as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drug

Ayahuasca was not administered in liquid form but as a freeze-dried encapsulated formulation. Based on previous

studies by our group (Riba et al. 2001a; 2003), the dose of ayahuasca administered was equivalent to 0.75 mg DMT/kg body weight. Lactose capsules were used as a placebo.

Study design

Volunteers participated in three experimental sessions at least 1 week apart. Each experimental session involved two administrations separated by 4 h. In the first experimental session, all participants received the treatment pair placebo–placebo in an open-label fashion. This first session was intended to familiarize the volunteers with the study setting and to minimize the stress associated with the experimental interventions.

Volunteers were informed that, in sessions 2 and 3, they would randomly receive any of the following treatment pairs: placebo–placebo, placebo–ayahuasca, ayahuasca–placebo, or ayahuasca–ayahuasca. In fact, only two of the four combinations were administered: placebo–ayahuasca and ayahuasca–ayahuasca. Treatment pairs were administered in a double-blind fashion. The designation and order of administration for each of the four individual treatments was as follows: (a) for the placebo–ayahuasca pair: at zero hours, the treatment denominated *placebo* and at 4 h an ayahuasca treatment, designated here as *Aya0*; and (b) for the ayahuasca–ayahuasca pair: at zero hours, an ayahuasca treatment, designated here *Aya1,* and, at 4 h, an ayahuasca dose, designated here *Aya2*.

This approach was chosen to reduce the number of times volunteers were exposed to ayahuasca from four to three. Since *Aya0* and *Aya2* were administered in the afternoon and *Aya1* in the morning, *Aya1* is not comparable with the other two treatments due to circadian changes and to the influence of the light meal served to the participants before administration of *Aya0* and *Aya2*. Consequently, values for *Aya1* are shown for illustrative purposes in the figures in the results section, but they were excluded from the statistical analyses and are omitted from the tables. To test whether a single repeated dose administration of ayahuasca leads to higher absolute effects and acute tolerance or sensitization, *Aya0* was compared vs. *Aya2*. Comparisons vs. the placebo administered in the placebo–ayahuasca pair were merely conducted to confirm that *Aya0* and *Aya2* were active (see the statistical analyses explanation below).

Volunteers were requested to abstain from any medication or illicit drug use in the 2 weeks before the experimental sessions and until after the study was completed. Volunteers also were requested to abstain from alcohol, tobacco, and caffeinated drinks in the 24 h before each experimental day. Urinalysis for alcohol and illicit drug use was performed on each experimental day. Urine samples were tested for alcohol, benzodiazepines, cannabis, amphetamine, opiates, and cocaine using automated homogeneous enzyme immunoassays (Multigent, Architect C16000 System, Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL, USA). Participants arrived at 7:00 AM under fasting conditions of at least 10 h and had a light breakfast before 10:00 AM. The first treatment was administered at approximately 11:00 AM and the second at 15:00 PM after a light meal. Throughout the experimental session, the volunteers remained seated in a comfortable reclining chair in a quiet, dimly lit room. Volunteers remained overnight in the laboratory and were discharged at 15:00 PM the following day.

Measurements

Subjective ratings

The subjective effects elicited by ayahuasca were measured by means of visual analog scales (VAS) and self-report questionnaires including the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI).

VAS were 100-mm horizontal lines anchored with the words "Not at all" and "Extreme" with the following labels: "any effect," indicating any physical or psychological modification that the volunteer attributed to the administered drug; "good effects," indicating any effect, physical or psychological, the volunteer valued as good; "bad effects," indicating any effect the volunteer valued as bad; "visual effects," indicating modifications in visual perception, including any variations in object shape, brightness, or color and any illusion, abstract or elaborate, seen with eyes either closed or open; "auditory effects," indicating modifications in auditory perception; "dizzy," indicating near-syncope or lightheadedness; "liking," reflecting that the volunteer liked the effects of the administered substance; "stimulated," indicating any increases in thought speed and/or content, or any increases in associations and/or insights; and "high," which reflected any positive psychological effect the volunteer attributed to the drug. The volunteers were requested to answer the VAS immediately before (baseline) and at 15, 30, 45 min, and 1, 1.5, 2, 2.5, 3, 4, 4.25, 4.5, 4.75, 5, 5.5, 6, 6.5, 7, 8, 10, and 12 h after administration of the first treatment.

The HRS includes six subscales: Somaesthesia, reflecting somatic effects; Affect, reflecting emotional and affective responses; Volition, indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; Cognition, describing modifications in thought processes or content; Perception, measuring visual, auditory, gustatory, and olfactory experiences; and Intensity, reflecting the strength of the overall experience (Strassman et al. 1994). A Spanish version of the questionnaire was used (Riba et al. 2001b). Scores for all subscales is 0 to 4.

The short version of the ARCI (Martin et al. 1971) consists of five scales or groups: MBG, the morphine–benzedrine group, measuring euphoria and positive mood; PCAG, the pentobarbital–chlorpromazine–alcohol group, measuring sedation; LSD, the lysergic acid diethylamide scale, measuring somatic–dysphoric effects; BG, the benzedrine group, measuring intellectual energy and efficiency; and the A scale, an empirically derived scale measuring amphetamine-like effects. Scores range from 0 to 16 for MBG, from −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG, and 0 to 11 for A. The questionnaire had been translated into Spanish and validated by Lamas et al. (1994). Volunteers were requested to answer the HRS and ARCI at 4 and 8 h after the first treatment.

Neurophysiological measures (EEG)

The EEG was recorded, preprocessed, and quantified following standard procedures as previously described (Riba et al. 2002). Recordings were obtained at 19 scalp locations according to the international 10/20 system by means of a Neuroscan SYNAMPS amplifier (Compumedics Neuroscan, Charlotte, NC, USA). A 3-min EEG with eyes closed was recorded at 0 (baseline) and 15, 30, 45 min, and 1, 1.5, 2, 2.5, 3, 4, 4.25, 4.5, 4.75, 5, 5.5, 6, 6.5, 7, and 8 h after administration of the first treatment. Following a fast Fourier transform, the target variables were calculated: relative power (expressed as percentage) in the beta (13–35 Hz) frequency band and in the beta-4 (25–30 Hz) and beta-5 (30–35 Hz) sub-bands. Modifications of these variables have been detected following acute ayahuasca administration (Riba et al. 2002; Santos et al., in press). Target variables were calculated at each electrode and time point. Averages for each variable in all 19 leads at each time point were used in the subsequent statistical analysis.

Cardiovascular measures

Systolic (SBP) and diastolic (DBP) blood pressures and heart rate (HR) were measured with the volunteer seated, before (baseline) and at 30 min, 1, 1.5, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, and 8 h after the first treatment using a Dinamap 8100 vital signs monitor (Critikon, Tampa, FL). The cuff was placed around the volunteer's left arm. Determination time was between 20 and 45 s.

Autonomic measures

Temperature and pupillary diameter were measured before administration (baseline) and at 30 min, 1, 1.5, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, and 8 h after the first treatment. Axillary temperature readings were obtained with a standard mercury-in-glass thermometer placed in the volunteer's armpit for at least 3 min. Pupillary diameter was measured with a portable pupillometer (NeurOptics Pupillometer; NeurOptics, Irvine, CA). The pupillometer was placed over the volunteer's eye immediately after turning the lights off and maintained in place until the pupillometer indicated that a valid reading had been obtained.

Neuroendocrine measures

Blood samples (3 mL, plain tubes without clot activator) were drawn before administration (baseline), and at 1, 2, 4, 5, 6, 8, and 28 h after administration of the first treatment and were allowed to stand at room temperature. Serum was separated by centrifugation and aliquots stored for the analysis of growth hormone (GH), prolactin, and cortisol.

Serum GH and prolactin concentrations were determined by a chemiluminescence immunoassay system (Immulite 2000®, Diagnostic Products Corp, EURO/Diagnostic Products Corporation, Llanberis, UK). The GH immunoassay, with a sensitivity of 0.06 mIU/L, uses the WHO 1st IRP 80/505, and shows intra- and interassay coefficients of variation (CV) of 5.3–6.1% and 5.7–6.5%, respectively. The prolactin immunoassay uses the 3rd IS 84/500, with an analytical sensitivity of 3.4 mIU/L, and intra-assay and total CV between 2.2–2.3% and 6.9–7.9%, respectively. Serum cortisol concentrations were measured by electrochemiluminescent immunoassay (Elecsys Modular Analytics E170®, Roche Diagnostics GmbH Manheim, Germany) with functional sensitivity <8 nmol/L, and intra-assay and total CVs of 1.7% and 2.8%, respectively, for mean human serum concentrations between 129 and 717 nmol/L.

Obtained values were transformed to nanograms per milliliter (prolactin and GH) and micrograms per deciliter (cortisol).

Lymphocyte subpopulations

Blood samples (3 mL, heparin tubes) were drawn before (baseline), and at 1, 2, 4, 5, 6, 8, and 28 h after administration and were subjected to lymphocyte immunophenotyping. The following lymphocyte subpopulations were quantified: CD8 T cells, CD4 T cells, CD3 T cells, CD19 B cells, and natural killer (NK) cells.

For lymphocyte immunophenotyping, blood samples were stained with the Lymphogram™ (Cytognos, Salamanca, Spain) reagent kit; each tube contains five different murine MoAbs with three fluorochromes: CD8 and CD19 with FITC (fluorescein isothiocyanate), CD3, and CD56 with PE (phycoerythrin). CD4 were labeled in tandem with PE and Cy5 (phycoerythrin−cyanate 5). The procedure has been detailed elsewhere (Bellido et

al. 1998). Lymphocyte subpopulations were expressed as percentage of all blood cells.

DMT plasma levels

Blood samples (10 ml, EDTA tubes) were drawn at 0 (baseline) and 30 min, and 1, 1.5, 2, 2.5, 3, 4, 4.25, 4.5, 5, 5.5, 6, 6.5, 7, 8, 10, and 12 h after administration for analysis of DMT. Samples were centrifuged at 2,000 rpm for 10 min at 4°C, and plasma was immediately frozen at −20°C. Frozen plasma samples were stored at −80°C until analysis. DMT plasma concentrations were determined as previously described (Yritia et al. 2002).

All measurements conducted at 4 h were performed prior to administration of the second treatment.

Statistical analysis

Data obtained in the first placebo−placebo acclimatization session were not included in the statistical analysis. The *placebo* used for the statistical comparisons was that administered in the placebo−ayahuasca session. The comparisons of interest were between *Aya0* and *Aya2*. These two ayahuasca treatments were fully equivalent in terms of time of the day and preceding meal. Nevertheless, *Aya0* and *Aya2* were compared with placebo to confirm that they were pharmacologically active.

For the HRS and ARCI questionnaires, scores on the different subscales were calculated and subjected to statistical analysis.

VAS, EEG, cardiovascular, neuroendocrine, immunological, temperature, and pupillary diameter measurements were transformed into differences from baseline (0 h). The following parameters were then calculated for each of the three treatments, i.e., placebo, *Aya0*, and *Aya2*: (1) the 0–4 h post-administration E_{max} ($E_{max(0–4h)}$) or peak effect (maximum absolute change from the 0 h baseline values); (2) the 0–4 h post-administration area under the curve ($AUC_{0–4h}$) of effect versus time; and (3) the 0–4 h post-administration area under the curve ($AUC_{0–4h}$) normalized by the respective $AUC_{0–4h}$ of the DMT plasma concentrations vs. time. These normalized AUCs were designated as AUC_{norm}. All AUCs were calculated using the trapezoidal rule. The comparison between AUC_{norm} after *Aya0* and AUC_{norm} after *Aya2* allowed making inferences regarding acute tolerance or sensitization development, taking the DMT plasma concentrations into account.

HRS and ARCI scores and the parameters described above for the pharmacodynamic variables were analyzed using paired Student's *t* tests.

For each ayahuasca treatment, the maximum DMT plasma concentration (C_{max}) was calculated and reported as mean±standard deviation (SD). The time to reach the maximum concentration (t_{max}) was reported as median and range. Areas under the concentration−time curves between treatment administration and 4 h ($AUC_{0–4h}$) were also calculated and reported as mean±SD. C_{max} and $AUC_{0–4h}$ were compared between ayahuasca treatments by means of Student's *t* test. T_{max} values were compared using non-parametric Wilcoxon's test.

In all tests performed, differences were considered statistically significant for *p* values lower than 0.05.

Results

Only nine of the 17 volunteers completed the study. One volunteer was excluded before the start of the acclimatization session due to a positive result for alcohol in the urinalysis. Two decided to voluntarily withdraw from the study, and five more were excluded due to vomiting. Vomiting was self-induced in one case; in another case, it occurred after the administration of the first ayahuasca dose in the ayahuasca−ayahuasca session, and in the remaining three cases, it occurred after the administration of the second ayahuasca dose in the ayahuasca–ayahuasca session. The data reported in the present paper refer only to the nine volunteers that completed all three experimental sessions. Participants in the final sample had a mean age of 32.8 (range, 24–41 years), a mean weight of 69.69 kg (range, 57–91), and a mean height of 177 cm (range, 170–186). Additionally, one subject showed no measurable DMT plasma levels after *Aya0*. Consequently, pharmacokinetic data are reported for eight volunteers only (see the "Pharmacokinetic analysis" section below). This also precluded the calculation of the normalized AUCs after *Aya0* for this participant. For this reason, the statistical comparison of normalized AUCs after *Aya0* vs *Aya2* was conducted for a sample of eight volunteers.

Subjective effects

Subjective effect results are shown in Fig. 1 and Table 1.

Both administered ayahuasca treatments proved psychoactive. Compared with placebo, the administration of both *Aya0* and *Aya2* led to significant increases in all subscales of the HRS and in the A and MBG subscales of the ARCI. Additionally, *Aya0* led to significant increases in the BG subscale and *Aya2* to significant increases in the LSD subscale. The effects of *Aya2* on the HRS subscales Somaesthesia and Volition were significantly higher than those of *Aya0*. The effects of *Aya2* on the HRS subscale Intensity showed a trend to significantly higher values than after *Aya0*. Higher increases were also observed in the Perception subscale, and these were marginally significant (p=0.050). No differences between doses were

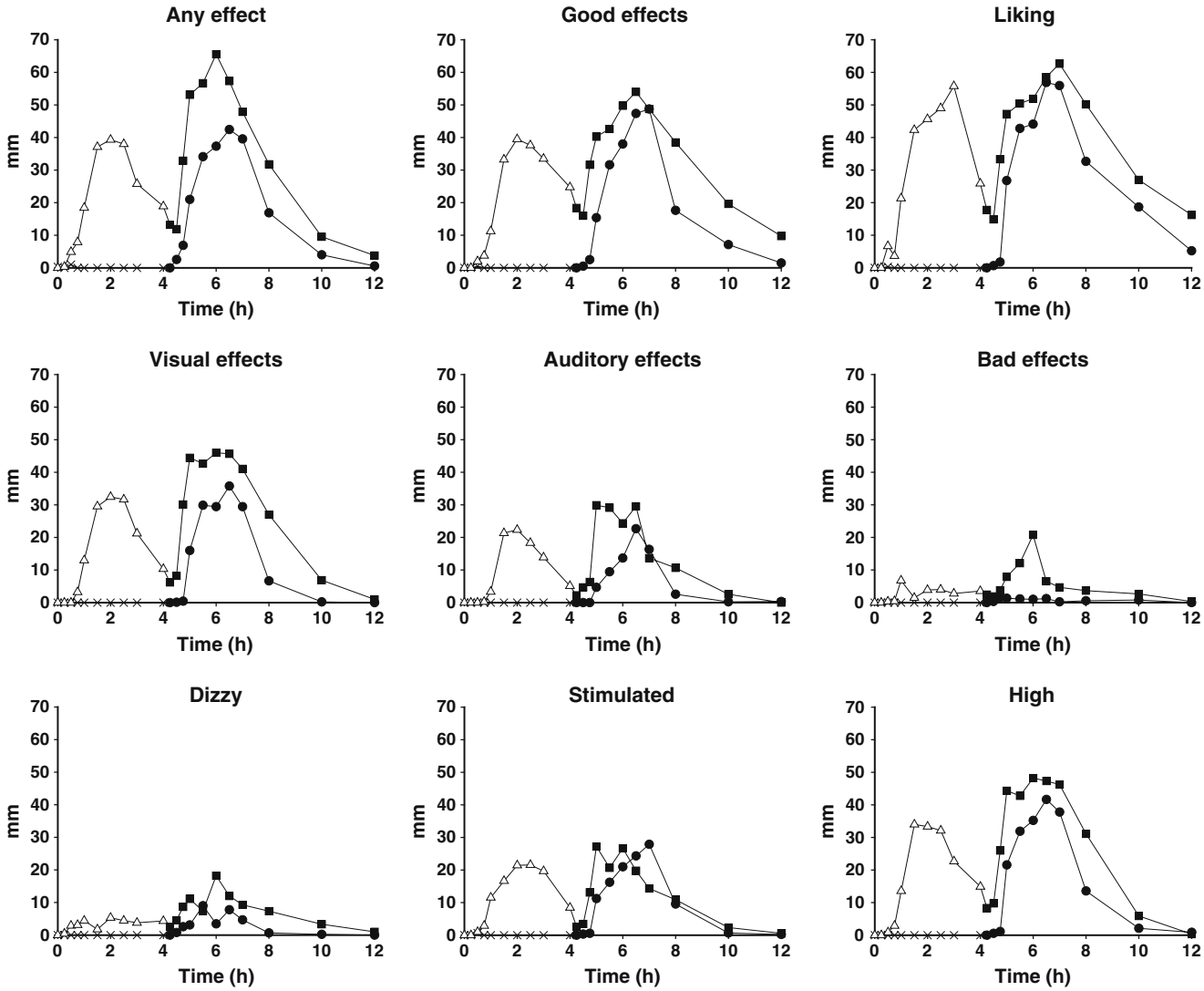


Fig. 1 Time course of scores on the nine VAS items (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*) and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo and *Aya2* was preceded 4 h by *Aya1*

found for Affect, Cognition, or ARCI subscales. Regarding the VAS items, both ayahuasca treatments produced significant increases relative to placebo in the peak values and $AUC_{0\text{-}4h}$ values of eight items (except for "bad effects", where only *Aya2* produced significant effects). For two VAS items ("any effect" and "bad effects"), values after *Aya2* were significantly higher than those after *Aya0*. The $AUC_{0\text{-}4h}$ for the "auditory effects" VAS item was higher for *Aya2* than for *Aya0*. The same parameter for the items "visual effects" and "dizzy" showed a trend to significantly higher values after *Aya2*. When DMT plasma levels were taken into account (AUC_{norm}), the VAS item "stimulated" showed a trend to lower effects after *Aya2* than after *Aya0*, while the item "bad effects" showed a trend for higher effects after *Aya2* than after *Aya0*.

EEG effects

Treatment effects on relative global beta power and relative beta-4 and beta-5 powers are presented in Fig. 2 and Table 2.

As shown therein, *Aya0* (AUC) induced significant increases in relative beta-4 and beta-5 powers and a marginally significant increase (p=0.050) in relative global beta power. However, no significant effects for *Aya0* peak values were observed in any of the EEG variables. On the other hand, *Aya2* (AUC and peak) induced significant increases in all three measures. There was only a trend in relative global beta power (peak) between ayahuasca treatments; and *Aya2* induced significantly larger increases in relative beta-4 power than *Aya0* (AUC and peak) and in

Table 1 Subjective effects induced by placebo, *Aya0* and *Aya2*

	Placebo	Ayahuasca0	Ayahuasca2	Pair-wise comparisons		
				PLA/AYA0	PLA/AYA2	AYA0/AYA2
HRS						
Somaesthesia	0.00 (0.00)	0.82 (0.53)	1.25 (0.55)	**	***	*
Affect	0.27 (0.66)	1.19 (0.55)	1.20 (0.53)	**	**	ns
Perception	0.00 (0.00)	1.41 (0.53)	1.81 (0.78)	***	***	0.050
Cognition	0.00 (0.00)	1.42 (0.71)	1.52 (0.68)	***	***	ns
Volition	0.19 (0.31)	1.05 (0.36)	1.51 (0.45)	***	***	**
Intensity	0.00 (0.00)	1.97 (0.65)	2.53 (0.78)	***	***	0.073
ARCI						
A	0.22 (0.67)	4.22 (2.86)	4.55 (3.68)	**	**	ns
BG	0.11 (0.78)	2.22 (2.39)	1.67 (3.12)	*	ns	ns
MBG	3.33 (0.71)	5.89 (4.48)	7.00 (5.57)	**	**	ns
PCAG	0.67 (1.41)	1.44 (3.28)	2.89 (4.25)	ns	ns	ns
LSD	−0.67 (1.12)	0.78 (2.49)	1.22 (2.17)	ns	*	ns
VAS						
Any effect_peak	1.33 (3.04)	51.67 (25.20)	76.33 (21.88)	***	***	*
Any effect_AUC	20.83 (54.57)	6,317.50 (4,326.05)	10,738.33 (5,313.29)	**	***	*
Any effect_AUC_norm	–	5.17 (3.63)	3.76 (3.05)	–	–	ns
Good effects_peak	0.44 (1.33)	61.33 (33.44)	68.55 (24.88)	**	***	ns
Good effects_AUC	6.67 (20.00)	7,503.33 (5,044.29)	10,231.67 (4,745.73)	**	***	ns
Good effects_AUC_norm	–	6.62 (5.96)	3.55 (2.63)	–	–	ns
Liking_peak	0.33 (1.00)	64.22 (31.76)	72.55 (28.86)	***	***	ns
Liking_AUC	5.00 (15.00)	8,479.17 (5,506.50)	10,995.00 (5,209.33)	**	***	ns
Liking_AUC_norm	–	7.52 (6.70)	3.67 (3.00)	–	–	ns
Visual effects_peak	0.00 (0.00)	46.78 (22.67)	65.89 (26.61)	***	***	ns
Visual effects_AUC	0.00 (0.00)	4,746.67 (2,841.53)	8,464.17 (6,172.66)	**	**	0.083
Visual effects_AUC_norm	–	3.91 (2.15)	2.67 (2.31)	–	–	ns
Auditory effects_peak	0.00 (0.00)	28.67 (24.50)	43.55 (29.81)	**	**	ns
Auditory effects_AUC	0.00 (0.00)	2,290.00 (2,419.08)	4,350.83 (3,952.53)	*	*	*
Auditory effects_AUC_norm	–	1.82 (1.88)	1.56 (2.22)	–	–	ns
Bad effects_peak	0.00 (0.00)	1.89 (3.51)	23.22 (22.24)	ns	*	*
Bad effects_AUC	0.00 (0.00)	183.33 (357.81)	1,829.17 (1,784.32)	ns	*	*
Bad effects_AUC_norm	–	0.17 (0.31)	0.48 (0.42)	–	–	0.057
Dizzy_peak	0.00 (0.00)	14.55 (12.03)	31.67 (25.11)	**	**	ns
Dizzy_AUC	0.00 (0.00)	956.67 (868.11)	2,295.83 (2,307.40)	*	*	0.064
Dizzy_AUC_norm	–	0.57 (0.38)	0.73 (0.43)	–	–	ns
Stimulated_peak	0.67 (2.00)	35.00 (21.30)	38.89 (25.22)	**	**	ns
Stimulated_AUC	10.00 (30.00)	3,654.17 (2,811.56)	3,955.83 (3,292.43)	**	**	ns
Stimulated_AUC_norm	–	2.64 (1.74)	1.41 (1.90)	–	–	0.064
High_peak	0.00 (0.00)	52.89 (23.76)	64.22 (28.76)	***	***	ns
High_AUC	0.00 (0.00)	5,880.00 (4,197.34)	8,940.83 (5,378.52)	**	**	ns
High_AUC_norm	–	4.79 (3.47)	2.63 (2.07)	–	–	ns

Mean (SD) of the scores obtained for the HRS and ARCI questionnaires subscales and for the VAS and results of the statistical analysis performed. $N=9$, except for normalized AUCs where $n=8$

PLA placebo, *AYA0* ayahuasca0, *AYA2* ayahuasca2, *A* amphetamine, *BG* benzedrine-group, *MBG* morphine–benzedrine-group, *PCAG* pentobarbital–chlorpromazine–alcohol-group, *LSD* lysergic acid diethylamide scale

*$p<0.05$, **$p<0.01$, ***$p<0.001$. Exact p values are given when $p<0.1$

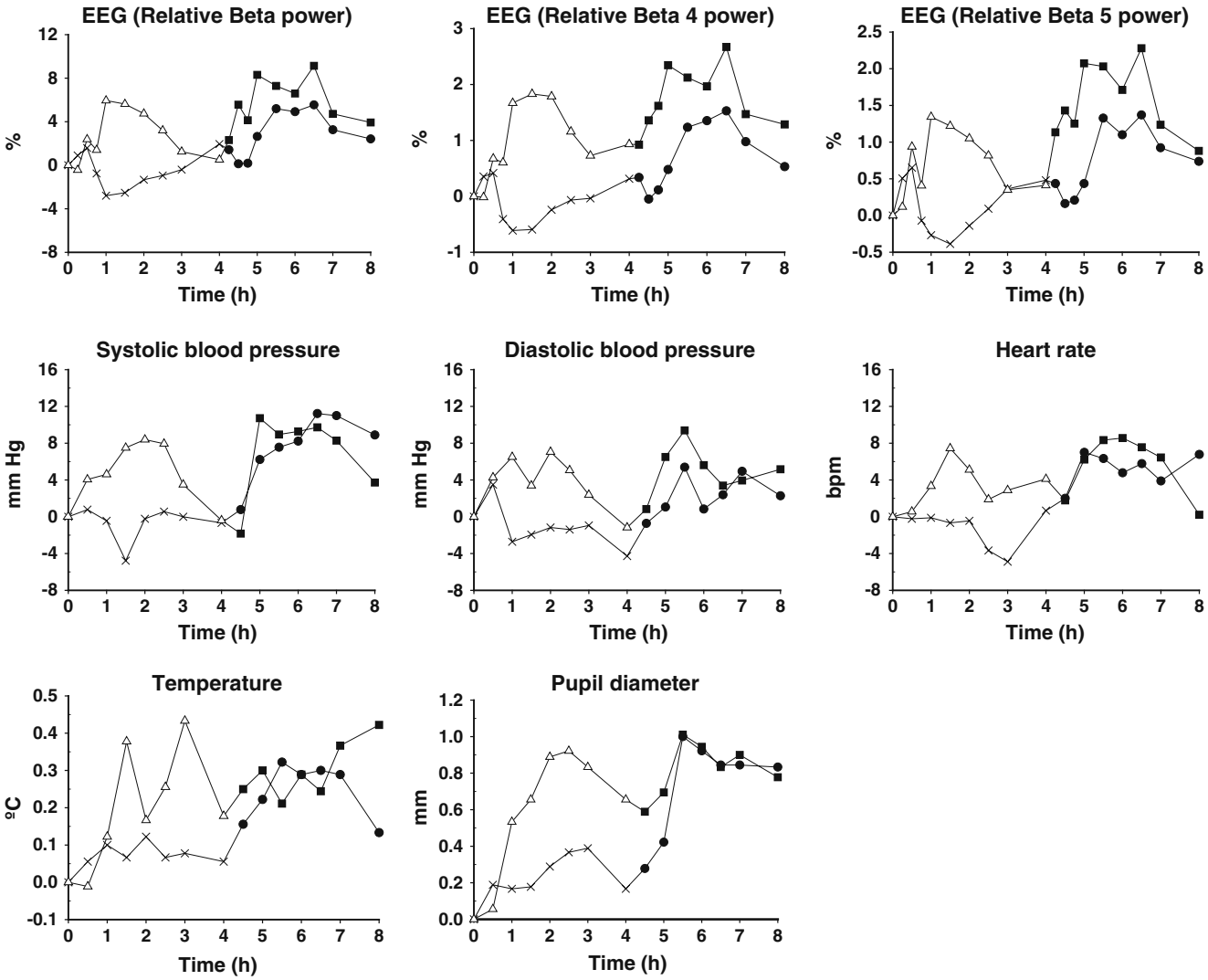


Fig. 2 Time course of electroencephalographic (EEG), cardiovascular and autonomic variables (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo and *Aya2* was preceded 4 h by *Aya1*

relative beta-5 power (peak). When DMT plasma levels were taken into account, no differences between active treatments appeared for any of the variables.

Cardiovascular effects

Cardiovascular effects are shown in Fig. 2 and Table 2. Peak and AUC values after both ayahuasca treatments were significantly higher than after placebo for SBP. No significant differences were found between active treatments. For peak DBP values, only *Aya2* produced increases significantly different from placebo. No significant differences were found between active treatments. In terms of AUC values, effects after both ayahuasca treatments were significantly larger than after placebo. No significant differences were found between ayahuasca treatments. For HR, *Aya0*, and *Aya2* produced significant increases compared with placebo, in terms of both peak and AUC values. No significant differences were found between active treatments. When DMT plasma levels were taken into account, there was a trend for lower values after *Aya2* for SBP and HR.

Occurrence of hypertension and/or tachycardia was examined for each participant. SBP rose above 140 mm Hg in three volunteers after *Aya0* (141 mm Hg; and 146 mm Hg, two volunteers) and in two volunteers after *Aya2* (147 mm Hg; and 142 mm Hg). Most of these events lasted 15–30 min. DBP values did not reach values above 90 mm Hg for any participant. HR rose above 100 beats/min (105 beats/min) in one volunteer after *Aya0*.

Table 2 Effects induced by placebo and *Aya0* and *Aya2* on EEG and cardiovascular and autonomic measures

	Placebo	Ayahuasca0	Ayahuasca2	Pair-wise comparisons		
				PLA/AYA0	PLA/AYA2	AYA0/AYA2
EEG measures						
Relative beta power_peak	−0.68 (8.37)	5.15 (12.85)	12.06 (18.07)	ns	*	0.074
Relative beta power_AUC	−140.86 (624.87)	790.33 (1,325.02)	1,391.50 (1,986.39)	0.050	*	ns
Relative beta power_AUC_norm	–	0.80 (1.23)	0.64 (0.87)	–	–	ns
Relative beta-4 power_peak	0.23 (2.08)	1.28 (3.44)	3.16 (3.52)	ns	**	**
Relative beta-4 power_AUC	−27.47 (191.92)	202.61 (327.20)	426.05 (334.23)	*	**	**
Relative beta-4 power_AUC_norm	–	0.20 (0.36)	0.16 (0.18)	–	–	ns
Relative beta-5 power_peak	0.41 (1.63)	1.21 (2.26)	4.17 (1.95)	ns	**	*
Relative beta-5 power_AUC	27.98 (113.85)	203.08 (188.09)	369.69 (374.27)	*	*	ns
Relative beta-5 power_AUC_norm	–	0.19 (0.18)	0.16 (0.20)	–	–	ns
Cardiovascular measures						
Systolic blood pressure_peak	−5.44 (14.49)	19.44 (7.99)	19.33 (10.96)	**	***	ns
Systolic blood pressure_AUC	−145.00 (868.58)	1,810.00 (1,088.56)	1,583.33 (904.40)	***	***	ns
Systolic blood pressure_AUC_norm	–	1.59 (1.56)	0.55 (0.63)	–	–	0.065
Diastolic blood pressure_peak	−1.33 (12.69)	4.67 (15.37)	12.22 (8.87)	ns	**	ns
Diastolic blood pressure_AUC	−301.33 (1,024.69)	501.67 (1,774.43)	1,113.33 (959.79)	*	**	ns
Diastolic blood pressure_AUC_norm	–	0.27 (2.00)	0.44 (0.33)	–	–	ns
Heart rate_peak	−0.78 (10.24)	12.55 (8.70)	14.67 (10.46)	*	*	ns
Heart rate_AUC	−365.00 (1,034.73)	1,186.67 (685.56)	1,371.67 (1,262.21)	**	*	ns
Heart rate_AUC_norm	–	0.88 (0.62)	0.45 (0.62)	–	–	0.096
Autonomic measures						
Temperature_peak	0.10 (0.61)	0.27 (0.59)	0.45 (0.69)	ns	ns	ns
Temperature_AUC	−12.50 (86.66)	27.67 (99.72)	81.54 (137.94)	*	0.055	ns
Temperature_AUC_norm	–	−0.01 (0.10)	0.02 (0.07)	–	–	ns
Pupillary diameter_peak	0.32 (0.70)	1.03 (0.65)	1.09 (0.40)	*	*	ns
Pupillary diameter_AUC	52.67 (77.98)	166.83 (100.40)	188.00 (86.68)	**	*	ns
Pupillary diameter_AUC_norm	–	0.14 (0.11)	0.05 (0.03)	–	–	ns

Means (SD) of the scores obtained and results of the statistical analysis performed. $N=9$, except for normalized AUCs where $n=8$

PLA placebo, *AYA0* ayahuasca0, *AYA2* ayahuasca2. Peak beta power expressed as percentage; Peak systolic blood pressure in mmHg; Peak diastolic blood pressure in mmHg; Peak heart rate in beats/minute; Peak body temperature in °C; Peak pupillary diameter in millimeters

*$p<0.05$, ** $p<0.01$, *** $p<0.001$. Exact p values are given when $p<0.1$

Autonomic effects

Autonomic effects are shown in Fig. 2 and Table 2. No significant differences between ayahuasca and placebo or between active treatments were found for temperature peak values. For pupillary diameter, *Aya0* and *Aya2* produced significant increases in peak values relative to placebo. No significant differences were found between active treatments. For AUC values, only *Aya0* produced statistically significant increases in temperature relative to placebo. *Aya2* only showed a trend for significantly higher AUC values than placebo. No significant differences were found between ayahuasca treatments. For pupillary diameter, *Aya0* and *Aya2* produced significant AUC increases relative to placebo. No significant differences were found between ayahuasca treatments. Finally, no differences were found between *Aya0* and *Aya2* in temperature and pupillary diameter when the normalized AUCs were compared.

Neuroendocrine effects

Neuroendocrine effects results are shown in Fig. 3 and Table 3.

Peak and AUC prolactin values after *Aya0* and *Aya2* were significantly increased relative to placebo. Increases after *Aya2* were significantly higher than after *Aya0*. For cortisol, only *Aya2* produced increases significantly different from placebo. A trend was seen for AUC values after *Aya0*. Increases after *Aya2* were significantly higher than after *Aya0* in terms of both peak and AUC values. For growth hormone, only *Aya0* produced increases in peak values significantly

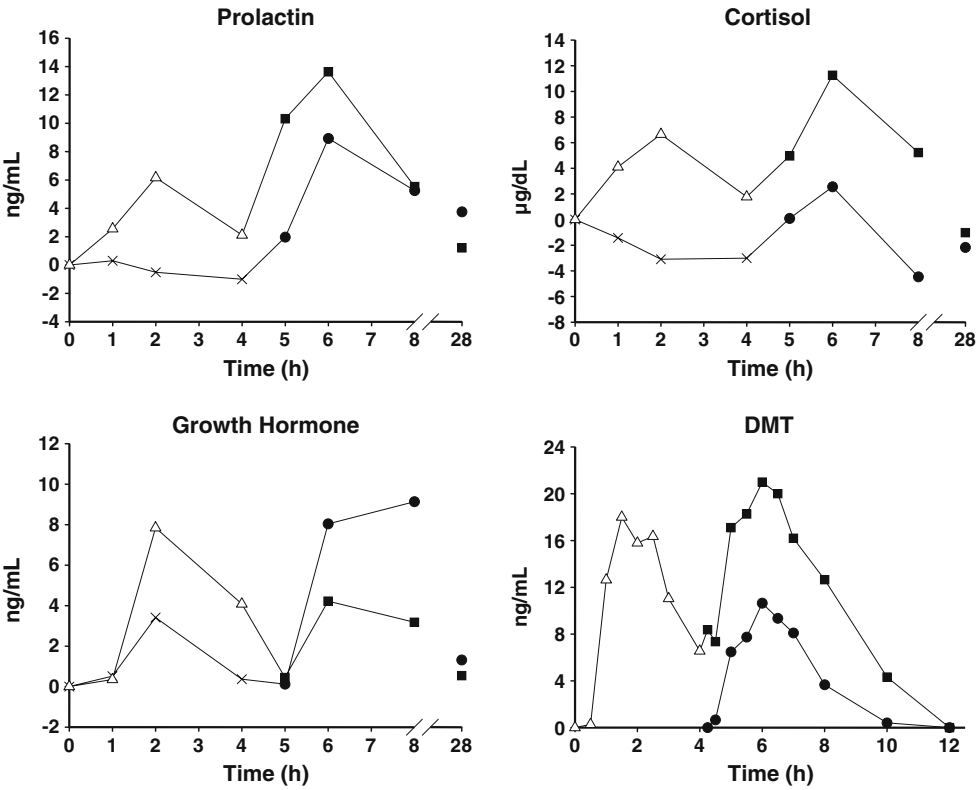


Fig. 3 Time course of neuroendocrine measures (means from nine volunteers) and DMT plasma concentrations (means from eight volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo, and *Aya2* was preceded 4 h by *Aya1*

different from placebo. A trend to lower peak values was found for *Aya2* when compared with *Aya0*. In terms of AUC values, *Aya2* produced significant increases from placebo, whereas a marginally significant effect (p=0.052) was observed for *Aya0*. No significant differences were observed between active treatments. The comparison of the normalized AUCs between active treatments yielded non-significant results for prolactin and cortisol and significantly lower values for growth hormone.

Lymphocyte subpopulations

Treatment effects on lymphocyte subpopulations are shown in Fig. 4 and Table 3.

The total lymphocyte percentage did not show any significant changes after either of the two ayahuasca treatments in terms of AUC or peak values. However, *Aya2* (AUC) decreased total lymphocyte percentage more than *Aya0*. CD3 lymphocyte levels were found to be decreased after *Aya0*, but not after *Aya2*. No differences were found between ayahuasca treatments. Peak CD4 levels showed a trend for a significant decrease after both ayahuasca treatments. Furthermore, CD4 AUC value decreases reached statistical significance after both ayahuasca treatments. But again, no differences were found between active treatments. No significant changes were found for CD8 lymphocytes (peak and AUC), but there was a trend for a significant reduction after *Aya0*. No differences were found between *Aya0* and *Aya2*. The analysis of CD19 levels yielded mixed results. Whereas *Aya0* produced a marginally significant reduction (p=0.050) in AUC, *Aya2* significantly reduced peak values. No differences were found in AUC between ayahuasca treatments, but *Aya2* produced a significantly higher reduction than *Aya0* in peak values. NK cells were significantly increased after both ayahuasca administrations (AUC) and after *Aya0* (peak value). There was a trend for a significant increase after *Aya2* (peak). No differences were found between ayahuasca treatments. The comparison of the normalized AUCs between active treatments yielded non-significant results for all lymphocyte subpopulations.

Pharmacokinetic analysis

The time course of DMT plasma concentrations is shown in Fig. 3. One volunteer did not show measurable levels of DMT after *Aya0* and was excluded from the pharmacokinetic analyses. The mean±SD of the maximum concentration values (C_{max}) was 13.97±9.35 ng/ml for *Aya0* and 32.57±20.96 ng/ml for *Aya2*. These values were statistically different [$t(7)=-2.92$, $p=0.022$]. The median (range) time at which the C_{max} was attained (t_{max})was 2.0 h (1–3) for *Aya0* and 2.0 h (1–3) for *Aya2*. These values were not statistically different [$z=-0.32$, $p>0.1$]. The AUC values were 1,703 mg/ml·min^{-1} for *Aya0* and 4,078 mg/ml·min^{-1} for *Aya2*. These values were statistically different. [$t(7)=-2.78$, $p=0.027$]. To test whether the higher DMT AUCs obtained

Table 3 Effects induced by placebo, *Aya0*, and *Aya2* on neuroendocrine parameters and lymphocyte subpopulations

	Placebo	Ayahuasca0	Ayahuasca2	Pair-wise comparisons		
				PLA/AYA0	PLA/AYA2	AYA0/AYA2
Hormones						
Prolactin_peak	−0.78 (3.03)	12.89 (9.41)	16.78 (10.11)	**	**	*
Prolactin_AUC	−88.37 (442.52)	1,206.35 (1,295.05)	2,241.37 (1,280.70)	*	**	*
Prolactin_AUC_norm	–	1.36 (2.24)	0.81 (0.73)	–	–	ns
Cortisol_peak	−2.89 (7.75)	−0.55 (9.59)	11.33 (6.30)	ns	**	**
Cortisol_AUC	−544.50 (1,286.94)	−114.07 (1,286.70)	1,678.33 (1,186.12)	0.078	**	**
Cortisol_AUC_norm	–	−0.08 (1.58)	0.53 (0.44)	–	–	ns
GH_peak	3.44 (5.70)	15.00 (11.77)	6.22 (4.18)	*	ns	0.076
GH_AUC	359.78 (539.55)	1,290.16 (1,024.17)	719.85 (421.33)	0.052	*	ns
GH_AUC_norm	–	1.30 (1.20)	0.26 (0.22)	–	–	*
Lymphocyte subpopulations						
Total lymphocytes_peak	−0.13 (0.35)	−0.02 (0.60)	−0.33 (0.88)	ns	ns	ns
Total lymphocytes_AUC	−21.50 (38.22)	−7.32 (69.26)	−64.15 (123.23)	ns	ns	*
Total lymphocytes_AUC_norm	–	0.00 (0.07)	−0.01 (0.03)	–	–	ns
CD3_peak	−4.22 (5.02)	−10.00 (7.24)	−11.11 (15.98)	*	ns	ns
CD3_AUC	−516.67 (790.43)	−1,460.00 (1,337.61)	−1,343.30 (2,009.07)	*	ns	ns
CD3_AUC_norm	–	−1.40 (2.06)	−0.29 (0.94)	–	–	ns
CD4_peak	−3.89 (4.23)	−8.55 (6.52)	−10.44 (8.40)	0.058	0.097	ns
CD4_AUC	−396.67 (741.37)	−1,190.00 (1,104.92)	−1,576.67 (1,382.53)	*	**	ns
CD4_AUC_norm	–	−1.01 (1.36)	−0.56 (0.63)	–	–	ns
CD8_peak	0.11 (3.29)	−1.67 (4.21)	−0.89 (3.95)	0.082	ns	ns
CD8_AUC	−50.00 (457.19)	−270.00 (681.96)	−93.95 (621.16)	ns	ns	ns
CD8_AUC_norm	–	−0.39 (1.03)	−0.01 (0.20)	–	–	ns
CD19_peak	1.67 (4.24)	0.89 (4.75)	−3.22 (1.79)	ns	*	*
CD19_AUC	250.00 (713.41)	126.67 (817.02)	−203.33 (160.00)	0.050	ns	ns
CD19_AUC_norm	–	0.06 (0.43)	−0.07 (0.08)	–	–	ns
NK_peak	2.33 (5.72)	8.33 (6.10)	8.78 (9.46)	*	0.096	ns
NK_AUC	293.33 (817.85)	1,293.33 (1,074.30)	1,433.33 (1,387.46)	**	*	ns
NK_AUC_norm	–	1.15 (1.66)	0.42 (0.55)	–	–	ns

Means (SD) of the values obtained and results of the statistical analysis performed. $N=9$, except for normalized AUCs where $n=8$

PLA placebo, *AYA0* ayahuasca0, *AYA2* ayahuasca2

*$p<0.05$, **$p<0.01$. Exact p values are given when $p<0.1$

after *Aya2* were larger than the mere superposition over the remaining DMT levels of the preceding ayahuasca dose (*Aya1*), the $AUC_{4\text{-}8h}$ of *Aya1* was calculated for each volunteer and subtracted from the AUC obtained after *Aya2*. The corrected values were again compared vs. the AUC values obtained after *Aya0*. The corrected AUC value was 2,993 mg/ml·min^{-1}. The comparison vs. *Aya0* yielded non-significant results [$t(7)=-1.71$, $p>0.1$].

Discussion

The aim of the present investigation was to study the pharmacology of two consecutive doses of ayahuasca and to test whether acute tolerance or sensitization phenomena occurred. To our knowledge, this is the first study of this nature conducted to date. In our view, it is important to gather this information considering the increasing popularity of ayahuasca preparations worldwide (Tupper 2008) and the common practice of ingesting several doses in a single session.

The administered dose of 0.75 mg DMT/kg was above the threshold of psychoactivity and proved physiologically active on many levels. Results for the individual ayahuasca treatments replicate and extend previous findings. Statistically significant psychological and physiological effects were observed when compared with placebo. This dose had been found to be psychoactive in a previous study (Riba et al. 2001a). In the present work, the administration of two

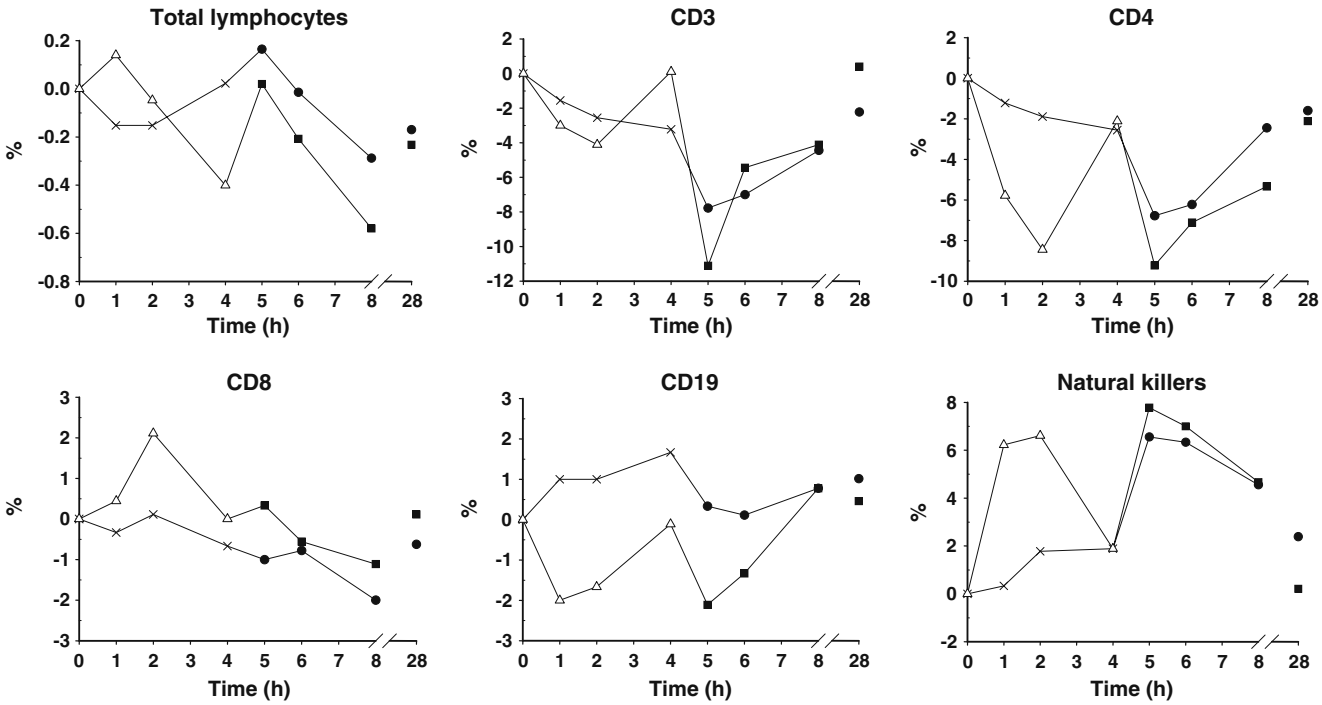


Fig. 4 Time course of effects on lymphocyte subpopulations (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo, and *Aya2* was preceded 4 h by *Aya1*

identical doses in succession with an interval of 4 h, led to virtually all subjective effects measures showing higher mean values after the second dose. Psychotropic effects were more intense, but unpleasant somatic effects and impairment also increased. In this respect, it is worth noting that several volunteers had to be excluded from the study due to vomiting after *Aya2*. Vomiting is commonly reported for liquid ayahuasca but rarely observed after single dose administration of the encapsulated freeze-dried formulation (Riba et al. 2001a; Riba et al. 2003).

The increase in psychotropic effects after the second dose can be explained by the significantly higher DMT levels attained. DMT was still present in blood at 4 h after *Aya1*, and DMT levels from the second dose were superimposed upon the first. The comparison of AUCs (see "Pharmacokinetic analysis" section) showed that the superimposition was linear, that is, no disproportionately higher DMT levels were attained after *Aya2*, when the DMT remaining from *Aya1* is taken into account.

At the subjective level, the results obtained are in line with those in the aforementioned study by Riba et al. (2001a) where researchers found that 0.75 mg DMT/kg produced significant increases in the same VAS items measuring overall psychotropic effects, perceptual modifications, and the VAS item "liking". The present time course of effects is also analogous to that previously reported, with effects peaking at 2 h after dosing. Furthermore, the pattern of responses in the HRS and ARCI are also equivalent. However, in the present study, ayahuasca significantly increased all HRS subscales including Volition, the only subscale that was not modified in the 2001 study. Here, both ayahuasca treatments consistently increased scores on the MBG and A scales of the ARCI. Identical findings were obtained by Riba et al. (2001a). In the present study, the comparison between ayahuasca treatments showed significantly higher somatic and unpleasant effects and impairment after *Aya2*. Auditory effects were also significantly enhanced. However, we did not obtain statistically robust evidence of sensitization. When VAS scores were normalized by DMT levels, we observed only a trend for increased unpleasant effects and for decreased stimulation. These results are in line with those by Strassman et al. (1996) who did not find differences in subjective scores (measured with the HRS) between the first and the fourth of four doses of intravenous DMT administered at 30-min intervals. However, contrary to the present study, the only significant effect observed was a reduction in Volition scores.

Similar to subjective measures, effects after *Aya2* on spontaneous brain electrical activity were larger than after *Aya0*. Ayahuasca increased relative power in the higher end of the beta EEG frequency band. This increase is an objective measure of the effects of ayahuasca on the CNS and has been reported in the past (Riba et al. 2002; Santos et al., in press). No tolerance or sensitization was observed when DMT levels were taken into account.

Unexpectedly, in contrast with subjective and EEG variables, increases in cardiovascular variables were not larger after *Aya2* than after *Aya0,* despite the fact that the 0.75 mg DMT/kg doses increased SBP, DBP, and HR significantly compared with placebo. The examination of the normalized AUCs showed that mean values after *Aya2* were lower than expected from the increased DMT levels present in plasma. The statistical comparison showed a trend towards a significantly lower response after *Aya2* in SBP and HR. This finding suggests that a certain acute tolerance might develop to the inotropic and chronotropic effects of ayahuasca. Strassman et al. (1996) had found non-significant reductions of mean arterial pressure after four closely spaced doses of DMT and a statistically significant reduction in heart rate. Decreases in these variables after repeated ayahuasca or DMT administration could be related to some level of desensitization (Roth et al. 1995; Romano et al. 2010), decreased signaling (Gresch et al. 2005), or downregulation of the 5-HT_{2A} receptor (Smith et al. 1999; Aloyo et al. 2001; Dougherty and Aloyo 2011).

Ayahuasca effects on autonomic variables appear to be more inconsistent than those of pure DMT. Temperature was affected by ayahuasca in the present study. However, despite larger mean values after *Aya2*, only *Aya0* produced statistically significant increases in temperature relative to placebo. A previous study in laboratory conditions did not find a clear-cut pattern of effects for this variable (Santos et al., in press). In contrast, Strassman and Qualls (1994) found increases in rectal temperature for intravenous DMT administered at doses of 0.2–0.4 mg/kg, an effect that was also observed previously for ayahuasca (Callaway et al. 1999) and for serotonergic compounds such as the mixed 5-HT agonist meta-chlorophenylpiperazine (*m*-CPP; Ghaziuddin et al. 2003), which suggests a non-specific hyperthermic effect of serotonergic stimulation. After repeated administration of DMT, Strassman and colleagues found non-significant reductions for this variable (Strassman et al. 1996).

In the present work, pupillary diameter was significantly increased after both ayahuasca doses. Mydriasis has been consistently described for DMT (Rosenberg et al. 1963; 1964; Strassman and Qualls 1994), whereas myosis has been observed for the 5-HT_2 antagonist ketanserin (Koudas et al. 2009). Increases in pupillary diameter were reported for ayahuasca by Callaway et al. (1999), who administered a lower dose (0.48 mg DMT/kg) and compared changes vs. baseline values, and by Santos et al. (in press) for a higher 1.0 mg DMT/kg dose. Increases in pupil diameter after *Aya2* were lower than expected when DMT levels were considered, but this effect did not reach statistical significance, and tolerance development cannot be concluded with certainty. A previous study in humans did not find any tolerance to the mydriatic effect of intramuscular DMT given twice daily for 5 days (Gillin et al. 1976).

At the neuroendocrine level, the observed increases in prolactin and cortisol also replicate previous findings (Santos et al., in press). Though increases after *Aya2* were significantly larger than after *Aya0* for prolactin and cortisol, when DMT levels were taken into account, no tolerance or sensitization was observed. However, the statistical comparison of the normalized AUCs suggests that tolerance develops to GH liberation after repeated exposure to ayahuasca. Strassman et al. (1996) had described acute tolerance development to the neuroendocrine effects of DMT.

Decreased GH after the second ayahuasca dose could be explained by changes at the 5-HT_{1A} receptor. Agonism at this site enhances GH release (Seletti et al. 1995; Pitchot et al. 2002), and consequently, higher GH levels would have been expected after the second ayahuasca dose. However, increased serotonergic tone and prolonged 5-HT_{1A} receptor stimulation have been found to decrease responsiveness at this level. Reduced GH secretion has been observed in humans given the 5-HT_{1A} agonists gepirone and buspirone after pretreatment with paroxetine and fluvoxamine, respectively (Sargent et al. 1997; Anderson et al. 1996). In another study, the same effect was observed for ipsapirone, also a 5-HT_{1A} agonist, after pretreatment with fluoxetine (Lerer et al. 1999). Furthermore, the repeated administration of selective 5-HT_{1A} agonists can lead to decreased receptor responsiveness in rats not only after several days (Assié et al. 2006), but also as early as 15 min after a single dose of 8-OH-DPAT (Riad et al. 2001). The rapid desensitization observed after 8-OH-DPAT was caused by the internalization of the 5-HT_{1A} receptor. Both prolonged activation of the 5-HT_{1A} receptor sites by DMT and increased serotonin caused by MAO inhibition (harmine and harmaline; McKenna et al. 1984) and serotonin reuptake inhibition (THH; Buckholtz and Boggan 1977) could have led to 5-HT_{1A} desensitization and decreased GH after the second dose.

The immunomodulatory effects of ayahuasca were analogous to those previously reported (Santos et al., in press), i.e., decreased CD4 and elevated NK subpopulations compared with placebo. Effects on CD19 and CD3 cells were less consistent and non-significant. No tolerance or sensitization was observed for any of the studied variables.

The absence of acute tolerance development for most of the variables assessed in the present investigation mimics results for DMT, the main active principle in ayahuasca. Clear tolerance has not been reported in animals (Cole and Pieper 1973; Gillin et al. 1973; Kovacic and Domino 1976) or in humans (Gillin et al. 1976) in the older literature. In the more recent study in humans by Strassman et al. (1996),

differential effects were observed depending on the studied variable. Subjective effects remained unchanged, but heart rate and ACTH, and prolactin levels, showed acute tolerance after repeated administration within a single experimental session. An analogous dissociation would be observed to a certain extent for ayahuasca.

The present study was limited by the small sample size. This was largely due to the adverse events associated with repeated ayahuasca intake. Five volunteers were excluded due to vomiting, which in three instances occurred after the administration of the second dose. Consequently, our results were obtained from those participants who tolerated ayahuasca better and may not be easily generalized.

In conclusion, the administration of two consecutive doses of ayahuasca led to higher DMT concentrations in plasma and increased psychotropic effects. The second dose was less well-tolerated leading to a higher incidence of unpleasant effects and vomiting. With regard to acute tolerance or sensitization development, a certain dissociation was observed. Whereas neither phenomenon was found for subjective, neurophysiological, autonomic, and immunological effects, tolerance was observed for GH and a trend for SBP and HR.

Acknowledgments We wish to thank Antoni Pastor and Rafael de la Torre (IMIM-Parc de Salut Mar) for the determination of DMT concentrations in plasma. This work was supported by grant SAF 2002–02746 from the Spanish Ministry of Education and Science and a private donation by Richard Wolfe. MV is supported by FIS through grant CP04/00121 from the Spanish Ministry of Health in collaboration with the Institut de Recerca de l'Hospital de Sant Pau.

Conflicts of interest and source of funding This work was supported by grant SAF 2002–02746 from the Spanish Ministry of Education and Science and a private donation by Richard Wolfe. The authors declare no conflict of interest. MV is supported by FIS through grant CP04/00121 from the Spanish Ministry of Health in collaboration with the Institut de Recerca de l'Hospital de Sant Pau.

References

Aloyo VJ, Dave KD, Rahman T, Harvey JA (2001) Selective and divergent regulation of cortical 5-HT_{2A} receptors in rabbit. J Pharmacol Exp Ther 299:1066–1072

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders (4th ed., text rev.). APA, Washington DC

Anderson IM, Deakin JF, Miller HE (1996) The effect of chronic fluvoxamine on hormonal and psychological responses to buspirone in normal volunteers. Psychopharmacology (Berl) 128:74–82

Assié MB, Lomenech H, Ravailhe V, Faucillon V, Newman-Tancredi A (2006) Rapid desensitization of somatodendritic 5-HT_{1A} receptors by chronic administration of the high-efficacy 5-HT_{1A} agonist, F13714: a microdialysis study in the rat. Br J Pharmacol 149:170–178

Bellido M, Rubiol E, Ubeda J, Estivill C, López O, Manteiga R, Nomdedéu JF (1998) Rapid and simple immunophenotypic characterization of lymphocytes using a new test. Haematologica 83:681–685

Buckholtz NS, Boggan WO (1977) Inhibition by β-carbolines of monoamine uptake into a synaptosomal preparation: structure-activity relationships. Life Sci 20:2093–2099

Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, Andrade EN, Andrade EO, Mash DC (1999) Pharmacokinetics of Hoasca alkaloids in healthy humans. J Ethnopharmacol 65:243–256

Cole JM, Pieper WA (1973) The effects of *N*,*N*-dimethyltryptamine on operant behavior in squirrel monkeys. Psychopharmacologia 29:107–112

Dougherty JP, Aloyo VJ (2011) Pharmacological and behavioral characterization of the 5-HT_{2A} receptor in C57BL/6 N mice. Psychopharmacology (Berl) 215:581–593

First MB, Spitzer RL, Gibbon M, Williams JB (1999) Entrevista Clínica Estructurada para los Trastornos del Eje I del DSM-IV: Versión Clínica (SCID-VC). Masson, Barcelona

Ghaziuddin N, Welch K, Greden J (2003) Central serotonergic effects of m-chlorophenylpiperazine (*m*CPP) among normal control adolescents. Neuropsychopharmacology 28:133–139

Gillin JC, Cannon E, Magyar R, Schwartz M, Wyatt RJ (1973) Failure of *N*,*N*-dimethyltryptamine to evoke tolerance in cats. Biol Psychiatry 7:213–220

Gillin JC, Kaplan J, Stillman R, Wyatt RJ (1976) The psychedelic model of schizophrenia: the case of *N*,*N*-dimethyltryptamine. Am J Psychiatry 133:203–208

Gresch PJ, Smith RL, Barrett RJ, Sanders-Bush E (2005) Behavioral tolerance to lysergic acid diethylamide is associated with reduced serotonin-2A receptor signaling in rat cortex. Neuropsychopharmacology 30:1693–1702

Isbell H, Belleville RE, Fraser WA, Logan CR (1956) Studies on lysergic acid diethylamide (LSD-25). I. Effects in former morphine addicts and development of tolerance during chronic intoxication. Arch Neurol Psychiatry 76:468–478

Koudas V, Nikolaou A, Hourdaki E, Giakoumaki SG, Roussos P, Bitsios P (2009) Comparison of ketanserin, buspirone and propranolol on arousal, pupil size and autonomic function in healthy volunteers. Psychopharmacology (Berl) 205:1–9

Kovacic B, Domino EF (1976) Tolerance and limited cross-tolerance to the effects of *N*,*N*-dimethyltryptamine (DMT) and lysergic acid diethylamide-25 (LSD) on food-rewarded bar pressing in the rat. J Pharmacol Exp Ther 197:495–502

Lamas X, Farré M, Llorente M, Camí J (1994) Spanish version of the 49-item short form of the Addiction Research Center Inventory. Drug Alcohol Depend 35:203–209

Lerer B, Gelfin Y, Gorfine M, Allolio B, Lesch KP, Newman ME (1999) 5-HT_{1A} receptor function in normal subjects on clinical doses of fluoxetine: blunted temperature and hormone responses to ipsapirone challenge. Neuropsychopharmacology 20:628–639

Martin WR, Sloan JW, Sapira JD, Jasinski DR (1971) Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. Clin Pharmacol Ther 12:245–258

McKenna DJ, Towers GHN, Abbott F (1984) Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of ayahuasca. J Ethnopharmacol 10:195–223

Pitchot W, Wauthy J, Hansenne M, Pinto E, Fuchs S, Reggers J, Legros JJ, Ansseau M (2002) Hormonal and temperature responses to the 5-HT_{1A} receptor agonist flesinoxan in normal volunteers. Psychopharmacology (Berl) 164:27–32

Riad M, Watkins KC, Doucet E, Hamon M, Descarries L (2001) Agonist-induced internalization of serotonin-1A receptors in the dorsal raphe nucleus (autoreceptors) but not hippocampus (heteroreceptors). J Neurosci 21:8378–8386

Riba J (2003) Human pharmacology of ayahuasca. Doctoral dissertation, Universitat Autònoma de Barcelona. Available at http://www.tdx.cesca.es/TDX-0701104-165104

Riba J, Rodriguez-Fornells A, Urbano G, Morte A, Antonijoan R, Monteiro M, Callaway JC, Barbanoj MJ (2001a) Subjective effects and tolerability of the South American psychoactive beverage ayahuasca in healthy volunteers. Psychopharmacology (Berl) 154:85–95

Riba J, Rodriguez-Fornells A, Strassman RJ, Barbanoj MJ (2001b) Psychometric assessment of the Hallucinogen Rating Scale. Drug Alcohol Depend 62:215–223

Riba J, Anderer P, Morte A, Urbano G, Jane F, Saletu B, Barbanoj MJ (2002) Topographic pharmaco-EEG mapping of the effects of the South American beverage ayahuasca in healthy volunteers. Br J Clin Pharmacol 53:613–628

Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanoj MJ (2003) Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J Pharmacol Exp Ther 306:73–83

Romano AG, Quinn JL, Li L, Dave KD, Schindler EA, Aloyo VJ, Harvey JA (2010) Intrahippocampal LSD accelerates learning and desensitizes the 5-HT_{2A} receptor in the rabbit. Psychopharmacology (Berl) 212:441–448

Rosenberg DE, Isbell H, Miner EJ (1963) Comparison of a placebo, *N*-dimethyltryptamine and 6-hydroxy-*N*-dimethyltryptamine in man. Psychopharmacologia 4:39–42

Rosenberg DE, Isbell H, Miner EJ, Logan CR (1964) The effect of *N*, *N*-dimethyltryptamine in human subjects tolerant to lysergic acid diethylamide. Psychopharmacologia 5:217–227

Roth BL, Palvimaki EP, Berry S, Khan N, Sachs N, Uluer A, Choudhary MS (1995) 5-Hydroxytryptamine$_{2A}$ (5-HT_{2A}) receptor desensitization can occur without down-regulation. J Pharmacol Exp Ther 275:1638–1646

Santos RG, Valle M, Bouso JC, Nomdedéu JF, Rodríguez-Espinosa J, McIlhenny EH, Barker SA, Barbanoj MJ, Riba J (in press) Autonomic, neuroendocrine and immunological effects of ayahuasca. A comparative study with *d*-amphetamine

Sargent P, Williamson DJ, Pearson G, Odontiadis J, Cowen PJ (1997) Effect of paroxetine and nefazodone on 5-HT_{1A} receptor sensitivity. Psychopharmacology (Berl) 132:296–302

Seletti B, Benkelfat C, Blier P, Annable L, Gilbert F, de Montigny C (1995) Serotonin$_{1A}$ receptor activation by flesinoxan in humans. Body temperature and neuroendocrine responses. Neuropsychopharmacology 13:93–104

Smith RL, Canton H, Barret RJ, Sanders-Bush E (1998) Agonist properties of *N*,*N*-dimethyltryptamine at 5-HT2A and 5-HT2C serotonin receptors. Pharmacol Biochem Behav 61:323–330

Smith RL, Barrett RJ, Sanders-Bush E (1999) Mechanism of tolerance development to 2,5-dimethoxy-4-iodoamphetamine in rats: down-regulation of the 5-HT_{2A}, but not 5-HT_{2C}, receptor. Psychopharmacology (Berl) 144:248–254

Strassman RJ, Qualls CR (1994) Dose-response study of *N*,*N*-dimethyltryptamine in humans. I. Neuroendocrine, autonomic and cardiovascular effects. Arch Gen Psychiatry 51:85–97

Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R (1994) Dose−response study of *N*,*N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch Gen Psychiatry 51:98–108

Strassman RJ, Qualls CR, Berg LM (1996) Differential tolerance to biological and subjective effects of four closely spaced doses of *N*,*N*-dimethyltryptamine in humans. Biol Psychiatry 39:784–795

Tupper KW (2008) The globalization of ayahuasca: harm reduction or benefit maximization? Int J Drug Policy 19:297–303

Yritia M, Riba J, Ortuno J, Ramirez A, Castillo A, Alfaro Y, De la Torre R, Barbanoj MJ (2002) Determination of *N*,*N*-dimethyltryptamine and β-carboline alkaloids in human plasma following oral administration of ayahuasca. J Chromatogr B 779:271–281

Autonomic, Neuroendocrine, and Immunological Effects of Ayahuasca

A Comparative Study With D-Amphetamine

Rafael G. dos Santos, MS,†‡, Marta Valle, PhD,†‡§, José Carlos Bouso, MS,*†‡, Josep F. Nomdedéu, MD,‖, José Rodríguez-Espinosa, MD, PhD,¶, Ethan H. McIlhenny, MS,#, Steven A. Barker, PhD,#, Manel J. Barbanoj, MD, PhD,††‡, and Jordi Riba, PhD*†‡*

Abstract: Ayahuasca is an Amazonian psychotropic plant tea combining the 5-HT_{2A} agonist *N*,*N*-dimethyltryptamine (DMT) and monoamine oxidase–inhibiting β-carboline alkaloids that render DMT orally active. The tea, obtained from *Banisteriopsis caapi* and *Psychotria viridis*, has traditionally been used for religious, ritual, and medicinal purposes by the indigenous peoples of the region. More recently, the syncretistic religious use of ayahuasca has expanded to the United States and Europe. Here we conducted a double-blind randomized crossover clinical trial to investigate the physiological impact of ayahuasca in terms of autonomic, neuroendocrine, and immunomodulatory effects. An oral dose of encapsulated freeze-dried ayahuasca (1.0 mg DMT/kg body weight) was compared versus a placebo and versus a positive control (20 mg D-amphetamine) in a group of 10 healthy volunteers. Ayahuasca led to measurable DMT plasma levels and distinct subjective and neurophysiological effects that were absent after amphetamine. Both drugs increased pupillary diameter, with ayahuasca showing milder effects. Prolactin levels were significantly increased by ayahuasca but not by amphetamine, and cortisol was increased by both, with ayahuasca leading to the higher peak values. Ayahuasca and amphetamine induced similar time-dependent modifications in lymphocyte subpopulations. Percent CD4 and CD3 were decreased, whereas natural killer cells were increased. Maximum changes occurred around 2 hours, returning to baseline levels at 24 hours. In conclusion, ayahuasca displayed moderate sympathomimetic effects, significant neuroendocrine stimulation, and a time-dependent modulatory effect on cell-mediated immunity. Future studies on the health impact of long-term ayahuasca consumption should consider the assessment of immunological status in regular users.

Key Words: ayahuasca, autonomic, neuroendocrine, immunity

(*J Clin Psychopharmacol* 2011;31: 00–00)

From *Human Experimental Neuropsychopharmacology, IIB Sant Pau; †Centre d'Investigació de Medicaments, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau; ‡Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM; §Pharmacokinetic and Pharmacodynamic Modelling and Simulation; and Servei ‖Laboratori d'Hematologia and ¶Servei de Bioquímica Clínica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; and #Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

Received December 17, 2010; accepted after revision June 13, 2011.

Reprints: Jordi Riba, PhD, Human Experimental Neuropsychopharmacology, Institut de Recerca, Hospital de la Santa Creu i Sant Pau, St Antoni Maria Claret, 167, Barcelona 08025, Spain (e-mail: jriba@santpau.cat).
†Dr Barbanoj is deceased.

This work was supported by grant SAF 2002-02746 from the Spanish Ministry of Education and Science and a private donation by Richard Wolfe.

ISSN: 0271-0749
DOI: 10.1097/JCP.0b013e31823607f6

Ayahuasca is a psychoactive beverage consumed throughout the Amazon Basin as the aqueous infusion of *Banisteriopsis caapi* and *Psychotria viridis*, 2 plants endemic to the region.[1,2] The tea is a central element of Amazonian shamanism, being used for magico-religious, ceremonial, and medicinal purposes.[2] Chemical analyses have shown that *P. viridis* contains the orally labile serotonergic agonist and monoamine oxidase (MAO) substrate *N*,*N*-dimethyltryptamine (DMT), whereas *B. caapi* contains several alkaloids with β-carboline structure (harmine, harmaline, and tetrahydroharmine) showing MAO inhibiting properties.[3] The β-carbolines present in ayahuasca reversibly block visceral MAO-A,[4,5] allowing the access of DMT to systemic circulation and the central nervous system (CNS). At the molecular level, DMT binds at 5-HT_{2A}, 5-HT_{1A}, and 5-HT_{2C} receptor sites,[6–8] eliciting psychedelic effects in humans.[9,10]

In recent years, ayahuasca use has spread from the indigenous to the general population not only in the Amazon but also to countries around the world. A relevant factor in this expansion is the beverage's use in syncretic religions. Several religious groups originating from Brazil and using ayahuasca as a sacrament in a ceremonial context have expanded their activities to Europe and North America. These groups typically ingest ayahuasca over extended periods on a bimonthly basis.[11,12] Based on the traditional uses of ayahuasca and both anecdotal and empirical data on potential health benefits derived from ayahuasca use, some authors have proposed its therapeutic use, especially in the field of drug addiction.[13] Two studies among Brazilian regular users of ayahuasca have found a decrease in illicit drug consumption after initiation of regular ayahuasca use.[14,15] However, despite its potential health benefits, data on the impact of ayahuasca on human physiology are still limited and warrant further investigation.

Previous clinical research has shown that ayahuasca displays distinct physiological and psychotropic effects. At doses equivalent to 0.6 to 0.8 mg DMT/kg body weight, ayahuasca leads to moderate increases in cardiovascular measures, with only diastolic blood pressure reaching statistical significance.[10] The effects of the drug on the CNS have been demonstrated by subjective effects measures and neurophysiological recordings. The analysis of self-report questionnaires has shown significant increases in scales measuring psychostimulant-like subjective activation and modifications of perception and thought processes.[10,16] Electroencephalographic (EEG) effects include an increase in relative power in the faster β band.[17] Central nervous system effects after ayahuasca administration have also been demonstrated by means of neuroimaging techniques (single-photon emission computed tomography)[18] and sleep recordings.[19] The time course of subjective effects and EEG measures runs parallel to DMT concentrations in blood.[3] Increases in diastolic blood pressure and normetanephrine excretion[10] suggest that ayahuasca exerts general sympathomimetic effects, together with

more specific serotonergic effects such as the aforementioned increases in EEG relative β[17] and rapid eye movement sleep suppression.[19]

To date, no controlled study has assessed the impact of acute ayahuasca on neuroendocrine measures and the immune function. In a noncontrolled study, researchers found increases in cortisol levels above preadministration values following a single ayahuasca dose.[20] Cortisol release is known to have an impact on cell immunity, leading to lymphocyte redistribution.[21] Lymphocytes are the main cellular components of the immune system. Lymphocyte deficiencies may predispose to infectious diseases. Some viral infections, such as that caused by the human immunodeficiency virus, may cause sustained and marked decreases in some T-cell subpopulations (CD4 lymphopenia). Substance use, such as alcohol intake and cigarette smoking, has shown detrimental effects on lymphocyte subpopulations.[22,23] Regarding psychedelics, a recent study found decreases in CD8 lymphocytes following the administration of 4-iodo-2, 5-dimethoxyphenyl-isopropylamine (DOI, a 5-HT_{2A} receptor agonist) to mice. This effect was antagonized by the 5-HT_{2A} antagonist ketanserin.[24]

In view of the expanding use of ayahuasca worldwide, in the present study we aimed to explore (*a*) the physiological impact of acute ayahuasca administration in terms of autonomic and neuroendocrine effects and (*b*) the potential effects of ayahuasca on cell-mediated immunity. As described below, autonomic variables, hormone levels, and distribution of lymphocyte subpopulations were evaluated. Cortisol was assessed for its direct role in lymphocyte regulation,[21] and prolactin and growth hormone (GH) were selected as measures of serotonergic stimulation.[25,26] Cell-mediated immunity changes were analyzed assessing the most relevant lymphocyte subpopulations: T lymphocytes (CD3, CD4, CD8), natural killer (NK) cells, and B lymphocytes (CD19). In addition, to verify alkaloid absorption and CNS effects, we also measured DMT plasma levels, subjective effects, and relative EEG β power. To gain greater insight into the specificity or generality of ayahuasca effects, D-amphetamine, a standard sympathomimetic drug, was used as an active comparator.

MATERIALS AND METHODS

Volunteers

Ten young healthy male volunteers were recruited. Mean age was 29.0 years (range, 20–38 years); mean weight was 67.0 kg (range, 60–85 kg); and mean height was 1.77 m (range, 1.69–1.96 m). Volunteers underwent a structured psychiatric interview (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*). Exclusion criteria included presence or history of Axis I disorders and alcohol or other substance dependence. Eligibility criteria included prior use of psychedelics on at least 10 occasions without sequelae derived thereof, that is, psychedelic-related disorders as described in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. A medical examination and laboratory tests were performed before study initiation to rule out any medical condition, allergies, and intolerances.

Participants had used psychedelics from 10 to 100 times. The most commonly used psychedelics were psilocybian mushrooms (10/10) and lysergic acid diethylamide (LSD) (9/10). Less commonly used were ketamine (5/10), peyote (4/10), and mescaline (1/10). None of the participants had used ayahuasca. Besides psychedelics, volunteers had consumed cannabis (10/10), cocaine (10/10), 3,4-methylenedioxymethamphetamine (MDMA) (8/10), and amphetamine (9/10). They reported moderate consumption of alcohol (7 drinks per week), cigarettes (fewer than 10 per day), and caffeinated drinks (<3 per day). Volunteers were in good health, confirmed by medical history, laboratory tests, and electrocardiogram. Prestudy examinations also included drug screening and serological testing (for hepatitis B and C and human immunodeficiency virus). The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans and was approved by the hospital's ethics committee and the Spanish Ministry of Health. The volunteers received detailed information on the nature of ayahuasca, the general psychological effects of psychedelics, and their possible adverse effects, as reported in the psychiatric literature. All volunteers gave their written informed consent to participate.

Drugs

The administered drugs were a placebo (lactose), 20 mg D-amphetamine, and a freeze-dried encapsulated formulation of ayahuasca equivalent to 1 mg DMT/kg body weight. The freeze-dried material was obtained from a Brazilian batch of ayahuasca and contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg tetrahydroharmine per gram. The ayahuasca dose administered was chosen based on an earlier work in which it had been proven to elicit full-blown psychotropic effects.[16] The administration of the placebo and the 2 active treatments in capsules allowed for the adequate masking of drug taste.

Study Design

The study was conducted according to a randomized, double-blind, placebo-controlled, crossover design and involved the participation on 3 experimental sessions at least 1 week apart. Volunteers were requested to abstain from any medication or illicit drug use in the 2 weeks before the experimental sessions and until study completion. Volunteers also abstained from alcohol, tobacco, and caffeinated drinks in the 24 hours before each experimental day. Urinalysis for illicit drug use was performed for each experimental session. On each experimental day, volunteers had a light breakfast before 10:00 AM, and at noon, they received capsules containing 1 of the 3 treatments. During measurements, the volunteers remained seated in a comfortable reclining chair in a quiet dimly lit room. All volunteers remained overnight in the laboratory and were discharged at noon of the following day.

Study Methods

Subjective Effects Measures

Subjective effects were measured by means of 2 self-report questionnaires: the Hallucinogen Rating Scale (HRS) and the Addiction Research Center Inventory (ARCI).

The HRS[9] measures psychedelic-induced subjective effects and includes 6 scales: *somaesthesia*, reflecting somatic effects; *affect*, sensitive to emotional and affective responses; *volition*, indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; *cognition*, describing modifications in thought processes or content; *perception*, measuring visual, auditory, gustatory, and olfactory experiences; and finally *intensity*, which reflects the strength of the overall experience. In the present study, a Spanish adaptation of the questionnaire was used.[27] The range of scores for all HRS scales is 0 to 4.

The short version of the ARCI[28] consists of 5 scales or groups: MBG, morphine-benzedrine group, measuring euphoria and positive mood; PCAG, pentobarbital-chlorpromazine-alcohol group, measuring sedation; LSD scale, measuring

somatic-dysphoric effects; BG, the benzedrine group, measuring intellectual energy and efficiency; and the A scale, an empirically derived scale measuring amphetamine-like effects. The range of scores is 0 to 16 for MBG, −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG, and 0 to 11 for A. The questionnaire had been translated into Spanish and validated by Lamas and coworkers.[29] Volunteers answered the ARCI immediately before drug administration and 4 hours after drug intake, whereas the HRS was answered only at 4 hours after administration.

EEG Measures

Spontaneous brain electrical activity was recorded, preprocessed, and quantified following standard procedures as previously described.[17] In brief, recordings were obtained at 19 scalp locations according to the international 10/20 system by means of a Neuroscan SYNAMPS amplifier. Three-minute EEGs with eyes closed were obtained at 0 (baseline) and 30 minutes and at 1, 1.5, 2, and 2.5 hours after administration. The EEG signal was recorded using high-pass and low-pass filters of 0.3 and 30 Hz, respectively, and digitized online with a sampling frequency of 100 Hz. Following ocular artifact rejection and correction steps, spectral analysis was performed using the fast Fourier transform. The target variable: relative power (expressed as percentage) in the β (13–30 Hz) frequency band was calculated from the spectral density curves at each electrode and time point. An average of relative β power in all 19 leads was used in the subsequent statistical analysis.

Autonomic Measures

Body temperature (°C) was measured by means of a mercury thermometer placed in the participant's armpit. Measurements were conducted at −15 (baseline 1), 0 (baseline 2), and 30 minutes and at 1, 1.5, 2, 4.5, 6, 8, and 10 hours after administration.

Pupillary diameter and pupillary light reflex (PLR) were determined using a Compact Integrated Pupillometer (AMTech GmbH, Weinheim, Germany), tested in darkness after a 5-minute dark adaptation period. Participants were instructed to fix their gaze on a target point located on the wall of the examination room at a distance of about 3 m to prevent pupillary near response or accommodation adjustments. Pupillary light reflexes were elicited by standardized light stimuli (whole-field stimulation) from a light-emitting diode with a duration of 200 milliseconds and using 3 increasing intensities (2.35×10^3, 4.7×10^3, and 9.4×10^3 cd/m^2), stimulus intensity being measured at the source. Changes in pupillary diameter were recorded for 2 seconds with a sampling rate of 250 Hz and stored on a personal computer.[30]

Three consecutive measures were made for each level of intensity, and the mean value was obtained. The different intensities were administered at 2-minute intervals. The target variables were as follows: initial pupillary diameter (in millimeters) and latency (in milliseconds) and amplitude (in millimeters) of the miotic light reflex response. The initial pupillary diameter is the diameter obtained just before light stimulation. The latency period is the interval between the light stimulation and the onset of the pupil contraction. The light reflex amplitude was determined as the difference between the initial and the minimum pupillary diameter after light stimulation.[31] The initial pupillary diameter reflects the sympathetic/parasympathetic balance, whereas latency and light reflex amplitude are parameters reflecting parasympathetic pupillary modulation.[31,32] Measurements were conducted at 0 (baseline) and 30 minutes and at 1, 2, 4, 6, 8, 10, and 24 hours after administration.

Respiration rate (in breaths per minute) was measured by means of a respiratory band placed around the participant's chest. The respiratory signal was digitized and recorded on a computer and later analyzed offline. The number of respiratory events in 1 minute was counted at each recording time point. Measurements were conducted at −15 (baseline 1), 0 (baseline 2), and 30 minutes and at 1, 1.5, 2, and 2.5 hours after administration.

Neuroendocrine Measures

Blood samples (3 mL, plain tubes without clot activator) were drawn at −40 (baseline 1), −10 (baseline 2), and 30 minutes and at 1, 1.5, 2, 4.5, and 6 hours after administration and were allowed to stand at room temperature. Serum was separated by centrifugation and aliquots stored for the analysis of GH, prolactin, and cortisol.

Serum GH and prolactin concentrations were determined by a chemiluminescence immunoassay system (Immulite 2000; Diagnostic Products Corp, EURO/Diagnostic Products Corporation, Llanberis, UK). The GH immunoassay, with a sensitivity of 0.06 mIU/L, uses the WHO first IRP 80/505 and shows intra-assay and interassay coefficients of variation (CVs) of 5.3% to 6.1% and 5.7% to 6.5%, respectively. The prolactin immunoassay uses the third IS 84/500, with an analytical sensitivity of 3.4 mIU/L and intra-assay and total CV between 2.2% to 2.3% and 6.9% to 7.9%, respectively. Serum cortisol concentrations were measured by electrochemiluminescent immunoassay (Elecsys Modular Analytics E170; Roche Diagnostics GmbH Manheim, Germany) with functional sensitivity of less than 8 nmol/L and intra-assay and total CVs of 1.7% and 2.8%, respectively, for mean human serum concentrations between 129 and 717 nmol/L.

Obtained values were transformed to nanograms per milliliter (prolactin and GH) and micrograms per deciliter (cortisol).

Lymphocyte Subpopulations

Blood samples (3 mL, heparin tubes) were drawn at baseline and at 1.5, 2, 4.5, and 24 hours after administration and were subjected to lymphocyte immunophenotyping. The following lymphocyte subpopulations were quantified: CD8 T, CD4 T, CD3 T, CD19 B, and NK cells.

For lymphocyte immunophenotyping, blood samples were stained with the Lymphogram (Cytognos, Salamanca, Spain) reagent kit; each tube contains 5 different murine MoAbs with 3 fluorochromes: CD8 and CD19 with fluorescein isothiocyanate, CD3 and CD56 with phycoerythrin. CD4 were labeled in tandem with PE and cyanate 5. The procedure has been detailed elsewhere.[33] Lymphocyte subpopulations were expressed as percentage of all blood cells.

DMT Plasma Levels

Blood samples (10 mL, EDTA tubes) were drawn at −10 (baseline) and 30 minutes and at 1, 1.5, 2, 2.5, 4.5, 6, and 10 hours after administration for analysis of DMT. Samples were centrifuged at 2000 revolutions per minute for 10 minutes at 4°C, and plasma was immediately frozen at −20°C. Frozen plasma samples were stored at −80°C until analysis. *N,N*-dimethyltryptamine was quantified by the method described by McIlhenny and coworkers,[34] which uses high-pressure liquid chromatography with electrospray ionization and tandem mass spectrometry. The method was adapted to quantify DMT in a plasma matrix using a protein precipitation/dilution protocol.

Protein precipitation 96-well plates (Thermo Scientific, Waltham, Mass) were used to prepare the samples. Analyses were conducted using a Thermo Open Autosampler and a Thermo Accela pumping system interfaced to a Thermo Velos linear ion trap-ion trap system with a heated electrospray ionization probe and operated in the positive ion mode as described.[35] The observed maximum concentration in plasma (C_{max}) and the time to reach this concentration (t_{max}) were determined for each individual.

Statistical Analyses

Subjective Effect measures

Before statistical analysis, ARCI scores were transformed to differences from preadministration values. The transformed ARCI scores and the raw HRS scores were analyzed using a 1-way repeated-measures analysis of variance (ANOVA) with drug (placebo, ayahuasca, amphetamine) as factor. When a significant effect was observed, pairwise comparisons were performed by means of Student *t* test.

EEG, Autonomic, Neuroendocrine, and Lymphocyte Measures

Preadministration values were subtracted from postadministration measures. Subsequently, we calculated peak variations (the maximum absolute change from baseline values). The obtained values were analyzed using a 1-way repeated-measures ANOVA with drug (placebo, ayahuasca, amphetamine) as factor. When a significant effect was observed, pairwise comparisons were performed by means of Student *t* test. In addition, a 2-way repeated-measures ANOVA was conducted, with drug (placebo, ayahuasca, amphetamine) and time point as factors to study the time course of effects. When this ANOVA yielded a significant drug or drug-by-time interaction, individual repeated-measures ANOVAs with drug as factor were performed at each postadministration time point followed by pairwise comparisons using Student *t* test.

DMT Plasma Levels

Descriptive statistics were used to report the characteristics of the time course of plasma DMT concentration. Maximum concentration (C_{max}) and time taken to reach the maximum concentration (t_{max}) were calculated and are reported as mean (SD) and as median and range, respectively.

In all tests performed, differences were considered statistically significant for $P < 0.05$. However, given the exploratory nature of the present study with regard to autonomic, neuroendocrine, and lymphocyte measures, pairwise comparisons between treatments are also reported in those cases where the main ANOVA yielded a $P < 0.1$.

RESULTS

Subjective Effects

Subjective effects results are shown in Table 1.

Compared with placebo, the administration of ayahuasca led to significant increases in all scales of the HRS and in the A, MBG, and LSD of the ARCI. On the other hand, differences from placebo were found for amphetamine in the affect, cognition, and intensity scales of the HRS and in the amphetamine scale of the ARCI. When the 2 active treatments were compared, ayahuasca led to significantly higher scores in the perception, cognition, volition, and intensity scales of the HRS. Regarding the ARCI, the comparison between active treatments found statistically significant differences in the BG scale, with amphetamine leading to increases and ayahuasca to decreases; in the PCAG scale where amphetamine showed decreases and ayahuasca increases; and finally in the LSD scale. Here both treatments led to increases that were much larger after ayahuasca.

EEG Effects

Treatment effects on relative β power are shown in Table 2 and Figure 1. As shown therein, ayahuasca induced significant increases in this variable, which was not modified by

TABLE 1. Subjective Effects Induced by Placebo, D-Amphetamine 20 mg, and Ayahuasca 1 mg DMT/kg

					Pairwise Comparisons		
	Placebo	D-Amphetamine	Ayahuasca	GLM	PLA:AMP	PLA:AYA	AYA:AMP
HRS							
Somaesthesia	0.07 (0.22)	0.5 (0.60)	1.23 (0.70)	<0.01	NS	*	NS
Affect	0.28 (0.09)	0.79 (0.52)	1.36 (0.69)	<0.01	†	*	NS
Perception	0.03 (0.09)	0.24 (0.45)	1.46 (0.97)	<0.001	NS	*	*
Cognition	0.01 (0.03)	0.49 (0.062)	1.58 (1.16)	<0.01	†	*	†
Volition	0.87 (0.74)	0.86 (0.43)	1.84 (0.75)	<0.01	NS	†	*
Intensity	0.00 (0.00)	1.10 (0.85)	2.23 (1.10)	<0.001	*	‡	†
ARCI							
A	0.30 (0.67)	2.20 (2.25)	3.30 (2.67)	<0.01	†	*	NS
BG	0.70 (0.82)	2.90 (2.85)	−0.70 (3.34)	<0.01	NS	NS	*
MBG	−0.10 (0.99)	2.20 (3.26)	3.10 (4.48)	0.069	NS	†	NS
PCAG	−1.60 (3.50)	−3.9 (3.38)	0.30 (5.38)	0.039	NS	NS	†
LSD scale	0.40 (1.65)	1.10 (1.52)	4.20 (2.25)	<0.01	NS	*	*

Values are mean (SD) of the scores obtained for the HRS and ARCI questionnaires subscales (n = 10) and results of the statistical analysis performed.

A indicates Amphetamine; AMP, D-amphetamine; AYA, ayahuasca; BG, benzedrine group; GLM, general linear model; MBG, morphine-benzedrine group; NS, not statistically significant; PCAG, pentobarbital-chlorpromazine-alcohol group; PLA, placebo.

*$P < 0.01$.

†$P < 0.05$.

‡$P < 0.001$.

amphetamine or placebo. The analysis of the time course of effects showed that ayahuasca-induced increases were significant, relative to placebo at 1.5 and 2 hours after dosing. In addition, ayahuasca was different from amphetamine at 1.5 hours.

Autonomic Measures

Autonomic effects results are shown in Table 2 and Figure 1.

Body Temperature

After placebo administration, body temperature showed a steady increase throughout the day. After amphetamine and ayahuasca administration, however, a biphasic pattern was observed; an initial decrease between 0 and 1 hour was followed by a gradual increase thereafter. This increase was larger for amphetamine. The overall analysis did not find any significant modification in peak values after either of the active treatments. However, the analysis of the time course of effects showed a significant decrease for ayahuasca as compared with placebo at 30 minutes after dosing. A significant increase relative to placebo was observed for amphetamine at 2 hours after administration.

Pupillometry

As shown in Figure 1, mean pupillary diameter values before light stimulation were larger for amphetamine and ayahuasca than for placebo. The overall statistical analysis showed a trend increase in peak values. Pairwise comparisons showed significant increases for amphetamine and no overall effect for ayahuasca. The analysis of the time course of effects showed significant elevations relative to placebo for amphetamine from 1 hour onward. Values remained significantly elevated at 24 hours at which time point they also differed significantly from those obtained after ayahuasca. This latter treatment significantly increased pupillary diameter relative to placebo between 0.5 and 2 hours after dosing.

Mean amplitude of the PLR was reduced to varying degrees by the 2 active treatments. The overall statistical analysis showed a trend effect in the general ANOVA for peak values. Pairwise comparisons showed a significant decrease after ayahuasca relative to placebo and no significant effect after amphetamine. The analysis of the time course of effects showed a significant decrease relative to placebo for ayahuasca at 30 minutes and a significant decrease relative to amphetamine at 2 hours.

Opposed patterns were seen between ayahuasca and amphetamine for pupillary reflex latency. Whereas placebo-like variations were observed for amphetamine, ayahuasca increased the mean values. Again, the overall ANOVA only showed a trend to significance. Pairwise comparisons showed trend increases relative to placebo for ayahuasca and amphetamine. Analysis of the time course of effects showed significant increases for ayahuasca relative to placebo and amphetamine at 2 hours.

TABLE 2. Effects Induced by Placebo, D-Amphetamine 20 mg, and Ayahuasca 1 mg DMT/kg on Peak Values for EEG and Autonomic Measures, Neuroendocrine Parameters, and Lymphocyte Subpopulations

					Pairwise Comparisons		
	Placebo	D-Amphetamine	Ayahuasca	GLM	PLA:AMP	PLA:AYA	AYA:AMP
EEG measures							
EEG relative β power	−1.41 (4.96)	−2.05 (4.46)	8.89 (10.56)	<0.01	NS	*	†
Autonomic measures							
Body temperature	0.33 (0.16)	0.49 (0.28)	0.43 (0.28)	>0.1	—	—	—
Pupillary diameter	0.58 (1.11)	1.66 (1.13)	1.50 (1.02)	0.053	†	NS	NS
PLR amplitude	0 (0.31)	−0.07 (0.26)	−0.33 (0.23)	0.065	NS	†	0.074
PLR latency	−3.5 (15.64)	−4.3 (13.67)	11.8 (17.63)	0.068	NS	0.093	0.051
Respiration rate	0.1 (2.86)	−0.45 (4.75)	0.05 (3.20)	>0.1	—	—	—
Hormones							
Prolactin	3.86 (5.07)	1.72 (5.06)	15.53 (12.03)	<0.01	NS	*	†
Cortisol	−5.28 (4.68)	5.31 (6.42)	11.64 (7.39)	<0.001	*	‡	0.070
GH	5.41 (4.74)	6.30 (9.34)	14.06 (15.25)	>0.1	—	—	—
Lymphocyte subpopulations							
Total lymphocytes	−2.40 (7.66)	−2.80 (7.10)	−4.40 (14.39)	>0.1	—	—	—
CD3	0.30 (6.22)	−7.30 (2.67)	−9.10 (6.61)	0.009	†	†	NS
CD4	−2.60 (6.87)	−8.90 (3.45)	−10.50 (6.52)	0.008	†	†	NS
CD8	−0.10 (3.84)	−1.00 (4.57)	−2.70 (4.08)	>0.1	—	—	—
CD19	−0.89 (4.01)	1.89 (6.51)	−1.44 (6.27)	>0.1	—	—	—
NK cells	1.70 (5.25)	7.70 (3.37)	11.60 (10.06)	0.014	†	†	NS

Mean (SD) of the scores obtained (n = 10), and results of the statistical analysis performed. Peak relative β power expressed as percentage; peak body temperature in °C; peak pupillary diameter in mm; peak PLR amplitude in mm; peak PLR latency in milliseconds; peak respiration rate in breaths/min; peak prolactin and peak GH in ng/mL; peak cortisol in μg/dL; peak lymphocyte subpopulations expressed as percentage.

*$P < 0.01$.

†$P < 0.05$.

‡$P < 0.001$.

AMP indicates D-amphetamine; AYA, ayahuasca; NS, not statistically significant; PLA, placebo.

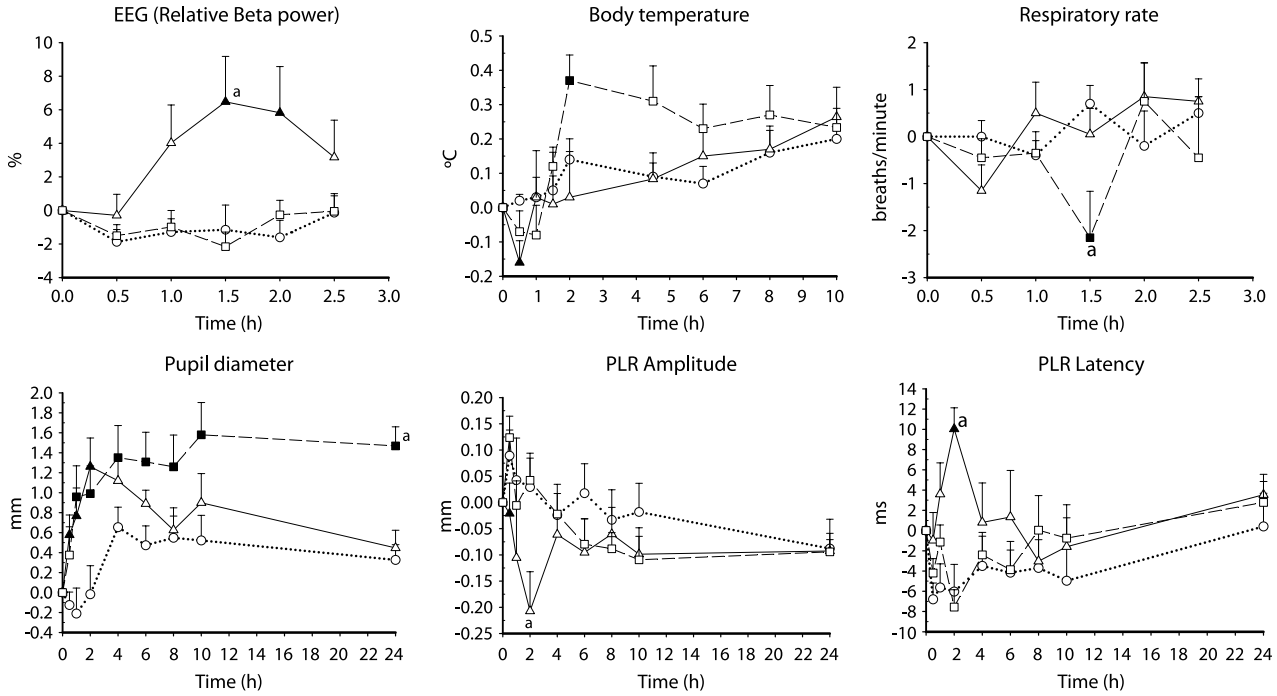


FIGURE 1. Time course of EEG and autonomic measures (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. Error bars denote 1 SEM.

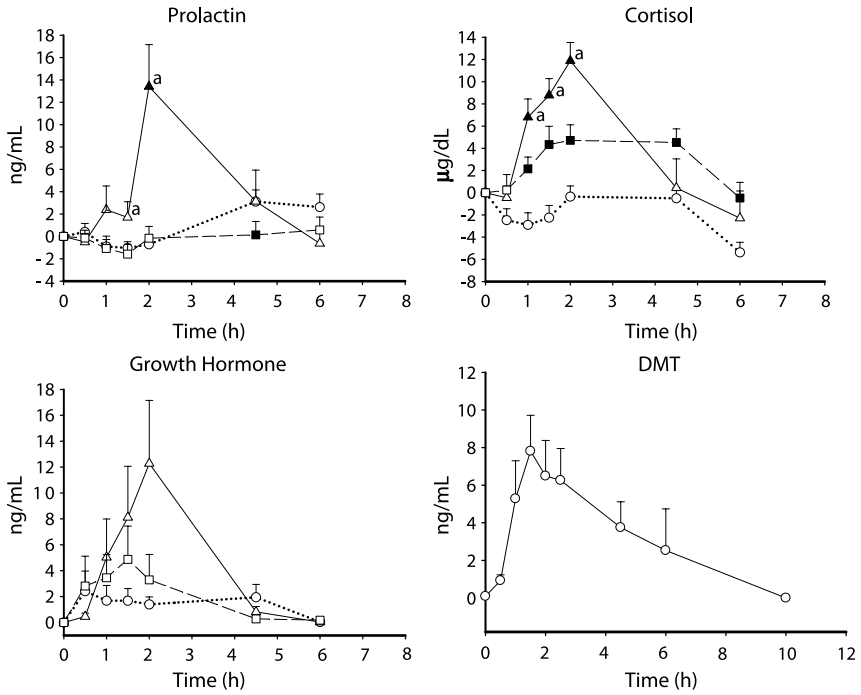


FIGURE 2. The upper panels and the lower left panel show the time course of neuroendocrine measures (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. The lower right panel shows the time course of DMT plasma concentrations after 1.0 mg DMT/kg body weight ayahuasca. Circles indicate means from 10 volunteers. Error bars denote 1 SEM.

Respiration Rate

No significant effect was observed for either active treatment in the overall ANOVA. The analysis of the time course of effects showed significant decreases for amphetamine relative to placebo and ayahuasca at 1.5 hours after dosing.

Neuroendocrine Measures

Neuroendocrine effects results are shown in Table 2 and Figure 2.

Prolactin peak levels after ayahuasca were significantly higher than those after placebo and amphetamine. The analysis of the time course of effects showed that ayahuasca significantly increased prolactin levels relative to placebo at 2 hours. Ayahuasca differed from amphetamine at 1.5 and 2 hours. Interestingly, although amphetamine did not modify prolactin in the peak value analysis, the time course analysis showed significantly reduced levels at 4.5 hours as compared with placebo.

Both active treatments significantly increased cortisol levels relative to placebo. Peak increases after ayahuasca showed a trend to be larger than those after amphetamine. Analyses at the different time points showed significant differences versus placebo in the time interval between 1 and 2 hours for ayahuasca and between 1 and 6 hours for amphetamine. Ayahuasca induced significantly higher cortisol levels than amphetamine at 1, 1.5, and 2 hours.

Regarding GH, although mean values after ayahuasca were higher than after placebo and amphetamine, no significant results were found in the ANOVA or at the individual time points.

Lymphocyte Subpopulations

Treatment effects on lymphocyte subpopulations are shown in Table 2 and Figure 3.

Total lymphocyte percentages in the 24-hour period did not show any significant changes after either of the 2 active treatments. However, the time course analysis showed an increase after ayahuasca relative to placebo at 1.5 hours and a decrease at 4.5 hours. The decrease was significant versus placebo and versus amphetamine. Interestingly, amphetamine nonsignificantly decreased total lymphocyte percentage at this time point. No differences were observed between treatments at 24 hours.

CD3 lymphocyte levels were found to be significantly decreased after ayahuasca and amphetamine. Time course analysis showed significant decreases at 1.5 and 2 hours after ayahuasca and at 1.5, 2, and 4.5 hours after amphetamine. No differences were found between treatments at 24 hours, although mean values were still lower than those after placebo.

Peak CD4 levels were significantly decreased after both active treatments. Time course analysis showed significant decreases at 1.5 and 2 hours for both ayahuasca and amphetamine. Again, no differences were found between treatments at 24 hours.

No significant changes were found for CD8 lymphocytes in the global and time course analyses. CD19 levels were also not found to be modified by any treatment in the ANOVA. However, the time course analysis found a significant decrease after ayahuasca at 2 hours.

Natural killer cells were significantly increased after ayahuasca and amphetamine. The time course analysis showed significant increases versus placebo at 1.5 and 2 hours after ayahuasca and at 1.5, 2, and 4.5 after amphetamine.

DMT Plasma Levels

The time course of DMT plasma concentrations is shown in Figure 2. The mean (SD) of the maximum concentration values

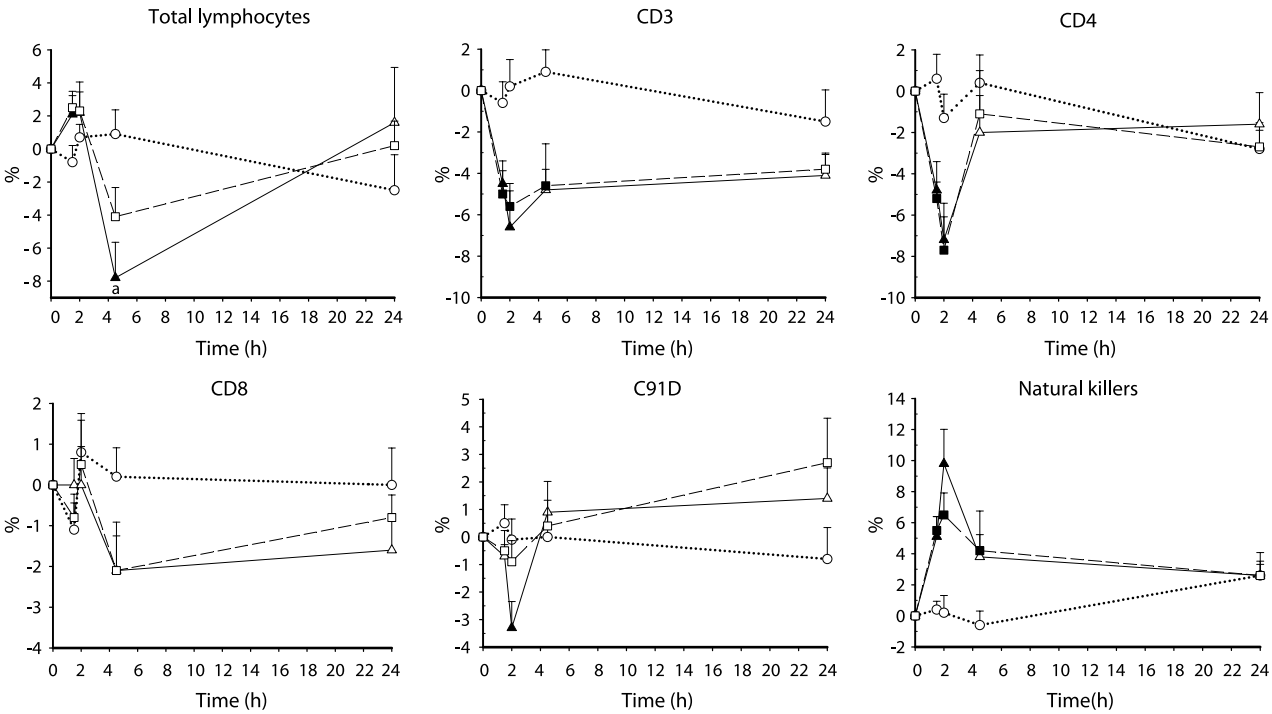


FIGURE 3. Time course of effects on lymphocyte subpopulations (means from 10 volunteers) after administration of placebo (circle, dotted line), 20 mg D-amphetamine (square, dashed line), and 1.0 mg DMT/kg body weight ayahuasca (triangle, solid line). Filled symbols indicate a significant difference from placebo. ''a'' indicates a significant difference between ayahuasca and amphetamine. Error bars denote 1 SEM.

(C_{max}) was 11.8 (SD, 6.4) ng/mL. The median (range) time at which the C_{max} was attained was 1.8 hours (range, 1–4.5 hours) after dosing.

DISCUSSION

The present investigation was undertaken to explore the autonomic, neuroendocrine, and immunomodulatory profile of ayahuasca. Considering the current expansion of ayahuasca use worldwide, we wished to further investigate the impact of this plant tea on human physiology. Results showed that ayahuasca induces relevant modifications in autonomic, neuroendocrine, and immune parameters as discussed below.

Systemic and CNS access of its main active principle was confirmed, respectively, by measurable DMT plasma levels and significant subjective effects. Maximum DMT concentrations were attained at 1.8 hours, in line with previously reported data.[10] Subjective effects included stimulant-like activation (ARCI-A), positive mood (ARCI-MBG), and somatic effects (ARCI-LSD, HRS-somaesthesia), in addition to perceptual modifications (HRS-perception), changes in thought processes and content (HRS-cognition), increased impairment (HRS-volition), and increased emotional lability (HRS-affect). These results replicate previous results after acute ayahuasca administration.[10,16]

The inclusion of amphetamine as an active control helped further characterize the psychotropic effect profile of ayahuasca. Thus, ayahuasca led to significantly higher scores than amphetamine in 4 of the 6 HRS scales (except HRS-somaesthesia and HRS-affect) and in the ARCI-LSD scale. On the other hand, as compared with ayahuasca, amphetamine significantly increased subjective feelings of intellectual energy and efficiency (ARCI-BG) and decreased scores on the sedative-sensitive ARCI-PCAG scale. Increases in the BG and decreases in the PCAG scale are known features of psychostimulants.[28] Although ayahuasca and amphetamine share some sympathomimetic properties, such as mydriasis and increases in blood pressure,[10] the divergences in subjective effects point to differential central mechanisms. Whereas amphetamine was perceived subjectively to increase intellectual energy, ayahuasca effects were rather felt as impairing. Differential effects on the CNS were further evidenced by the statistically significant increases observed in EEG relative β power. This effect was absent after amphetamine and replicates previous findings.[17] Although $5HT_{2A}$ receptor activation has repeatedly been found to cause physical signs of sympathetic activation,[36,37] the serotonergic mechanism leads to a pattern of CNS effects, which clearly differs from that by dopamine- and noradrenaline-enhancing drugs.

Ayahuasca effects on autonomic measures were not particularly robust. Body temperature showed a biphasic time course after ayahuasca and amphetamine. Mean temperature decreased below placebo levels between drug administration and the first hour and gradually rose thereafter. The initial decrease was significant for ayahuasca, but the subsequent increase was not. Previous studies involving the parenteral administration of DMT found inconsistent results for this measure, with 1 study reporting increases and 3 others reporting no change or ambiguous results.[36–39] Amphetamine, on the other hand, caused a nonsignificant initial decrease in body temperature followed by an increase that was larger than after ayahuasca, attaining statistical significance at 2 hours after dosing. Interestingly, a similar biphasic pattern had been previously described for body temperature after amphetamine and after the amphetamine derivative and serotonin releaser MDMA, although changes were statistically different from placebo only for the latter drug.[40,41] Weak changes were also found in the present study for respiration, which was reduced after amphetamine but only at 1 time point. Ayahuasca did not modify this variable. Early controlled studies with parenteral DMT did not find significant changes for this variable,[38] and it has not been measured in more recent studies.

Effects on pupillary diameter were more intense than on other autonomic variables, and a mydriatic effect was observed for both ayahuasca and amphetamine. However, whereas the effect of amphetamine was long lasting (still significant at 24 hours after administration), the effect after ayahuasca was significant only until 2 hours after dosing; mean values had fallen back to placebo levels at 8 hours. These findings are consistent with many previous studies. Mydriasis has been demonstrated in several controlled studies for parenteral DMT[36,38,39] and also for oral amphetamine and other psychostimulants.[28,40] As pupillary diameter is controlled by a balance between sympathetic and parasympathetic tone, based on our present findings ayahuasca seems to display sympathomimetic properties like those of amphetamine. However, ayahuasca was also found to decrease the amplitude and increase the latency of the PLR, effects typically ascribed to anticholinergic drugs. However, none of the components of ayahuasca seem to display affinity at muscarinic receptor sites.[3] A potential explanation is that the observed effects could be due to the noradrenergic inhibition of parasympathetic neurotransmission in the Edinger-Westphal nucleus, the CNS nucleus that controls constriction of the iris. The mixed serotonin/noradrenaline reuptake inhibitor venlafaxine has also been found to increase PLR latency and decrease PLR amplitude in the absence of any affinity of the compound for muscarinic receptors.[42,43] Results in those studies were also interpreted in terms of parasympatholytic effects mediated by noradrenergic inhibition of the Edinger-Westphal nucleus.

This is the first study in which the aforementioned autonomic variables have been measured after ayahuasca in a controlled clinical trial. In the only previous study known to us, the authors reported increases in respiration rate, pupillary diameter, and oral temperature.[20] However, as they did not include a nondrug (placebo) condition, the reported effects cannot be directly compared with ours. The autonomic effects of ayahuasca suggest sympathetic activation of lower intensity than that elicited by parenteral DMT in a dose range of 0.05 to 0.4 mg/kg.[36]

Ayahuasca and amphetamine produced significant time-dependent modifications in neuroendocrine variables and lymphocyte subpopulations. Ayahuasca, which contains the direct serotonergic agonist DMT, increased prolactin levels, whereas amphetamine, which increases noradrenergic and dopaminergic neurotransmission, did not. These results are in line with published data showing prolactin increases after DMT and serotonergic drugs such as MDMA, fenfluramine, and citalopram but not after amphetamine.[40,44] It is well known that dopamine is a potent inhibitor of prolactin secretion.[45] On the other hand, stimulation of the serotonin $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, and $5\text{-}HT_{2C}$ receptors, which are all targets of DMT, increases prolactin release.[26] Neither of the active treatments produced significant changes in GH levels, but mean values were increased after ayahuasca. Growth hormone secretion is stimulated by selective $5\text{-}HT_{1A}$ agonists.[25] The lack of a significant result could be due to the lower affinity of DMT for the $5\text{-}HT_{1A}$ receptor.[7,8] Both ayahuasca and amphetamine produced significant increases in cortisol and variations in lymphocyte subpopulations. Activation of the sympathetic nervous system (SNS) and cortisol release have a well-known modulatory effect on lymphocytes.[21] Thus, besides a potentially direct receptor-mediated action, either

mechanism could be responsible for the changes observed in the present study, that is, reductions in CD3 and CD4 and enhanced NK cell levels. These changes appeared to be transient, with baseline values recovered after 24 hours.

A recent study found DOI reduces CD8 lymphocytes in mice via activation of the 5-HT_{2A} receptor.[24] Although no significant changes in CD8 percentages were found in the present study, data from the study in mice indicate that serotonergic psychedelics can modulate the immune function using a direct mechanism. Nevertheless, the remarkable similarity between the effects of ayahuasca and amphetamine on lymphocyte distribution in the present study suggests that changes may rather have been caused by an indirect mechanism common to both treatments rather than by specific drug-target interactions with immune cells. As mentioned above, this indirect mechanism could involve the hypothalamic-pituitary-adrenal axis and the SNS. Experimental evidence shows that despite their different molecular mechanisms of action; both DMT and amphetamine stimulate the hypothalamic-pituitary-adrenal axis, as reflected by increases in adrenocorticotropic hormone release.[36,46] Regarding the SNS, there is abundant literature on the procatecholaminergic effects of amphetamine,[28] and there is also evidence for increased urine excretion of noradrenaline metabolites after ayahuasca, suggesting an increased activity of the SNS after both drugs.[10] Further evidence on the nonspecific nature of ayahuasca effects is the similarity with the profile of changes induced by MDMA. Effects by this drug have been consistently replicated in many studies and basically involve decreases in CD3 and CD4 T lymphocytes and increases in NK cells.[47,48] Other psychoactive substances, such as cocaine, cannabis, alcohol, nicotine, and opiates, have also been found to modify the status of the immune system, both after acute intake and following chronic exposure.[23,48–50]

The health impact of ayahuasca ingestion in terms of susceptibility to disease is difficult to ascertain with the present data. Reductions in CD3 and CD4 are usually interpreted as detrimental. CD4 cells regulate cytotoxic T cells such as CD8 lymphocytes, which in turn destroy cells infected with intracellular microbes. CD4 cells also regulate B lymphocytes (CD19), which are responsible for antibody secretion.[51] On the other hand, increases in NK cells could be beneficial, these cells being involved in fighting virally infected and cancerous cells.[52,53] However, the overall time-dependent neuroendocrine and immunological profile observed in the present study mimics that observed in humans under stress.[54,55] Increased glucocorticoid levels and lymphocyte redistribution in acute stress have traditionally been regarded as immunosuppressant.[21] However, more recent views emphasize that, contrary to chronic stress, acute stress may have modulatory rather than inhibitory effects on immunity.[56] Considering the increasing popularity of ayahuasca and that ingestion of the tea on a regular basis is a central feature of the ayahuasca religions, the long-term impact of regular use on immunity warrants further investigation.

To conclude, the present findings indicate that acute ayahuasca has a moderate impact on the autonomous nervous system and a more robust activation of the hypothalamic-pituitary-adrenal axis. In addition, acute ayahuasca administration shows modulatory capacity on cell-mediated immunity, inducing a time-dependent redistribution of lymphocyte subtypes. A limitation of this study is the use of single doses only of the administered active drugs, thus precluding the assessment of dose-response relationships for the studied variables. Future studies should evaluate both the acute impact of different ayahuasca doses on immune function and the effects of chronic exposure in frequent users.

AUTHOR DISCLOSURE INFORMATION

The authors declare no conflicts of interest.

REFERENCES

1. Schultes RE, Hofmann A. *The Botany and Chemistry of Hallucinogens*. Springfield, IL: Charles C. Thomas; 1980.
2. Schultes RE, Hofmann A. *Plants of the Gods: Origins of Hallucinogenic Use*. New York: A. van der Marck Editions; 1987.
3. Riba J. *Human Pharmacology of Ayahuasca* [doctoral thesis]. Universitat Autònoma de Barcelona, 2003. Available at: http://www.tdx.cesca.es/TDX-0701104-165104/. Accessed April 28, 2011.
4. Buckholtz NS, Boggan WO. Monoamine oxidase inhibition in brain and liver produced by β-carbolines: structure-activity relationships and substrate specificity. *Biochem Pharmacol*. 1977;26:1991–1996.
5. McKenna DJ, Towers GHN, Abbott F. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of ayahuasca. *J Ethnopharmacol*. 1984;10:195–223.
6. Glennon RA, Dukat M, Grella B, et al. Binding of β-carbolines and related agents at serotonin (5-HT(2) and 5-HT(1A)), dopamine (D(2)) and benzodiazepine receptors. *Drug Alcohol Depend*. 2000;60:121–132.
7. Pierce PA, Peroutka SJ. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. *Psychopharmacology.* 1989;97:118–122.
8. McKenna DJ, Repke DB, Lo L, et al. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. *Neuropharmacology.* 1990;29:193–198.
9. Strassman RJ, Qualls CR, Uhlenhuth EH, et al. Dose-response study of *N*,*N*-dimethyltryptamine in humans, II. Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry.* 1994;51:98–108.
10. Riba J, Valle M, Urbano G, et al. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *J Pharmacol Exp Ther.* 2003;306:73–83.
11. Tupper KW. The globalization of ayahuasca: harm reduction or benefit maximization. *Int J Drug Policy.* 2008;19:297–303.
12. Labate BC, Rose IS, Santos RG. *Ayahuasca Religions: A Comprehensive Bibliography and Critical Essays*. Santa Cruz, CA: Multidisciplinary Association for Psychedelic Studies; 2009.
13. McKenna DJ. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacol Ther.* 2004;102:111–129.
14. Grob CS, McKenna DJ, Callaway JC, et al. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *J Nerv Ment Dis*. 1996;184:86–94.
15. Fábregas JM, González D, Fondevila S, et al. Assessment of addiction severity among ritual users of ayahuasca. *Drug Alcohol Depend*. 2010;111:257–261.
16. Riba J, Rodríguez-Fornells A, Urbano G, et al. Subjective effects and tolerability of the South American psychoactive beverage ayahuasca in healthy volunteers. *Psychopharmacology.* 2001;154:85–95.
17. Riba J, Anderer P, Morte A, et al. Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage ayahuasca in healthy volunteers. *Br J Clin Pharmacol*. 2002;53:613–628.
18. Riba J, Romero S, Grasa E, et al. Increased frontal and paralimbic activation following ayahuasca, the pan-Amazonian inebriant. *Psychopharmacology.* 2006;186:93–98.
19. Barbanoj MJ, Riba J, Clos S, et al. Daytime ayahuasca administration modulates REM and slow-wave sleep in healthy volunteers. *Psychopharmacology.* 2008;196:315–326.

20. Callaway JC, McKenna DJ, Grob CS, et al. Pharmacokinetics of Hoasca alkaloids in healthy humans. *J Ethnopharmacol*. 1999;65: 243–256.
21. Friedman EM, Irwin MR. Modulation of immune cell function by the autonomic nervous system. *Pharmacol Ther*. 1997;74:27–38.
22. Schaberg T, Theilacker C, Nitschke OT, et al. Lymphocyte subsets in peripheral blood and smoking habits. *Lung*. 1997;175:387–394.
23. Friedman H, Newton C, Klein TW. Microbial infections, immunomodulation, and drugs of abuse. *Clin Microbiol Rev.* 2003;16:209–219.
24. Davydova SM, Cheido MA, Gevorgyan MM, et al. Effects of 5-HT_{2A} receptor stimulation and blocking on immune response. *Bull Exp Biol Med*. 2010;150:219–221.
25. Seletti B, Benkelfat C, Blier P, et al. Serotonin1A receptor activation by flesinoxan in humans. Body temperature and neuroendocrine responses. *Neuropsychopharmacology.* 1995;13:93–104.
26. Freeman ME, Kanyicska B, Lerant A, et al. Prolactin: structure, function, and regulation of secretion. *Physiol Rev.* 2000;80: 1523–1631.
27. Riba J, Rodríguez-Fornells A, Strassman RJ, et al. Psychometric assessment of the Hallucinogen Rating Scale. *Drug Alcohol Depend*. 2001;62:215–223.
28. Martin WR, Sloan JW, Sapira JD, et al. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. *Clin Pharmacol Ther*. 1971;12:245–258.
29. Lamas X, Farré M, Llorente M, et al. Spanish version of the 49-item short form of the Addiction Research Center Inventory. *Drug Alcohol Depend*. 1994;35:203–209.
30. Katz B, Mueller K, Helmle H. Binocular eye movement recording with CCD arrays. *Neuro-ophthalmology.* 1987;7:81–91.
31. Dütsch M, Hilz MJ, Rauhut U, et al. Sympathetic and parasympathetic pupillary dysfunction in familial dysautonomia. *J Neurol Sci*. 2002;195:77–83.
32. Wilhelm H, Wilhelm B. Clinical applications of pupillography. *J Neuroophthalmol*. 2003;23:42–49.
33. Bellido M, Rubiol E, Ubeda J, et al. Rapid and simple immunophenotypic characterization of lymphocytes using a new test. *Haematologica*. 1998;83:681–685.
34. McIlhenny EH, Riba J, Barbanoj MJ, et al. Methodology for and the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. *Biomed Chromatogr.* 2011;25(9):970–984.
35. McIlhenny EH, Riba J, Barbanoj MJ, et al. Methodology for determining major constituents of ayahuasca and their metabolites in blood. *Biomed Chromatrogr*. 2011 [Epub ahead of print]. Doi: 10.1002/bmc.1657.
36. Strassman RJ, Qualls CR. Dose-response study of *N*,*N*-dimethyltryptamine in humans, I. Neuroendocrine, autonomic and cardiovascular effects. *Arch Gen Psychiatry.* 1994;51:85–97.
37. Strassman RJ, Qualls CR, Berg LM. Differential tolerance to biological and subjective effects of four closely spaced doses of *N*,*N*-dimethyltryptamine in humans. *Biol Psychiatry.* 1996;39: 784–795.
38. Rosenberg DE, Isbell H, Miner EJ. Comparison of a placebo, *N*-dimethyltryptamine and 6-hydroxy-*N*-dimethyltryptamine in man. *Psychopharmacologia*. 1963;4:39–42.
39. Rosenberg DE, Isbell H, Miner EJ, et al. The effect of *N*,*N*-dimethyltryptamine in human subjects tolerant to lysergic acid diethylamide. *Psychopharmacologia*. 1964;5:217–227.
40. Mas M, Farré M, de la Torre R, et al. Cardiovascular and neuroendocrine effects and pharmacokinetics of 3,4-methylenedioxymethamphetamine in humans. *J Pharmacol Exp Ther.* 1999;290:136–145.
41. de la Torre R, Farré M, Roset PN, et al. Pharmacology of MDMA in humans. *Ann N Y Acad Sci*. 2000;914:225–237.
42. Bitsios P, Szabadi E, Bradshaw CM. Comparison of the effects of venlafaxine, paroxetine and desipramine on the pupillary light reflex in man. *Psychopharmacology.* 1999;143:286–292.
43. Siepmann T, Ziemssen T, Mueck-Weymann M, et al. The effects of venlafaxine on autonomic functions in healthy volunteers. *J Clin Psychopharmacol*. 2007;27:687–691.
44. Flory JD, Manuck SB, Perel JM, et al. A comparison of D,L-fenfluramine and citalopram challenges in healthy adults. *Psychopharmacology.* 2004;174:376–380.
45. Fitzgerald P, Dinan TG. Prolactin and dopamine: what is the connection? A review article. *J Psychopharmacol*. 2008;22(suppl 2):12–19.
46. Armario A. Activation of the hypothalamic-pituitary-adrenal axis by addictive drugs: different pathways, common outcome. *Trends Pharmacol Sci*. 2010;31:318–325.
47. Pacifici R, Zuccaro P, Farré M, et al. Immunomodulating activity of MDMA. *Ann N Y Acad Sci*. 2000;914:215–224.
48. Pacifici R, Zuccaro P, Hernandez López C, et al. Acute effects of 3,4-methylenedioxymethamphetamine alone and in combination with ethanol on the immune system in humans. *J Pharmacol Exp Ther.* 2001;296:207–215.
49. Irwin MR, Olmos L, Wang M, et al. Cocaine dependence and acute cocaine induce decreases of monocyte proinflammatory cytokine expression across the diurnal period: autonomic mechanisms. *J Pharmacol Exp Ther.* 2007;320:507–515.
50. Pacifici R, Zuccaro P, Farré M, et al. Combined immunomodulating properties of 3,4-methylenedioxymethamphetamine (MDMA) and cannabis in humans. *Addiction*. 2007;102:931–936.
51. Chaplin DD. Overview of the immune response. *J Allergy Clin Immunol*. 2010;125(2 suppl 2):S3–S23.
52. Caligiuri MA. Human natural killer cells. *Blood*. 2008;112:461–469.
53. Lanier LL. Evolutionary struggles between NK cells and viruses. *Nat Rev Immunol*. 2008;8:259–268.
54. Breznitz S, Ben-Zur H, Berzon Y, et al. Experimental induction and termination of acute psychological stress in human volunteers: effects on immunological, neuroendocrine, cardiovascular, and psychological parameters. *Brain Behav Immun*. 1998;12:34–52.
55. Van de Kar LD, Blair ML. Forebrain pathways mediating stress-induced hormone secretion. *Front Neuroendocrinol*. 1999;20:1–48.
56. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation*. 2009;16:300–317.

Mid-Term Effects

Literature research conducted by José Carlos Bouso for
The International Center for Ethnobotanical Education, Research & Service

A Six-Month Prospective Evaluation of Personality Traits, Psychiatric Symptoms and Quality of Life in Ayahuasca-Naïve Subjects

Paulo Cesar Ribeiro Barbosa, M.Sc.*; Irene Maurício Cazorla, Ph.D.**;
Joel Sales Giglio M.D., Ph.D.*** & Rick Strassman, M.D****

Abstract—The authors assessed 23 subjects immediately before and six months (27.5 weeks) after their first ayahuasca experience in an urban Brazilian religious setting, either Santo Daime (N = 15) or União do Vegetal (N = 8). Measures included scores on instruments assessing psychiatric symptoms, personality variables and quality of life. Independent variables were the frequency of ayahuasca use throughout the period and the length of ayahuasca wash-out after six months. Santo Daime subjects had a significant reduction of minor psychiatric symptoms, improvement of mental health, and a change in attitude towards more confidence and optimism. The União do Vegetal group had a significant decrease in physical pain, and attitude change towards more independence. Independence was positively correlated with the frequency of ayahuasca use and negatively correlated with the wash-out period. We discuss possible mechanisms by which these changes may occur and suggest areas for future research.

Keywords—ayahuasca, mental health, psychometric, religion

Ayahuasca is a hallucinogenic beverage produced from decocting parts of two Amazonian plants. One is the root bark and sometimes the stem cortex of the liana *Banisteriopsis caapi,* which contains the beta-carboline harmala alkaloids harmine, harmaline, and tetrahydroharmine. The second group comprises plants containing N,N-dimethyltryptamine (DMT), such as the leaves of the bush *Psychotria viridis* (McKenna & Towers 1984). Archeological data suggest its use among Amazonian pre-Columbian cultures dates from at least 2000 B.C. (Naranjo 1986). Contemporary culturally sanctioned ritual use of the beverage for magico-religious purposes throughout the western Amazon Basin by Amerindian and mestizo populations has been described (Luz 2002; Dobkin de Rios 1989; Reichel-Dolmatoff 1975). Ethnological data suggest that the rituals accompanying the use of ayahuasca in these societies can minimize social and psychological problems associated with the use of psychoactive substances in western societies (Dobkin de Rios & Smith 1977).

Throughout the twentieth century, migrant people from northeastern Brazil have combined the local Amerindian/

*Professor of Science Methods and Mental Health, Departamento de Filosofia e Ciências Humanas, Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia, Brazil.

**Professor of Statistics, Departamento de Ciências Exatas e Tecnológicas, Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia, Brazil.

***Professor of Psychiatry, Departamento de Psicologia Médica e Psiquiatria, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil.

****Clinical Associate Professor of Psychiatry, University of New Mexico School of Medicine; Cottonwood Research Foundation, Taos, New Mexico USA

Please address correspondence and reprint requests to Paulo Cesar Ribeiro Barbosa, Rua 02, N.101 ap. 305, Ilhéus, Bahia State, Brazil, CEP 45654-010; email: pcesarr@yahoo.com.br

mestizo-Amazonian ayahuasca use with their previous religious beliefs. The result of this process has been the establishment of modern urban Brazilian ayahuasca religions, such as *União do Vegetal* (also known as UDV), *Santo Daime* and *Barquinha,* who use ayahuasca as a key element in their doctrines and ceremonial meetings (Senna Araújo 1999; Brissac 1999; Labigalini Jr. & Dunn 1995; MacRae 1992). During the last three decades Santo Daime and UDV churches have spread from the Amazon to major cities in Brazil as well as to Europe and North America (Tupper 2008; Labate 2002).

Based on the right of religious freedom and respect for cultural diversity, lawsuits against the religious use of ayahuasca were decided in favor of Santo Daime in Holland and Spain (Tupper 2008) and UDV in the United States (Groisman & Dobkin de Rios 2007). In Brazil, where thousands of urban, ritual ayahuasca users practice, a recent federal regulatory commission upheld a favorable decision for the ritual use of ayahuasca (CONAD 2006; CONFEN 1986). As these judicial decisions are expected to significantly increase the religious use of ayahuasca throughout the world, studies on mental health of ayahuasca users within these religious contexts are needed.

Grob and colleagues (1996) began the systematic assessment of the mental health of urban ayahuasca users within a modern syncretic religious setting. They reported, in this case-control study, that long-term regular users of ayahuasca within the UDV suffered from less psychopathology, and had better scores with respect to some measures of memory function and personality traits compared to non-ayahuasca users.

Subsequently, a more comprehensive case-control study was conducted to evaluate the effects of ayahuasca among adolescent users in UDV regarding neurocognitive function, psychiatric symptomatology, and drug use (Da Silveira et al. 2005; Doering-Silveira et al. 2005a, b). This study found a lower incidence of some psychiatric symptoms and alcohol use among ayahuasca-using adolescents compared to in the control group (Da Silveira et al. 2005; Doering-Silveira et al. 2005a). Furthermore, neurocognitive assessments showed no significant differences between groups (Doering-Silveira et al. 2005b).

All of these studies focused on subjects who already had formal institutional ties to UDV after a long and regular period of ayahuasca use at the time of the assessments. Leading researchers in the field have pointed to the need for longitudinal studies that elicit the health status of subjects before their first ritual experience with ayahuasca and throughout the process of becoming involved with the framework of the ayahauasca religion (McKenna, Callaway & Grob 1998; Grob et al. 1996). Furthermore, systematic psychological evaluations of ayahuasca users in other Brazilian ayahuasca religions, such as Santo Daime, are still lacking.

This report is the second phase of our study designed to address these issues. The first phase described psychological evaluations of ayahuasca-naive subjects one to four days before and one to two weeks after their first ayahuasca experience in UDV or Santo Daime (Barbosa, Giglio & Dalgalarrondo 2005). The present phase presents the evaluations of these subjects six months after that initial experience.

BACKGROUND INFORMATION: SANTO DAIME AND UDV

The study was conducted in two large cities in southeastern Brazil: São Paulo (one UDV and two Santo Daime temples) and Campinas (two UDV temples). Santo Daime and UDV define themselves as religions aiming to contribute to human moral and spiritual development. Both groups ascribe to Christian principles of goodness, fraternity, harmony and justice. They also believe in spiritual evolution through successive reincarnations of the soul. Behavioral patterns consistent with those principles (e.g, helpfulness, respect, serenity and diligence towards life and other church members) are encouraged; while behavior inconsistent with these principles (e.g., substance abuse and violence) are discouraged.

Within these religions ayahuasca is not regarded merely as a psychoactive substance. Rather, this psychedelic beverage is viewed as a divine gift related to the mythic origins of these religions. It is regarded as the tool of spiritual evolution *par excellence* because of its property of opening people's receptivity to the "spiritual world" (Santo Daime 2008; CEBUDV 1989).

The use of ayahuasca is thoroughly integrated into these religions' rituals. These rituals are characterized by the constant preaching of Santo Daime's and UDV's religious ethos (e.g., goodness, harmony, and justice) and worldview (e.g., the divine nature of ayahuasca and the reality of the spiritual world).

In Santo Daime, the preaching takes place through the collective performance of hymns and a synchronized dance called a *bailado*, both of which are accompanied by vigorous percussive and musical instruments. All participants are required to sing and dance during the ceremonies, which last four to 12 hours.

In UDV, the preaching occurs through questions directed to the session leader by the participants, through popular songs played on stereo equipment, and also through hymns performed by single participants. During some periods silence predominates. The rituals invariably last four hours and the participants remain seated in a relaxed position throughout the ceremony.

Santo Daime and UDV beliefs and practices concerning ayahuasca direct the psychedelic experience towards positive social and individual outcomes by means of the collective ceremonial performance and preaching. These external structures reinforce the religions' values and social cohesion, providing conceptual and behavioral guidelines that minimize confusion or disorientation during the visionary

experiences and maximize a particular manner of interpreting and integrating the hallucinogenic state (Baker 2005; MacRae 1992).

This well-established religious conceptual and ceremonial framework is the key feature that has lead researchers to characterize the religious use of ayahuasca as a sacrament similar to those of the Christian churches (e.g., Eucharist), in contrast to more idiosyncratic use of psychedelic drugs that lack well-established concepts and practices (Baker 2005).

Besides attending ayahuasca ceremonies regularly, membership in these religions involves participation in other communal activities such as maintenance of the church organization: e.g., collective cleaning of the shrines and fund raising activities. In addition, participants usually are actively involved in the social support network of the group. This network is used to further integrate religious values into psychosocial spheres, including offering services or goods at a lower price than the market value, and amusement activities.

Despite the Amazonian origins of Santo Daime and UDV among the working classes, the religious use of ayahuasca in larger southern Brazilian cities seems to be predominantly a middle- and educated-class phenomenon (Barbosa, Giglio & Dalgalarrondo 2005). In this urban context, the novices of Santo Daime tend to be recruited through New Age religious social circles, and those of UDV through relatives and close friends who belong to the organization (Barbosa 2001).

People who are about to experience ayahuasca are instructed by a senior member of Santo Daime or UDV in the protocol to be adopted during the ritual. Once the novice undergoes his/her first ritual ayahuasca experience, he/she is free to attend regularly scheduled ceremonies without establishing formal affiliation with the religion. Santo Daime and UDV regard this period as an opportunity for the individual to learn about the doctrine, the religious community, and the effects of ayahuasca before deciding to formally join the religion. Santo Daime advises novices to try ayahuasca at least two times after the first experience. No such recommendation exists in UDV. Usually this period lasts from a few weeks to one year in an individual who attends the ceremonies regularly. The formal affiliation (called *fardamento* in Santo Daime and *associação* in UDV) is symbolized by the wearing of a uniform, which means that from this point on the individual is is referred to as *fardado* (Santo Daime) or *associado* (UDV) and must accept the guidelines of the religion

METHODS

Design

Twenty three subjects were assessed prospectively one to four days before their first ritual experience with ayahuasca (T0); and between seven and 14 days (T1) and six months (mean 27.5 weeks; SD 3.2; range 26-34) after their first experience with ayahuasca (T2). Fifteen participants experienced ayahuasca in the Santo Daime and eight in the União do Vegetal.

Subjects

Recruiting naive subjects to make a group analysis was difficult because first ritual ayahausca experiences are relatively rare, and require rather demanding logistics to be evaluated. Therefore, it was decided to use whatever individuals were available. Novices were invited to participate in the research by elder members of Santo Daime or UDV who were responsible for instruction in the behavior to be adopted during the ritual. Those who accepted the invitation to participate in the research were directed to the researcher. Twenty-eight subjects were evaluated in the first phase of the study, between T0 and T1 (Barbosa, Giglio & Dalgalarrondo 2005). Five subjects dropped out between T1 and T2: four in Santo Daime dropped out—one suffered an intercurrent myocardial infarction, one failed to appear for the scheduled interview, one moved abroad, and one was lost to follow-up. Follow-up revealed that none of the three former subjects contacted described adverse effects from their ayahuasca experience. One from UDV dropped out because of an unrelated stressor (a recent mugging).

Of the 23 subjects who completed the study, 15 (65%) were female (Santo Daime 11; UDV 4). The mean age was 37 years (SD 13.3; range 18-57), [Santo Daime: 34.3 years (SD 13.7; range 18-57), UDV: 42.3 (SD: 11.8; range 27-57)]. Eleven subjects (48%) were married (Santo Daime 4; UDV 7), nine (39%) were single (Santo Daime 8; UDV 1), and three (13%) were separated (all from Santo Daime). Eleven subjects (48%) possessed a bachelor's degree (Santo Daime 5; UDV 6), 10 (43%) finished high school (Santo Daime 8; UDV 2), and two (9%) were college students (both from Santo Daime). One subject from the Santo Daime reported marijuana use along with ayahuasca during Santo Daime ceremonies. All subjects lived in the São Paulo and Campinas areas, two large and modern cities located in southwestern Brazil.

At T2 one of the 15 Santo Daime and four of the eight UDV subjects (21.7% of the 23 subjects) subjects had formally become members of the religious organization.

Number of ayahuasca experiences and length of wash-out period. Subjects were asked how many times they had attended Santo Daime or UDV rituals after their first session with ayahuasca, and the wash-out period (length of time abstinent) between their last use and T2.

Clinical Interview Schedule-Revised Edition (CIS-R). The CIS-R is a semistructured interview that measures the intensity of minor psychopathological symptoms. The scale comprises 14 sections: somatic symptoms, fatigue, difficulties in concentration, sleep problems, irritability, preoccupation with body functions, depression, depressive ideas, worry, anxiety, compulsions, obsessions, panic and

phobias. The score for each section ranges from 0 to 4 with the exception of "depressive ideas" which ranges from 0 to 5. The total CIS-R score ranges from 0 to 57; the higher the score, the more distressed is the subject. A psychiatric disorder is considered present if a subject's cumulative score is 12 or greater. We used a Brazilian version of the CIS-R (Freitas & Botega 2002; Botega et al. 1995). Subjects were asked to answer items for the week-long period preceding the evaluation day. The scale was administered at all three time points.

Short Form-36 Health Survey (SF-36). The SF-36 is a self-rated questionnaire with 36 questions designed to measure eight dimensions of general health and wellbeing: physical functioning (10 questions), role-physical (four questions), bodily pain (two questions), general health (five questions), vitality (four questions), social functioning (two questions), role-emotional (three questions) and mental health (five questions). Scores for each of the eight dimensions ranges from 0 to 100. The higher the score the less distressed is the subject. The SF-36 also includes an additional question regarding personal health status during the previous year (McHorney, Ware & Raczek 1993; Ware & Sherbourne 1992). We used a Brazilian Portuguese version of the SF-36 (Ciconelli et al. 1999).

Since this instrument is intended to assess the subject's status during the month before the evaluation day we administered it only twice. This was in order to compare baseline scores at T0 with those at the six-month evaluation (T2), and we therefore excluded the shorter follow-up time point (T1).

Temperament and Character Inventory—125 items (TCI-125). The TCI-125 is a self-rated questionnaire of 125 true-or-false items generating scores for four domains of "temperament" and three domains of "character." Temperament domains are postulated to be determined by neural systems and genetic inheritance, while domains of character may reflect individual differences in self-concepts more susceptible to change than those belonging to the temperament dimensions (Cloninger, Svrakic & Przybeck 1993). The temperament dimensions are: novelty seeking (20 items), reward dependence (15 items), harm avoidance (20 items), and persistence (five items). The character dimensions are: self-directedness (25 items), cooperativeness (25 items), and self-transcendence (15 items). A Brazilian Portuguese version of the TCI-125 was developed for this study through a process of translation and back-translation (available by request). As this scale is designed to assess personality traits that are believed not amenable to change within short term periods, we administered it only at T0 and T2, and excluded the shorter follow-up time point T1.

The study was conducted in accordance with the Declarations of Helsinki and Tokyo, and Brazilian laws concerning studies on humans. It was approved by the ethics committee at the Universidade Estadual de Campinas, Brazil (#114/2002).

Analysis

As in the previous phase of the study, we analyzed the variables for the total sample, and also compared the Santo Daime with the UDV subsamples (Barbosa, Giglio & Dalgalarrondo 2005). Five analyses were done with these groups.

1) Scores between groups (Santo Daime versus UDV) for each time point (T0, T1[CIS-R only] and T2) were analyzed using Fisher's Exact test and Mann-Whitney U-tests.
2) Scores for changes in the CIS-R, SF-36 and TCI-125 for each group (total sample, Santo Daime and UDV) at T0, T1 [CIS-R only] and T2 were analyzed using McNemar's and Wilcoxon's Signed Rank tests.
3) If significant effects were found for Santo Daime, UDV, or the total sample between T0 and the final follow-up time point T2 we used Spearman's correlation analyses to determine the relationship between these significant effects and the number of ayahuasca experiences the subjects underwent throughout the six-month period.
4) These significant effects were also correlated with the length of wash-out from their last ayahuasca experience to the time of the evaluation (T2).
5) We also used Spearman's analyses to determine correlations between significant changes among CIS-R, TCI-125 and SF-36 between T0 and T2. The correlations were done in parts, two by two (e.g. significant change for CIS-R was correlated with significant change of a specific dimension of SF-36; significant change of a specific dimension of TCI-125 was correlated with a significant change of CIS-R, and so on).

In the current phase of the study, we also compared data for subjects by the criterion of intensity of ayahuasca use throughout the six-month evaluation period. Subjects who underwent more than nine ayahuasca experiences (including the first one) and a wash-out period of four weeks or less were placed into the "regular-use" group. Subjects who underwent nine or less ayahuasca experiences and a washout period of more than four weeks were placed into the "irregular-use" group. We used Fisher's Exact test and Mann-Whitey tests to analyze differences between regular-use and irregular-use groups at each follow-up time point. McNemar's and Wilcoxon's Signed Rank tests were used to analyze differences between groups throughout the follow-up time points.

SPSS 10.0 software was used for analysis of CIS-R, TCI-125 and SF-36 data. We considered $p < 0.05$ as statistically significant. Dropouts were excluded from the analyses.

RESULTS

Intensity of Ayahuasca Use throughout the Follow-up Period

Seventeen of 23 subjects (73.9%) were irregular ayahuasca users (i.e., less than 10 experiences and more than four weeks washout at T2). Thirteen (87.7%) of the 15 Santo Daime subjects and four (50%) of the eight UDV subjects were irregular ayahuasca users. All regular users were formally initiated members of the religions, except for one uninitiated Santo Daime subject.

Clinical Interview Schedule – Revised Edition (CIS-R)

The Fisher's Exact test revealed a significantly higher incidence of CIS-R-defined psychiatric cases in Santo Daime (9/15, 60%) than in UDV (0/8) at T0 ($p = 0.007$). Due to the decreased incidence of Santo Daime cases over time this difference was no longer significant at T2 (2/15, 13%). There was also no significant difference between the incidence of cases in the total sample at T0 and T2. No significant differences were found between regular and irregular ayahuasca users at T0 and T2.

A significant reduction in the intensity of minor psychiatric symptoms as measured by the CIS-R was found in the Santo Daime group between T0 and T1 (11.6 ± 8.3 vs 5.1 ± 4.6; $p = 0.005$), a difference that remained significant at T2 (5.1 ± 5.7; $p = 0.024$ compared to T0). No significant difference was observed between T1 and T2.

No significant differences were found in CIS-R scores at any time points for the UDV group. No significant differences in scores were found between the Santo Daime and UDV groups at T2, whereas scores were significantly higher in the Santo Daime vs. the UDV at T0 (11.6 ± 8.3 vs 2.3 ± 2.4; $p = 0.01$). Interestingly, scores in the regular user group dropped significantly after their first ayahuasca session (T0 = 6.5 ± 6.1 vs T1 = 1.5 ± 2.3; $p = 0.042$). Scores were lower among the regular compared to irregular ayahuasca users at T1 only (1.5 ± 2.3 vs 5.7 ± 5.0 ; $p = 0.043$). Total sample scores showed a significant improvement only between T0 and T1 (8.4 ± 8.1 vs 4.6 ± 4.8; $p = 0.018$).

In the Santo Daime group, no significant correlation was found between improvement in CIS-R scores between T0 and T2 and intensity of use of ayahuasca or length of the wash-out period.

Short Form-36 Health Survey Questionnaire (SF-36)

Significant improvements in the mental health dimension in the Santo Daime group (T0 = 58.4 ± 23.8 vs T2 = 74.7 ± 13.8; $p = 0.027$) and in bodily pain in the UDV group (T0 = 78.3 ± 14.1 vs T2 = 90.7 ± 18.5; $p = 0.044$) were found between T0 and T2. At T0 the UDV group scores were higher than those of the Santo Daime group for the social functioning (92.9 ± 14.2 vs 76.7 ± 16.3; $p = 0.022$), emotional role (85.7 ± 26.2 vs 42.2 ± 38.8; $p = 0.017$), and mental health dimensions (77.7 ± 14.6 vs 58.4 ± 23.8; $p = 0.037$).

In the UDV group, we found no significant correlation between bodily pain improvement (T0 – T2) and intensity of ayahuasca use or length of wash-out. In the Santo Daime group, no significant correlation was found between improvement in mental health (T0 – T2) and the intensity of use of ayahuasca or length of wash-out period.

At T2 regular users scored higher than irregular users in the dimensions of emotional role (94.4 ± 13.6 vs 52.1 ± 40.3; $p = 0.023$) and social functioning (95.8 ± 6.5 vs 70.3 ± 23.7; $p = 0.019$).

Temperament and Character Inventory-125 items (TCI-125)

Between T0 and T2, significantly lower scores for harm avoidance were found in the Santo Daime group (9.6 ± 4.2 vs 6.9 ± 2.9; $p = 0.035$), and for reward dependence in the UDV group (9.9 ± 2.9 vs 7.6 ± 2.0; $p = 0.017$) as well as in the total sample (9.5 ± 2.9 vs 8.4 ± 2.4; $p = 0.028$) between T0 and T2. The Santo Daime group scored significantly higher (10.9 ± 3.8) than did the UDV ($7.9 + 1.1$) in novelty seeking at T2 ($p = 0.025$).

In the Santo Daime group we found no correlation between the decrease in the harm avoidance score (T0 – T2) and either the intensity of use of ayahuasca or the length of wash-out period.

In the UDV group a significant positive correlation was found between the decrease in reward dependence scores between T0 and T2 and intensity of ayahuasca use [r_s ($N = 8$) = .759, $p = 0.029$]. The lower reward dependence score was also negatively correlated with length of wash-out period [r_s ($N = 8$) = -.843, $p = 0.009$].

In the total sample, we found a significant positive correlation between the decrease in reward dependence scores between T0 and T2 and intensity of ayahuasca use [r_s ($N = 23$) = .505, $p = 0.014$]. The decrease in reward dependence score was not correlated with length of wash-out period [r_s ($N = 23$ = -.391 $p = 0.065$].

Regular users showed a significant decrease in reward dependence scores between T0 (12.2 ± 1.7) and T2 (8.8 ± 2.6; $p = 0.026$). Regular users scored significantly higher than the irregular users on reward dependence scores (12.2 ± 1.7 vs 8.6 ± 2.7) at T0 ($p = 0.005$) and in self-directedness scores at T2 (regular 23.0 ± 2.1 vs irregular 18.2 ± 4.2; $p = 0.009$).

Correlation between CIS-R, SF-36 and TCI-125 Significant Results

In the Santo Daime group, we found a significant negative correlation between the decrease of CIS-R psychiatric symptoms and improvement of SF-36 mental health domain

[r_s (N = 15) = -0.780, p = 0.001]. There was a significant positive correlation between CIS-R improvement and the decrease of TCI-125 harm avoidance scores [r_s (N = 15) = 0.601, p = .018].

DISCUSSION

Careful six-month follow-up of newly initiated ayahuasca users of the two most popular Brazilian ayahuasca religions, the Santo Daime and the UDV, demonstrated no adverse effects of participation in either group on scores regarding quality of life as measured by the SF-36, nor minor psychiatric symptoms as measured by the CIS-R. In addition, regular ayahuasca users (more than nine sessions during the evaluation period) scored significantly higher in both emotional role and social functioning domains of SF-36 than did the irregular users (nine or fewer sessions).

The Santo Daime group demonstrated significant improvement on CIS-R-measured minor psychiatric symptoms as well as significant improvement in SF-36-assessed mental health from baseline to either one or both follow-up time points.

These six-month data confirm and extend our findings from the first phase of this study in which we described that the motivations of the Santo Daime sample to participate in ayahuasca rituals included a search for "healing" and "equilibrium." This group demonstrated positive mood and behavioral changes two weeks following their first ayahuasca experience (Barbosa, Giglio & Dalgalarrondo 2005).

Our current findings demonstrate maintenance of improvements in the Santo Daime sample at six-month follow-up. Anthropological literature has emphasized the role of spontaneous remission to explain health improvement related to "religious healing" (Kleinman 1980). As some subjects sought participation in the Santo Daime during episodes of psychological distress (Barbosa, Giglio & Dalgalarrondo 2005) it is possible that a spontaneous drop-off of distress that may have occurred during the six-month period contributed to our observed benefits. However, growing evidence of benefits from the administration of psychedelics within a structured setting (Winkelman 2007) reinforces the suggestion that the religious use of ayahuasca itself influenced the positive mental changes. Our data suggest that either administration of hallucinogens in a clinical research setting (Griffiths et al. 2006; Grinspoon & Bakalar 1986) or in a naturalistic religious setting may result in some psychological benefit.

The reduction of SF-36-assessed somatic pain complaints in the UDV group over the six-month observation period is consistent with reports that hallucinogens may alleviate pain syndromes (Grinspoon & Bakalar 1979).

Our proposing that hallucinogen use in naturalistic religious settings may lead to similar benefits as those purported to occur in clinical research settings is supported by theoretical models which propose transcultural and universal brain mechanisms for hallucinogen-assisted healing (Winkelman 2007). However, absence of a significant correlation between intensity of ayahuasca use and time between last use and positive changes at six months does not lend itself to simply suggesting acuity or intensity of ayahuasca use determines long-term effects.

Despite the suggestion that the effects of regular use of ayahuasca on the serotonergic system may have positive psychological effects (McKenna 2007), other variables concerning the use of a powerful DMT-containing psychedelic within a social religious setting should be taken into account. Social support and encouragement of healthy behavior have been described as contributing to mental health improvement because they provide a sense of belonging to a fellowship that helps deal with painful affect and discourages unhealthy behavior such as drug abuse and attitudes related to stress, such as competitiveness and anger (Moreira-Almeida, Lotufo Neto & Koenig (2006). Perhaps these mechanisms are enhanced in these Brazilian ayahuasca religions by the enhancement of suggestibility resulting from ayahuasca use. Hyper-suggestibility, a marked characteristic of the psychedelic experience, would facilitate the acceptance of the Santo Daime and UDV ethos and worldview which are integrated during the ritual (see Dobkin de Rios, Grob & Baker 2002). In addition, the ability of psychedelics to enhance the psychotherapeutic process may play a role in participants' working through difficult personal issues (Grinspoon & Bakalar 1986). Finally, the spiritual experiences brought on by psychedelics (Griffiths et al. 2006; Pahnke 1966) can also elicit a spectrum of personally and socially beneficial consequences. Therefore, we suggest that the religious use of ayahuasca acts at a number of focal points, exerting a constellation of psychological, social, spiritual, and pharmacological factors.

Unlike the Santo Daime group, the total sample's significant improvement in CIS-R-assessed psychiatric symptoms between T0 and T1 was no longer significant at T2. At T1 all subjects were evaluated within one to two weeks after their first ayahausca experience. At T2 73.9% of subjects were evaluated more than four weeks after their last ayahuasca experience (range one to 28 weeks). This may reflect that this longer hiatus between the last ritual and T2 contributed to the fading of affective improvement in the total sample.

The finding that the regular-use group had already undergone a significantly better improvement in CIS-R-assessed psychiatric symptoms between T0 and T1 compared to the irregular-use group may reflect a selective process that seems to occur after the first ayahuasca experience. Such a process would influence the intensity of subsequent ayahuasca use, i.e., better responses to the first ayahausca experience would prompt people to attend ayahuasca sessions more often than unpleasant responses.

An intriguing finding concerns the data on personality change. TCI-125 dimensions that are proposed to be more

amenable to change (Cloninger, Svrakic & Przybeck's "character" dimensions) did not change. However, two theoretically less malleable "temperament" dimensions, reward dependence and harm avoidance, did.

Change scores for reward dependence were positively correlated with the intensity of ayahuasca use in the total sample, whereas irregular ayahuasca users showed no significant effects. Within the UDV group change scores for this factor were positively correlated with intensity of ayahuasca use and negatively correlated with the length of time elapsing between one's last ayahuasca session and T2. These data, as well as those demonstrating a drop-off of beneficial effects in the total group on CIS-R-assessed psychiatric symptoms between T1 and T2 suggest that some short-lived therapeutic processes require a particular number of, and intervals between, sessions for optimal benefit.

With respect to reward dependence, Cloninger's model defines this trait as contributing to the maintenance of previously rewarded behavior. According to this model, the lowering of reward dependence scores suggests a decrease in such behavior: e.g., from sentimentality, eagerness to please others, and sensitivity to signs of social of approval towards detachment, coolness, emotional independence, and less sensitivity to signals of social approval (Cloninger 1987). Pharmacological studies have hypothesized that ayahuasca has short-term effects on the norepinephrine system (Riba et al. 2003), which is a major determinant of the reward dependence domain (Cloninger 1987). These pharmacological effects may relate to our current findings.

The significant reduction in harm avoidance scores within the Santo Daime group is identical to that seen in Grob and colleagues' 1996 research which found that regular long-term UDV ayahuasca users scored lower than matched controls on this dimension. As that study assessed regular and long-term users of ayahuasca (bimonthly use for at least 15 years), it is not known at what point subjects and controls began to diverge from each other along this dimension. Our data suggest this may take place rather early, at least among Santo Daime ayahuasca users.

According to Cloningers's model of personality, low harm avoidance is related to low anxiety levels (Cloninger 1987). The decrease in the harm avoidance domain correlates with CIS-R measured improvement of psychiatric symptoms. Perhaps this improvement in the Santo Daime sample mediated the lowering of the observed scores on harm avoidance. This hypothesis is consistent with the findings of the first phase of our study, which reported positive affective changes such as increased serenity, joy, relaxation, and assertiveness (Barbosa, Giglio & Dalgalarrondo 2005); this corresponds, to some extent, to our observed lowering of the harm avoidance domain.

This current report is limited by a relatively small sample size. In addition, its naturalistic nature limited our control of variables such as the concentration of the relevant psychoactive alkaloids in the brew and their cumulative doses.

In summary, we extend our findings from this first naturalistic study comparing the psychological states and traits of subjects before and after their use of ayahuasca in a religious setting. Our use of ayahuasca-naïve subjects as their own controls also is more reliable than previous case-control studies. Our follow-up showed that most subjects did not use ayahuasca regularly and did not formally adhere to religious observance throughout the follow-up period. As Grob and colleagues' previous case-control studies evaluated only regular ayahuasca users, they missed an important population that uses ayahuasca irregularly and often discontinues use.

Due to recent favorable judicial decisions in the United States and Western Europe on religious use of ayahuasca, we expect its use to increase significantly. Naturalistic studies are necessary to more fully explicate the complex relationships between the larger social world and mental health responses to ayahuasca use. Also, mental health studies should be done comparing the ayahuasca religions with other religions, in order to clarify the specific role of the psychedelic beverage on psychological status of the adepts.

In conclusion, this study demonstrates no adverse, and some beneficial, effects resulting from the use of ayahuasca within a religious setting in the urban Brazilian context. Future studies should consider following up this project by recruiting larger sample sizes of ayahuasca-naïve subjects who are about to try the beverage for the first time, and to discriminate among the multiple set and setting factors that may influence outcome.

REFERENCES

Baker, J.R. 2005. Psychedelic sacraments. *Journal of Psychoactive Drugs* 37 (2): 179-87.

Barbosa, S.C.T. 2001. *Psiquiatria Cultural do Uso Ritualizado de um Alucinógeno no Contexto Urbano: Uma Investigação dos Estados de Consciência Induzidos pela Ingestão da Ayahuasca no Santo Daime e União do Vegetal em Moradores de São Paulo* (dissertation). Campinas (SP): Unicamp.

Barbosa, P.C.R.; Giglio, J.S. & Dalgalarrondo, P. 2005. Altered states of consciousness and short-term psychological after-effects induced by the first time ritual use of ayahuasca in an urban context in Brazil. *Journal of Psychoactive Drugs* 37 (2): 193-202.

Botega, N.J.; Pereira, W.A.B.; Bio, M.R.; Garcia Jr., C. & Zomignani M.A. 1995. Psychiatric morbidity among medical inpatients: A standardized assessment (GHQ-12 and CIS-R) using lay interviewers in a Brazilian hospital. *Social Psychiatry and Psychiatric Epidemiology* 30 (3): 127-31.

Brissac, S.C.T. 1999. *A Estrela do Norte Iluminando até o Sul: Uma Etnografia da União do Vegetal em um Contexto Urbano* (dissertation). Rio de Janeiro (RJ): Museu Nacional.

Centro Espírita Beneficente União do Vegetal (CEBUDV). 1989. *Hoasca, Fundamentos e Objetivos*. Brasília (DF): Sede Geral.

Ciconelli, R.M.; Ferraz, M.B.; Santos, W.; Meinão, I., Quaresma, M.R. 1999. Tradução para a língua portuguesa e validação do questionário genérico de avaliação de qualidade de vida SF-36 (Brasil SF-36). *Revista Brasileira de Reumatologia* 39 (3): 143-50.

Cloninger, C.R. 1987. A systematic method for clinical description and classification of personality variants. *Archives of General Psychiatry* 44 (6): 573-88.

Cloninger, C.R.; Svrakic, D.M. & Przybeck, T.R. 1993. A psychobiological model of temperament and character. *Archives of General Psychiatry* 50 (12): 975-90.

Conselho Federal de Entorpecentes (CONFEN). 1986. *Resolução Número 6 do CONFEN*. Available at: http://www.ayahuascabrasil.org.

Conselho Nacional Antidrogas (CONAD). 2006. *Grupo Multidisciplinar de Trabalho – GMT – Ayahuasca: Relatório Final*. Available at: http://www.ayahuascabrasil.org.

Da Silveira, D.X.; Grob C.S.; De Rios, M.D.; Lopez, E.; Alonso, L.K.; Tacla, C. & Doering-Silveira, E .2005. Ayahuasca in adolescence: A preliminary psychiatric assessment. *Journal of Psychoactive Drugs* 37 (2): 129-33.

Dobkin de Rios, M. 1989. A modern-day shamanistic healer in the Peruvian Amazon: Pharmacopoeia and trance. *Journal of Psychoactive Drugs* 21 (1): 91-99.

Dobkin de Rios M & Smith, D.E .1977. Drug use and abuse in cross cultural perspective. *Human Organization* 36 (1): 14-21.

Dobkin de Rios, M.; Grob, C.S. & Baker, J.R. 2002. Hallucinogens and redemption. *Journal of Psychoactive Drugs* 34 (3): 239-48.

Doering-Silveira E.; Lopez, E., Grob, C.S.; Dobkin de Rios, M.; Alonso, L.K.; Tacla, C.; Shirakawa, I; Bertolucci, P.H. & Da Silveira, D.X. 2005a. Ayahuasca in adolescence: A neuropsychological assessment. *Journal of Psychoactive Drugs* 37 (2): 123-28.

Doering-Silveira, E.; Grob, C.S.; Dobkin de Rios, M.; Lopez, E.; Alonso, L.K. & Tacla, C. 2005b. Report on psychoactive drug among adolescents using ayahuasca within a religious context. *Journal of Psychoactive Drugs* 37 (2): 141-44.

Freitas, G.V. & Botega, N.J. 2002. Gravidez na adolescência: prevalência de depressão, ansiedade e ideação suicida. *Revista da Associação Medica Brasileira* 48 (3): 245-49.

Griffiths, R.R.; Richards, W.A.; McCann, U. & Jesse, R. 2006. Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. *Psychopharmacology* 187 (3): 268-83; discussion 284-92.

Grinspoon, L. & Bakalar, J.B. 1986. Can drugs be used to enhance the psychotherapeutic process? *American Journal of Psychotherapy* 40 (3): 393-404.

Grinspoon, L. & Bakalar, J.B. 1979. *Psychedelic Drugs Reconsidered*. New York: Basic Books.

Grob, C.S.; McKenna, D.J.; Callaway, J.C.; Brito, G.S.; Neves, E.S.; Oberlaender, G.; Saide, O.L.; Labigalini, E.; Tacla, C.; Miranda, C. Strassman, R.J. & Boone, K.B. 1996. Human psychopharmacology of hoasca: A plant hallucinogen used in ritual context in Brazil. *Journal of Nervous and Mental Disease* 184 (2): 86-94.

Groisman, A. & Dobkin de Rios, M. 2007. Ayahuasca, the U.S. Supreme Court and the UDV-US government case: Culture, religion and implications of a legal dispute. In: M.J. Winkelman & T.B. Roberts (Eds.) *Psychedelic Medicine: Social, Clinical and Legal Perspectives. Vol 1*. Westport, CT: Praeger.

Kleinman, A. 1980. *Patients and Healers in the Context of Culture: An Exploration of the Borderland Between Anthropology, Medicine, and Psychiatry*. Berkeley, CA: University of California Press.

Labate, B.C. 2002. A literatura brasileira sobre as religiões ayahuasqueiras. In: B.C. Labate & W.S. Araújo (Eds.). *O Uso Ritual da Ayahuasca*. Campinas, SP: Mercado de Letras.

Labigalini Jr., E. & Dunn, J. 1995. The Union of Vegetable: The ritualized use of hoasca tea. *Psychiatric Bulletin* 19 (5): 313-14.

Luz, P. 2002. O uso ameríndio do caapi. In: B.C. Labate & W.S. Araújo (Eds.) *O Uso Ritual da Ayahuasca*. Campinas (SP), Brazil: Mercado de Letras.

MacRae, E. 1992. *Guiado Pela Lua: Xamanismo e Uso Ritual da Ayahuasca no Culto do Santo Daime*. São Paulo (SP), Brazil: Brasiliense.

McHorney, C.A.; Ware, J.J. & Raczek, A.E. 1993. The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. *Medical Care* 31 (3): 247-63.

McKenna, D.J. 2007. The healing vine: Ayahuasca as a medicine in the 21st century. In: M.J. Winkelman & T.B. Roberts (Eds.) *Psychedelic Medicine: Social, Clinical and Legal Perspectives. Vol 1*. Westport, CT: Praeger.

McKenna, D.J. & Towers, G.H.N. 1984. Biochemistry and pharmacology of tryptamines and beta-carbolines. *Journal of Psychoactive Drugs* 16 (4): 347-58.

McKenna, D.J.; Callaway, J.C. & Grob, C.S.1998. The scientific investigation of ayahuasca: A review of past and current research. *Heffter Review of Psychedelic Research* 1: 65-76.

Moreira-Almeida, A.; Lotufo Neto, F. & Koenig, H.G. 2006. Religiousness and mental health. *Revista Brasileira de Psiquiatria* 28 (3): 242-50.

Naranjo, P. 1986. El *ayahuasca* in la arqueología ecuatoriana. *America Indigena* 46 (1): 117-27.

Pahnke, W.N. 1966. Drugs and mysticism. *International Journal of Parapsychology* 8 (2): 295-313.

Reichel-Dolmatoff, G. 1975. *The Shaman and the Jaguar: A Study of Narcotic Drugs Among the Indians of Colombia*. Philadelphia, PA: Temple University Press.

Riba, J.; Valle, M.; Urbano, G.; Yritia, M.; Morte, A. & Barbanoj, M.J. 2003. Human pharmacology of ayahuasca: Subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *Journal of Pharmacology and Experimental Therapeutics* 306 (1): 73-83.

Santo Daime. 2008. *Santo Daime: A Doutrina da Floresta*. Available at: http://www.santodaime.org.

Senna Araújo. 1999. *Navegando nas Ondas do Daime: História, Cosmologia e Ritual na Barquinha*. Campinas, São Paulo: Editora da Unicamp.

Tupper, K.W. 2008. The globalization of ayahuasca: Harm reduction of benefit maximization. *International Journal of Drug Policy* 19 (4): 297-303.

Ware, J.E. & Sherbourne, C.D. 1992. The MOS 36-Item Short-Form Health Survey (SF-36): I. Conceptual framework and item selection. *Medical Care* 30 (6): 473-83.

Winkelman, M. J. 2007. Therapeutic bases of psychedelic medicines: Psychointegrative effects. In: M.J. Winkelman & T.B. Roberts (Eds.) *Psychedelic Medicine: Social, Clinical and Legal Perspectives. Vol 1*. Westport, CT: Praeger.

Long Term Effects

Literature research conducted by José Carlos Bouso for
The International Center for Ethnobotanical Education, Research & Service

The Journal of Nervous & Mental Disease vol 184 No. 2

Volume 184(2) February 1996 pp 86-94

Human Psychopharmacology of Hoasca, A Plant Hallucinogen Used in Ritual Context in Brazil

GROB, CHARLES S. M.D[1].; McKENNA, DENNIS J. Ph.D.[2]; CALLAWAY, JAMES C. Ph.D.[3]; BRITO, GLACUS S. M.D.[4]; NEVES, EDISON S. M.D.[4]; OBERLAENDER, GUILHERME M.D.[4]; SAIDE, OSWALDO L. M.D.[5]; LABIGALINI, ELIZEU M.D.[6]; TACLA, CRISTIANE Ph.D.[6]; MIRANDA, CLAUDIO T. M.D.[6]; STRASSMAN, RICK J. M.D.[7]; BOONE, KYLE B. Ph.D.[1]

A multinational, collaborative, biomedical investigation of the effects of hoasca (ayahuasca), a potent concoction of plant hallucinogens, was conducted in the Brazilian Amazon during the summer of 1993. This report describes the psychological assessment of 15 long-term members of a syncretic church that utilizes hoasca as a legal, psychoactive sacrament as well as 15 matched controls with no prior history of hoasca ingestion. Measures administered to both groups included structured psychiatric diagnostic interviews, personality testing, and neuropsychological evaluation. Phenomenological assessment of the altered state experience as well as semistructured and open-ended life story interviews were conducted with the long-term use hoasca group, but not the hoasca-naive control group. Salient findings included the remission of psychopathology following the initiation of hoasca use along with no evidence of personality or cognitive deterioration. Overall assessment revealed high functional status. Implications of this unusual phenomenon and need for further investigation are discussed.

J Nerv Ment Dis 184:86-94, 1996

Hoasca is a hallucinogenic concoction of potent psychoactive plants that are indigenous to the Amazon basin of South America. It has been known under a variety of names, including ayahuasca, caapi, yage, mihi, dapa, natema, pinde, daime, and vegetal. Hoasca is the Portuguese transliteration for ayahuasca and is the accepted term utilized throughout Brazil. Prior to the European conquest, domination, and acculturation of South America, beginning in the 16th century, hoasca was widely used by the native peoples for purposes of magic and religious ritual, divination, sorcery, and the treatment of disease (Dobkin de Rios, 1972). In spite of prolonged and savage attempts by the European conquerors to repress and eradicate native culture and belief systems (Taussig, 1986), sacramental and medicinal use of hoasca remained extant.

[1] Department of Psychiatry, Harbor-UCLA Medical Center, Box 498, 1000 West Carson Street, Torrance, California, 90509. Send reprint requests to Dr. Grob
[2] Botanical Dimensions, Occidental, California.
[3] Department of Pharmacology and Toxicology, University of Kuopio, Finland
[4] Centro De Estudos Medicos, Sao Paulo, Brazil.
[5] Departmento de Psiquitria, Universidade Estadual do Rio De Janeiro, Brazil
[6] Departamento de Psiquitria, Escola Paulista de Medicina, Sao Paulo, Brazil
[7] Department of Psychiatry, University of New Mexico, Albuquerque, New Mexico.
The authors acknowledge the support of the Heffter Research Institute, Botanical Dimensions, and Jeffrey Bronfman.

Scientific study of hoasca began with the renowned English botanist Richard Spruce, who from 1849 to 1864 traveled extensively throughout the Brazilian, Venezuelan, and Ecuadorian Amazon to compile an inventory of the varieties of plant life found there (Schultes and Raffauf, 1992). Spruce made a number of valuable discoveries, including Hevea, the genus of the rubber tree, and cinchona, from which quinine is derived. He also identified one of the primary sources of a powerful hallucinogenic brew used by the Mazan and Zaparo Indians, called ayahuasca (Quechua for "vine of the souls" or "vine of the dead"), and previously described by the Ecuadorian Manuel Villavicencio (1858), as a large woody vine that would later be given the formal botanical designation of Banisteriopsis caapi (Ott, 1994; Spruce, 1908). Subsequent laboratory analysis would reveal the presence of the psychoactive beta-carboline alkaloids harmine, harmaline, and tetrahydroharmine, although when first isolated during the early 20th century they would receive the rather exotic appellation of telepathine. As identified by early field observers of hoasca use, additional psychoactive admixtures were often added to the cooking B. caapi preparations, most notably highly potent and hallucinogenic tryptamine-containing plants, including, such vision-inducing plants as Psychotria viridis (McKenna and Towers, 1984).

Throughout the Amazon basin, the use of hoasca remained so deeply rooted in tribal mythology and philosophy that modern investigators have been able to confidently conclude that its use extended back to the earliest aboriginal inhabitants of the region (Schultes and Hofmann, 1992). They have recorded the tradition of hoasca use by the indigenous peoples of the region for the purpose of freeing the soul from corporeal confinement and facilitating access to realms of alternate reality, allowing for a variety of magical experiences, including accessing communication with the spirits of the ancestors. Anthropologists who have conducted ethnographic studies of the native inhabitants of the Amazon Basin have described such common hoasca-induced phenomena as visions of jaguars, snakes and other predatory animals, visions of distant persons, "cities" and landscapes, the sensation of "seeing" the detailed enactment of recent mysterious events, and the sense of contact with the supernatural (Harner, 1973).

Hoasca, as is the case with other plant hallucinogens, has a prehistoric tradition of use by native aboriginal peoples as shamanic sacraments or catalysts (Bravo and Grob, 1989; Furst, 1976). It is considered a "great medicine" and is used to both diagnose and treat illness (Schultes and Hofmann, 1992). Its use is fully sanctioned by societal customs and laws and, in fact, is the core experience upon which tribal and collective consciousness rests. Utilization of such potent plant hallucinogens as hoasca typically occurs within a ritualized context, including the traditional rites of initiation (Grob and Dobkin de Rios, 1992). The powerful hypersuggestible effects induced by the hallucinogenic plant drug reinforce collective belief systems, strengthen group cohesion, and facilitate culturally conditioned and syntonic visions which provide revelation, blessing, healing, and ontological security (Dobkin de Rios and Grob, 1994).

Use of hoasca for purposes of healing and religious sustenance has, during the centuries of European acculturation of Amazonia, emerged from the exclusive tribal domains of the rain forest and been incorporated into the contemporary fabric of rural and urban society, particularly among the indigenous Mestizo populations of Peru, Colombia, and Ecuador. Identified as a valuable adjunct to folk healing practices, hoasca is ritually administered by "ayahuasqueros" to carefully selected groups

of patients (Dobkin de Rios, 1972). Scrupulously adhering to the shamanic models practiced by the aboriginal peoples, these folk healers similarly use the sacramental hoasca for purposes of medical diagnosis and healing, divination, and as a path of access to the realms of the supernatural.

During the 20th century, the use of hoasca within the context of modern syncretic religious movements, particularly in Brazil, has arisen. One such church, and the object of the current study, is the Uniao do Vegetal (UDV), whose translation from the Portuguese means "union of the plants." The UDV originated in the early 1950s when its founder, Gabriel de Costa, a rubber tapper who had first experienced the effects of hoasca with the native Indians of Bolivia, returned to the rapidly expanding Brazilian Amazon settlement of Rio Branco with his visions of spiritual revelation and personal mission. Gathering a group of loyal followers, Maestre Gabriel, as he came to be known, elaborated a mythology and structure for his new religion. Spreading first through the Brazilian Amazon and then to the more densely populated and urbanized South, the UDV grew over the subsequent four decades to achieve an eventual size of approximately 7000 members nationwide, drawing adherents from across the socioeconomic and professional spectra. Organized along the lines of an early Christian parish, local "nucleos," or congregations, are centers where sacramental hoasca is consumed in large bimonthly ritual ceremonies which are presided over by local "maestres," leaders of the religious sect. Although not the only Brazilian syncretic church to use hoasca as a ritual sacrament, the Santo Daime sect being the largest and most widely known, the UDV does have the strongest organizational structure as well as the most highly disciplined membership. Of all the hoasca churches in Brazil, the UDV was also most pivotal in convincing the government narcotics commission to remove hoasca from the list of banned drugs, which was accomplished in 1987 for use within religious ceremonial contexts.

Although achieving some attention and even notoriety in North American literature and the popular press, most notably the work of William Burroughs and Allen Ginsberg (1963), the psychological pheno menon induced by hoasca has been subjected to virtually no rigorous study. Various travelers to the Amazon Basin have reported their own first-hand accounts of experiences with hoasca (Weil, 1980), while both formal and informal anthropological narratives have excited the public imagination (Lamb, 1971; Luna and Amaringo, 1991). Indeed, interest in the exotic Amazonian traditions and effects of hoasca have sparked a steady stream of North American tourists, often attracted by articles and advertisements in popular and New Age magazines (Krajick, 1992; Ott, 1993). Concern over possible adverse psychological health effects incurred by such naive travelers has also been raised by a noted anthropological authority of hoasca use in the Amazon (Dobkin de Rios, 1994). Contrasted with testimonials of improved psychological and moral functioning by adherents of the syncretic hoasca churches in Brazil, a formal study exploring the effects of long-term use of this unusual hallucinogenic beverage would appear to be indicated.

During the summer of 1993 a multinational group of biomedical researchers from the United States, Finland, and Brazil met in Manaus, the capital city of the Brazilian state of Amazonia, to conduct an examination of the psychological and biochemical effects of hoasca. Prior to the actual performance of the study, an invitation had been extended by the Uniao do Vegetal to conduct an investigation of the toxicity of their hoasca "tea." Given the long history of repression of their religious movement and use of the hoasca sacrament prior to government sanction in 1987, the leaders of the UDV had surmised that the

conclusions of a fair and objective scientific study might be of some protective value in the future if the political winds in Brazil were to shift. Consequently, and upon consultation with the North American research group, a decision was made to utilize the oldest nucleo outside of Rio Branco, in Manaus, where a large percentage of the membership had been ritually consuming hoasca on a regular basis for more than 10 years. Given the complicated logistics and demands placed upon subjects in this study, the tightly organized structure of the UDV and its highly disciplined membership proved to be invaluable in the successful completion of the project's goals. Part 1 of this report will detail the results of our investigation of the effects of the hoasca tea on psychological function and Part 2 will discuss our examination of the effects of hoasca on human biochemistry. conditions as well as limitations of time, it was not feasible to completely analyze all demographic data until after initiation of the actual study. At that time it was also identified that control subjects had significantly higher yearly incomes than experimental subjects. In endeavoring to explain this discrepancy we noted that the method of control subject recruitment had called for each of the experimental subjects to provide for the study a close friend or associate who had never participated in UDV ceremonies nor had consumed hoasca under any other circumstances. It was noted in retrospect that several experimental subjects had asked their supervisors at their places of employment to volunteer for the study.

Methods

Fifteen members of the syncretic church, Uniao do Vegetal, living in the Brazilian Amazon city of Manaus, were randomly selected from a larger group of volunteers. Criteria for inclusion into the study included membership in the UDV for at least 10 years. Members of the UDV participate in church rituals utilizing hoasca as a psychoactive sacrament a minimum of twice monthly, but often as frequently as several times per week, although always within ritual context. In addition to regular participation in ceremonial consumption of hoasca, the UDV requires of its membership complete abstinence from all other psychoactive substances, including alcohol, tobacco, marijuana, cocaine, and amphetamines.

Fifteen control subjects who had never consumed hoasca were also recruited, with the objective of matching them on all demographic parameters. Because of the relatively small sample size, and the need to limit the number of variables, all experimental and control subjects were men. Controls were compatibly matched to experimental subjects along the parameters of age, ethnicity, marital status, and level of education. Although attempts were made to control for diet and current consumption of alcohol, complete compliance was not possible to achieve. Because of difficult field

A variety of parameters were utilized to assess past and current levels of psychological function. Both experimental and control subject groups were administered structured psychiatric diagnostic interviews (Composite International Diagnostic Interview [CIDI]), personality testing (Tridimensional Personality Questionnaire [TPQ]), and neuropsychological testing (WHO-UCLA Auditory Verbal Learning Test). Experimental subjects, but not control subjects, were asked to fill out an additional questionnaire (Hallucinogen Rating Scale [HRS]) following a hoasca session. Each of the experimental subjects was also interviewed in a semistructured format designed to ascertain their life stories.

All subjects were monolingual speakers of Portuguese. Portuguese versions of the CIDI and the TPQ were readily

available for this study, having been translated previously and validated in Portuguese by the creators of these instruments. Portuguese versions of the WHO-UCLA Auditory Verbal Learning Test and the HRS were developed for this study by Brazilian collaborators, who translated the instruments first into Portuguese, then back into English, and finally back once again into Portuguese. The CIDI and the WHO-UCLA Auditory Verbal Learning Test sessions were conducted by collaborating Brazilian mental health professionals instructed in their administration. The TPQ and HRS are self-report questionnaires. The semistructured life story interviews were conducted by an English-speaking psychiatrist assisted by an interpreter fluent in both English and Portuguese. All life story interviews were audiotaped.

Composite International Diagnostic Interview

The CIDI is a comprehensive, fully standardized diagnostic interview for the assessment of mental disorders according to the definitions and criteria of ICD-10 and DSM-III-R (Robbins et al., 1988). The CIDI was conceived for use in a variety of cultures and settings. Although its primary application has been for epidemiological studies of mental disorders, the CIDI has also been utilized for clinical and research purposes. In the course of its development, the CIDI was subjected to a variety of tests in different settings, countries, and cultures for feasibility, diagnostic coverage, test-retest reliability, and procedural reliability (Wittchen et al., 1991).

Tridimensional Personality Questionnaire

The TPQ is a 100-item, self-administered, paper-and-pencil, true/false instrument which takes approximately 15 minutes to complete (Cloninger, 1987a). The questionnaire measures the three higher order personality dimensions of novelty seeking, harm avoidance, and reward dependence, each of which measures four lower order dimensions
(Cloninger, 1987b). The novelty seeking domain measures the spectrums of exploratory excitability versus stoic rigidity (9 items), impulsiveness versus reflection (8 items), extravagance versus reserve (7 items), and disorderliness versus regimentation (10 items). The harm avoidance domain measures the spectrums of anticipatory worry versus uninhibited optimism (10 items), fear of uncertainty versus confidence (7 items), shyness with strangers versus gregariousness (7 items), and fatigability and asthenia versus vigor (10 items). The reward dependence domain measures the spectrums of sentimentality versus insensitiveness (5 items), persistence versus irresoluteness (9 items), attachment versus detachment (11 items), and dependence versus independence (5 items). The TPQ is based on a unified biosocial model of personality integrating concepts focused on the neuroanatomical and neurophysiological basis of behavioral tendencies, styles of learning, and the adaptive interaction of the three personality dimensions (Cloninger et al., 1991).

WHO-UCLA Auditory Verbal Learning Test

The WHO-UCLA Auditory Verbal Learning Test is a simple list-learning task similar to the Rey Auditory Verbal Learning Test (Rey, 1964), but which also is suitable for use in cross-cultural contexts and is sensitive to mild degrees of cognitive dysfunction. To be familiar to a variety of cultures, the test comprises a list of items carefully selected from categories such as parts of the body, tools, household objects, and common transportation vehicles (Maj et al., 1993). Subjects are read a list of 15 items at the rate of approximately one word per second, following which they are asked to recite as many words as they can recall. The same list is read to subjects a total of five

successive times, and on each occasion subjects are asked to recite as many words as they can remember. This is followed by an interference test where subjects are read 15 words from a second list and asked to recite as many as they can from the second list, following which they are asked to again recall the words from the first list. For the final trial, subjects are read from a list of 30 words, half of which (in random order) are from the original list. Subjects then are asked to indicate after each word whether they recognize it as part of the original list of 15 words.

Hallucinogen Rating Scale

The HRS is a 126-item questionnaire originally developed to assess the range of effects induced by intravenous administration of synthetic dimethyltryptamine (Strassman et al., 1994). A 0 to 4 scale is utilized for most questions, with 0 = not at all, 1 = slightly, 2 = moderately, 3 = quite a bit, and 4 = extremely. Responses to items are analyzed according to six conceptually coherent "clusters": somesthesia (interoceptive, visceral, and cutaneous/tactile effects), affect (emotional/affective responses), perception (visual, auditory, gustatory, and olfactory experiences), cognition (alterations in thought processes or content), volition (a change in capacity to willfully interact with themselves, the environment, or certain aspects of the experience), and intensity (strength of the various aspects of the experience).

Life Story Interview

Each of the 15 experimental subjects agreed to submit to an approximate hour-long interview conducted by a psychiatrist (C. S. G.). The interview addressed various facets of their lives related to their experience as members of the Uniao do Vegetal and their frequent participation in rituals utilizing the psychoactive sacrament, hoasca. The interviews were conducted, with the aid of a translator, in a semistructured and open-ended manner. Each subject was asked to "tell the story of your life from the time before you first drank the hoasca tea... to how you first became acquainted with the UDV and the effects of the hoasca... to how your life has developed since the time you became a part of the UDV."

Results

Psychiatric Diagnoses

A structured psychiatric interview was conducted with each of the 15 experimental subjects and each of the 15 normal control subjects. Administration of the CIDI identified that whereas none of the UDV experimental subjects had a current psychiatric diagnosis, active diagnoses of alcohol abuse disorder and hypochondriasis were present in two of the matched control subjects. However, assessment of past (although no longer active) psychiatric diagnoses indicated that, according to ICD-10 and DSM-III-R criteria, five of the UDV experimental subjects had prior formal alcohol abuse disorders, two had past major depressive disorders, and three had past phobic anxiety disorders. On the other hand, among the 15 control subjects, only one subject had a past psychiatric disorder that was no longer present-an alcohol abuse disorder that had remitted 2 years before the study.

Personality Testing

The TPQ, measuring the three domains of novelty seeking, harm avoidance, and reward dependence, was administered to the 15 experimental long-term hoasca-drinking subjects and to the 15 hoasca-naive control subjects. Means and standard deviations and results of t-test comparisons

are shown in Table 1. Significant findings on the novelty seeking domain included UDV subjects having greater stoic rigidity versus exploratory excitability ($p < .049$) and greater regimentation versus disorderliness ($p < .016$). A trend toward group difference was found along the spectrum of greater reflection versus impulsivity ($p < .1$). No group differences were found along the spectrum of reserve versus extravagance ($p < .514$). Summation of all four spectrums of the novelty seeking domain identified a highly significant difference between the two groups ($p < .0054$).

Analysis of the harm avoidance domain of the TPQ also identified significant differences between the two groups. The UDV experimental subjects were found to have significantly greater confidence versus fear of uncertainty ($p < .043$) with a trend toward greater gregariousness versus shyness with strangers ($p < .067$) and greater uninhibited optimism versus anticipatory worry ($p < .098$). Totaling the four spectrums of the harm avoidance dimension yielded a significant difference between the two groups ($p < .011$).

Analysis of the final TPQ domain of reward dependence did not demonstrate any significant difference between the two groups in total score and any of the subdomain scores.

Neuropsychological Testing

All 15 experimental subjects and 15 control subjects were administered the WHO-UCLA Auditory Learning Verbal Memory Test (Table 2). Experimental subjects performed significantly better than control subjects on their recall of words on the fifth learning trial ($p < .038$). Experimental subjects also performed better than control subjects, although to a non-statistically significant degree, on the following tests: number of words recalled ($p < .253$), delayed recall ($p < .248$), and words recalled after interference ($p < .158$). There was no difference between the two groups in their collective capacities on the test involving the number of false-positive errors on the recognition task ($p < .602$).

Phenomenological Assessment

The Hallucinogen Rating Scale was completed by each of the 15 UDV subjects within 1 hour following the close of the experimental hoasca session, where a variety of medical and biochemical parameters had been assessed. Analysis of the 126-item HRS yielded findings placing the hoasca experience in the mild end of the spectrum when contrasted to the highly potent, short-acting intravenous dimethyltryptamine (DMT) experience. Whereas the highly intense DMT experience is over in less than 30 minutes, the full hoasca experience lasts on average 4 hours. The analysis of data revealed that the clinical clusters of the HRS for the hoasca subjects scored in the relatively mild range when contrasted with prior investigations of the effects of intravenous DMT (Strassman et al., 1994). The clusters of intensity (1.633 ±.533), affect (.947 ±.229), cognition (.908 ±.494), and volition (1.309 ±.429) were compatible to an intravenous DMT experience between a dosage level of.1 and.2 mg/kg, whereas the cluster of perception (.484 ±.501) was comparable to an intravenous DMT experience of.1 mg/kg and the cluster of somatesthesia (.367 ±.256) was less appreciable than the lowest intravenous DMT dose (.05 mg/kg) used.

Life Story Interviews

All 15 experimental subjects provided detailed information about their personal histories, with particular emphasis on how their involvement with the UDV and experience with hoasca had impacted the course of their lives. Their age range at the time of the study was from 26 to 48 years,

with a mean age of 37. Two had been born into the UDV, whereas the other 13 had formally been members for 10 to 18 years, with a mean duration of membership of 14 years. Three were currently maestres (church leaders), two were sons of senior maestres, and one was the son-in-law of a senior maestre.

Many of the subjects reported a variety of pervasive dysfunctional behaviors prior to their entry into the UDV. Eleven subjects reported having a history of moderate to severe alcohol use prior to entering the UDV, with five of them reporting episodes of binding associated with violent behavior. Two had been jailed beacuse of their violence. Four subjects also related prior involvement with other drugs of abuse, including cocaine and amphetamine. Eight of the 11 subjects with prior histories of alcohol and other drug use and misuse were addicted to nicotine at the time of their first encounter with the UDV and ritual hoasca use. Additional self-descriptions prior to entry into their church included impulsive, disrespectful, angry, aggressive, oppositional, rebellious, irresponsible, alienated, and unsuccessful.

All 15 of the UDV subjects reported that their experience with ritual use of hoasca as a psychoactive ritual sacrament had had a profound impact on the course of their lives. For many of them, the critical juncture was their first experience under the influence of the hoasca. A common theme was the perceived belief while in the induced altered state of consciousness that they were on a self-destructive path that would inevitably lead to their ruin and even demise unless they embarked on a radical change in their personal conduct and orientation. Some examples included: "I had a vision of myself in a car going to a party. There was a terrible accident and I could see myself die." "I was at a carnival, on a carousel, going around and around and around without ever stopping. I didn't know how to get off. I was very frightened." "I could see where I was going with the life I was leading. I could see myself ending up in a hospital, in a prison, in a cemetery." "I saw myself arrested and taken to prison. They were going to execute me for a horrible crime I had committed." Subjects also reported that while in the throes of their nightmarish visionary experience, they would encounter the founder of the UDV, Maestre Gabriel, who would deliver them from their terrors: "I saw these horrible, ugly animals. They attacked me. My body was disassembled, different parts were lying all over the ground. Then I saw the Maestre. He told me what I would need to do to put all my body parts back together." "I ran through the forest terrified that I was going to die. Then I saw the Maestre. He looked at me. I was bathed in his light. I knew I would be okay." "I was in a canoe, out of control, going down the river. I thought I would die. But then I saw Maestre Gabriel in a canoe in front of me. I knew that as long as I stayed with the Maestre I was safe."

Subjects reported that since entering the UDV their lives had gone through profound changes. In addition to entirely discontinuing alcohol, cigarettes, and other drugs of abuse, subjects emphatically stated that their daily conduct and orientation to the world around them had undergone radical restructuring: "I used to not care about anybody, but now I know about responsibility. Every day I work on being a good father, a good husband, a good friend, a good worker. I try to do what I can to help others.... I have learned to be calmer, more self confident, more accepting of others.... I have gone through a transformation." Subjects emphasize the importance of "practicing good deeds," watching one's words, and having respect for nature. Finally, subjects report experiencing improvement in their memory and concentration, continual positive mood states, fulfillment in their day-to-day interactions, and a sense of meaning and coherence to their lives.

Subjects unequivocally attributed the positive changes in their lives to their involvement in the UDV and their participation in the ceremonial ingestion of hoasca. They saw the hoasca as a catalyst in their psychological and moral evolution, but were quick to point out, however, that it was not the hoasca alone that was responsible, but rather taking the hoasca within the context of the UDV ritual structure. Several of the subjects were in fact quite critical of other Brazilian groups which utilize hoasca in less controlled and less focused settings. Subjects described the UDV as a "vessel" that enables them to safely navigate the often turbulent states of consciousness induced by hoasca ingestion. The UDV is their "mother... family... house of friends," providing "guidance and orientation" and allowing them to walk the "straight path." They emphasized the importance of "uniao," or union, of the plants and of the people. Without the structure of the UDV, the subjects contended, hoasca experiences may be unpredictable and lead to an inflated sense of self. Within the "house of the UDV," however, the hoasca-induced state is controlled and directed "down the path of simplicity and humility."

Discussion

As this investigation was a first attempt to study the phenomenon of hoasca use from a biomedical perspective, and as the setting for the study was relatively primitive (the Brazilian Amazon), these results need to be viewed as preliminary and tentative. Nevertheless, the findings presented are intriguing and to some degree unexpected. Psychiatric diagnostic assessments revealed that although an appreciable percentage of our long-term hoasca-using subjects had had alcohol, depressive, or anxiety disorders prior to their initiation into the hoasca church, all disorders had remitted without recurrence after entry into the UDV. Such change was particularly noticeable in the area of excessive alcohol consumption, where in addition to the five subjects who had CIDI diagnoses of prior alcohol abuse disorders, six additional subjects reported moderate patterns of alcohol consumption that fell short of achieving actual psychiatric diagnostic status on formal structured interview. All 11 of these subjects with prior involvement with alcohol achieved complete abstinence shortly after affiliating with the hoasca church. In addition to their chronic substance use problems, subjects were also quite emphatic that they had undergone radical transformations of their behavior, attitudes toward others, and outlook on life. They are convinced that they had been able to eliminate their chronic anger, resentment, aggression, and alienation, as well as acquire greater self-control, responsibility to family and community, and personal fulfillment through their participation in the hoasca ceremonies of the UDV. Although the salutary effects of a strong group support system and religious affiliation cannot be minimized, it is not inconceivable that the long-term use of the hoasca itself may have had a direct positive and therapeutic effect on our subjects' psychiatric and functional status. Prior biochemical analyses of hoasca preparations have identified significant monoamine oxidase inhibitor action (McKenna et al., 1984), and may be relevant to these clinical findings.

Personality evaluation utilizing the Tridimensional Personality Questionnaire revealed significant differences between the UDV subjects and normal controls on both the novelty seeking and harm avoidance domains, but not on the reward dependence domain. The UDV subjects scored significantly lower on both the novelty seeking and harm avoidance dimensions as compared with control subjects. Individuals who had relatively low scores on novelty seeking have been described in the

psychiatric literature as reflective, rigid, loyal, stoic, slow-tempered, frugal, orderly, and persistent (Cloninger, 1987b). Low novelty seeking scores are also associated with overall behaviors consistent with high social desirability and emotional maturity (Cloninger et al., 1991). Individuals with low harm avoidance scores are described as confident, relaxed, optimistic, carefree, uninhibited, outgoing, and energetic (Cloninger, 1987b). The association of low novelty seeking with low harm avoidance has been identified with the traits of hyperthymia, cheerfulness, stubbornness, and overconfidence (Cloninger, 1987b). As the personality dimensions measured on the TPQ are thought to be heritable tendencies, a pertinent question arising from these results is whether the personality attributes as measured here have been influenced by long-term ceremonial consumption of hoasca or rather are they factors predictive specifically for individuals becoming involved with such a process as the UDV?

A similar problem arises with the interpretation of the neuropsychological data. Although long-term UDV hoasca-imbibing subjects scored significantly higher on neuropsychological testing compared with their hoasca-naive controls, as measured on the WHO-UCLA Auditory Learning Verbal Memory Test, the lack of retrospective data makes it impossible to determine whether the hoasca "tea" has had a cognitive enhancing effect or not. Although our UDV subjects spoke at length of how the hoasca had improved their powers of memory and concentration, the current methodology was not designed to definitively substantiate this connection. Only with comparative evaluation to neuropsychological performance prior to their very first experience with hoasca consumption can a comprehensive understanding of the long-term effects of hoasca on cognitive status be established. Also, only by administering such measures on naive subjects, and then following them prospectively over time with serial evaluations as they became involved with the UDV and ritual use of hoasca, can we definitively ascertain whether the hoasca does indeed improve cognitive status. The methodological approach utilized for the present study was only intended to be preliminary and exploratory, and did not possess the necessary logistics which would have allowed for such a prospective study. Indications are, however, that given the presented data analyses, the long-term consumption of hoasca within the structured UDV ceremonial setting does not appear to exert a deleterious effect on neuropsychological function.

This study has been an initial attempt to rigorously apply contemporary research models and tools to the little-studied phenomenon of ceremonial use of the plant hallucinogen hoasca. Although with a long tradition of use among the indigenous peoples of the Amazon Basin, widespread medicinal application by the mixed race mestizo populations, and 20th century development of the syncretic churches of Brazil, medical and psychiatric researchers have up to now failed to address the question of what are the effects of this highly unusual psychoactive botanical. Testimonials of its putative health-enhancing and restorative effects need to be explored, as do allegations of its potential for deleterious outcome. The establishment of legal sanctions within a religious context in Brazil provides an important and necessary prerequisite for future objective and comprehensive investigations. The ceremonial use of hoasca, as studied within the framework of this research project, is clearly a phenomenon quite distinct from the conventional notion of "drug abuse." Indeed, its apparent impact upon the subjects evaluated in the course of our inquiries appears to have been positive and therapeutic, both in self-report and objective testing. There is clearly a need to pursue rigorous and comprehensive follow-up studies to the preliminary explorations

reported here, not only to further elucidate the unique phenomenon of hoasca use within a highly structured ceremonial setting but also because of growing interest and use of hoasca in North America and Europe. It will be imperative to carefully delineate the potential for adverse effects as well as to establish the optimal safety parameters within which hoasca might be taken. In this light, careful study of the ceremonial structure and safe-guards of such groups could facilitate future research development. It is our hope that subsequent endeavors to investigate the hoasca phenomenon will explore these matters, and determine whether our preliminary findings can be replicated. Regardless of whether these results will ultimately be corroborated, we believe we have demonstrated that this fascinating, albeit neglected, phenomenon can be rigorously studied utilizing state of the art tools of research investigation.

TABLE 1

Personality Testing in 15 Long-Term Hoasca Users and 15 Matched Controls

TPQ	*Subjects*	*Controls*	*t*	*p*
Novelty seeking				
NS1: exploratory excitability vs. stoic rigidity	3.78+-1.12	5.00+-1.79	-2.08	0.049**
NS2: impulsiveness vs. reflection	1.57+-1.34	2.81+-2.27	-1.71	0.100*
NS3:extravagance vs. reserve	3.00+-1.30	3.36.+-1.43	-0.66	0.514
NS4: disorderliness vs.regimentation	2.00+-1.ll	3.64+-2.01	-2.59	0.016**
NS total:NS1 + NS2 + NS3 + NS4	10.36+-2.27	14.82+-4.81	-3.07	0.0054**
Harm avoidance				
HA1: anticipatory worry vs. unihibited optimism	1.21+-1.37	2.36+-1.97	-1.72	0.098*
HA2: fear of uncertainty vs. confidence	2.93+-0.73	4.09+-1.87	-2.14	0.043**
HA3: shyness with strangers vs. gregariousness	1.93+-1.77	3.27+-1.68	-1.92	0.067**
HA4: fatigability and asthenia vs. vigor	1.93.+-0.92	3.00+-2.45	-1.51	0.144
HA total: HA1 + HA2 + HA3 + HA4	8.00+-3.57	12.45+-4.55	-2.75	0.011**
Reward dependence				
RD1: sentimentality vs. insensitiveness	4.21+-0.89	3.90+-1.58	0.61	0.547
RD2: persistence vs. irresoluteness	4.43+-1.74	4.45+-1.86	-0.04	0.972
RD3: attachment vs. detachment	4.71+-1.94	4.27+-2.41	0.51	0.616
RD4: dependence vs. independence	1.93+-1.21	1.73+-1.62	0.36	0.725
RD Total: RD1 + RD2 + RD3 + RD4	15.29+-2.76	14.36+-3.91	0.69	0.496

TABLE 2

Neuropsychological Testing in 15 Long-Term Hoasca Users and 15 Matched Controls

WHO-UCLA Auditory Verbal Learning Test	Subjects	Controls	*t*	*p*
Words recalled on 5th learning trial	11.21+-1.93	9.50+-2.07	2.19	0.038**
Words recalled after interference	9.53+-2.72	8.16+-1.99	1.45	0.158
Dellayed recall	9.53+-2.64	8.41+-1.62	1.28	0.248
No. of words recalled	14.33+-0.72	13.75+-1.176	1.17	0.253
No. of false-positive errors on recognition task	1.06.+-1.10	0.083+-1.19	0.53	0.602

References

Bravo G, Grob CS (1989) Shamans, sacraments, and psychiatrists. *J Psychoactive Drugs* 21:123-128.

Burroughs WS, Ginsberg A (1963) *The Yage letters*. San Francisco: City Lights Books.

Cloninger CR (1987a) The Tridimensional Personality Questionnaire, version iv. St. Louis, MO: Department of Psychiatry, Washington University School of Medicine.

Cloninger CR (1987b) A systematic method for clinical description and classification of personality variants. *Arch GenPsychiatry* 44:573-588.

Cloninger CR, Przybeck TR, Svrakic DM (1991) The Tridimensional Personality Questionnaire: U.S. normative data. *Psychol Rep* 69:1047-1057.

Dobkin de Rios M (1972) *Visionary vine: Hallucinogenic healing in the Peruvian Amazon*. San Francisco: Chandler Publishing.

Dobkin de Rios M (1994, January) *Drug tourism in the Amazon*. Omni, p. 20.

Dobkin de Rios M, Grob CS (1994) Hallucinogens, suggestibility, and adolescence in cross-cultural perspective. *Yearbook of Ethnomedicine and the Study of Consciousness* 3:113-132.

Furst PT (1976) *Hallucinogens and culture*. Novato, CA: Chandler and Sharp Publishing.

Grob CS, Dobkin de Rios M (1992) Adolescent drug use in cross-cultural perspective. *J Drug Issues* 22:121-138.

Harner MJ (1973) Common themes in South American Indian yage experiences, In MJ Harner (Ed), *Hallucinogens and shamanism* (pp. 155-175). London: Oxford University Press.

Krajick K (1992, June 15) Vision quest. *Newsweek,* pp. 44-45.

Lamb FB (1971) *Wizard of the Upper Amazon: The story of Manuel Cordova-Rios*. Boston: Houghton-Miflin.

Luna LE, Amaringo P (1991) Ayahuasca visions: *The religious iconography of a Peruvian shaman*. Berkeley, CA: North Atlantic Books.

Maj M, D'Elia L, Satz P, Janssen R, Zaudig M, Uchiyama C, Starace F, Galderisi S, Chervinsky A (1993) Evaluation of three new neuropsychological tests designed to minimize cultural bias in the assessment of HIV-1-seropositive persons: A WHO study. *Arch Clin Neuropsychol* 8:123-135.

McKenna DJ, Towers GHN (1984) Biochemistry and pharmacology of tryptamines and beta-carbolines. *J Psychoactive Drugs* 16:347-358.

McKenna DJ, Towers GHN, Abbott F (1984) Monamine oxidase inhibitors in South American hallucinogenic plants: Tryptamine and beta-carboline constituents of Ayahuasca. *J Ethnopharmacol* 10:195-223.

Ott J (1993) *Pharmacotheon. Entheogenic drugs: Their plant sources and history*. Kennewick, WA: Natural Products.

Ott J (1994) *Ayahuasca analogues: Pangaean entheogens*. Kennewick, WA: Natural Products.

Rey A (1964) *L'Examen clinique en psychologie*. Paris: Presses Universitaires de France.

Robbins LN, Wing J, Wittchen HU (1988) The Composite International Diagnostic Interview: An epidemiological instrument used in conjunction with different diagnostic systems in different cultures. *Arch Gen Psychiatry* 45:1069-1077.

Schultes RE, Hofmann A (1992) *Plants of the gods: Their sacred, healing and hallucinogenic powers*. Rochester, VT: Healing Arts Press.

Schultes RE, Raffauf RF (1992) *Vine of the soul: Medicine men, their plants and rituals in the Colombian Amazonia.* Oracle, AZ: Syngertic Press.

Spruce R (1908) *Notes of a botanist on the Amazon and Andes*. London: MacMillan.

Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R (1994) Dose-response study of N,N-dimethyltryptamine in humans. II: Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry* 51:98-108.

Taussig M (1986) *Shamanism, colonialism and the wild man: A study in terror and healing*. Chicago: University of Chicago Press.

Villavicencio M (1858) *Geografia de la Republica del Ecuador*. New York: R Craigshead.

Weil AT (1980) In the land of Yage. In *The marriage of the sun and moon: A quest for unity in consciousness*. Boston: Houghton-Miflin.

Wittchen HU, Robins LN, Cottler LB, Sartorius N, Burke JD, Regier D (1991) Cross-cultural feasibility, reliability and sources of variance of the Composite International Diagnostic Interview (CIDI). *Br J Psychiatry* 159:645-653.

Ayahuasca in Adolescence: A Preliminary Psychiatric Assessment†

Dartiu Xavier Da Silveira, M.D., Ph.D.*; Charles S. Grob, M.D.**; Marlene Dobkin de Rios, Ph.D.***; Enrique Lopez, Psy.D.****; Luisa K. Alonso, Ph.D.*****; Cristiane Tacla, Psy.****** & Evelyn Doering-Silveira, M.Sc.*******

Abstract—Ayahuasca is believed to be harmless for those (including adolescents) drinking it within a religious setting. Nevertheless controlled studies on the mental/ psychiatric status of ritual hallucinogenic ayahuasca concoction consumers are still lacking. In this study, 40 adolescents from a Brazilian ayahuasca sect were compared with 40 controls matched on sex, age, and educational background for psychiatric symptomatology. Screening scales for depression, anxiety, alcohol consumption patterns (abuse), attentional problems, and body dysmorphic disorders were used. It was found that, compared to controls, considerable lower frequencies of positive scoring for anxiety, body dismorphism, and attentional problems were detected among ayahuasca-using adolescents despite overall similar psychopathological profiles displayed by both study groups. Low frequencies of psychiatric symptoms detected among adolescents consuming ayahuasca within a religious context may reflect a protective effect due to their religious affiliation. However further studies on the possible interference of other variables in the outcome are necessary.

Keywords—adolescence, ayahuasca, hallucinogen, psychopathology, religion, scales

Ayahuasca is a hallucinogenic concoction of plants used as a psychoactive ritual sacrament in ceremonies of the syncretic churches União do Vegetal (UDV) and Santo Daime. In Brazil, law has sanctioned the use of ayahuasca within the context of religious practice since 1987. Ayahuasca is consumed only during religious ceremonies, which last approximately four hours, being regularly scheduled twice monthly and often attended by multigenerational families. Within the UDV, adolescents are offered the opportunity to voluntarily join their parents and participate in ritual ceremonies where ayahuasca is consumed, and it is a common belief among members of the UDV that ayahuasca presents no risk for adolescents as long as they take it within a religious context. Nevertheless, to date there have been no controlled studies on the effects of periodic ritual ayahuasca use on adolescents.

In 1993, a comprehensive research investigation of ayahuasca use in long-term adult members of the UDV called the Hoasca Project was conducted in the Brazilian Amazon city of Manaus (Callaway et al. 1999, 1996 1994;

†This research was funded in part with a grant from the Heffter Research Institute, Santa Fe, New Mexico and from the Fundação de Amparo a Pesquisa do Estado de Sao Paulo, Brazil. Special thanks are due to Dr. Glacus Da Souza Brito and Dr. Otavio Castillo Campos Derrera, who served as liaison between the União do Vegetal Church and the Research Team.

*Professor, Department of Psychiatry, School of Medicine, and Director, Addiction Unit (PROAD), Department of Psychiatry, Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

**Director, Division of Child and Adolescent Psychiatry, Harbor/ UCLA Medical Center, Torrance, California.

***Associate Clinical Professor, Department of Psychiatry and Human Behavior, University of California, Irvine.

****Clincial Assistant Professor, Department of Psychiatry and Behavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, California.

*****Professor, Brasilia Catholic University Graduate School, Brazil.

******Psychologist, Department of Psychiatry, Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

*******Neuropsychologist, Addiction Unit (PROAD), Department of Psychiatry, Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

Please address correspondence and reprint requests to Dartiu Xavier Da Silveira, Rua Florida 320, 04565-000, São Paulo, Brazil; email:dartiu@psiquiatria.epm.br

TABLE 1
Demographic Characteristics of Adolescent Study (N = 80)

	Ayahuasca Group (N = 40)	Comparison Group (N = 40)
Age	16.52 years (SD = 1.34)	16.62 years (SD = 1.0)
Sex: Male	N = 22 (55.0 %)	N = 22 (55.0 %)
Female	N = 18 (45.0 %)	N = 18 (45.0 %)
Civil status: Single	N = 38 (95.0 %)	N = 37 (92.5 %)
Residence:		
Living with Parents	N = 37 (92.5 %)	N = 39 (97.5 %)
Ethnic group: White	N = 30 (75.0 %)	N = 33 (82.5 %)

McKenna et al. 1998; Grob et al. 1996). Phase I evaluations of pharmacokinetics, neuroendocrine assays, serotonin function, and psychiatric and medical health were then conducted. Contrasting the findings on 15 subjects from the UDV for at least 10 years with matched controls who had never consumed ayahuasca, this pilot investigation concluded that there was no evidence of injurious effect induced by ritual use of ayahuasca. Indeed, UDV subjects appeared to have experienced a remission of severe psychiatric disorders, including drug and alcohol abuse, following their entry into this religion.

Currently, the membership of the UDV in Brazil is estimated at close to 9,000, including approximately 1,200 adolescents. Considering the proportion of this age group within the population that uses these psychoactive substances on a regular basis, it is advisable to investigate the adolescents' psychiatric status and behavioral functioning.

The main objective of this study is to evaluate the mental condition of these adolescents through screening instruments for psychiatric disorders.

METHOD

Sample and Procedure

The study involved 40 adolescents, from both sexes, ages ranging from 15 to 19 years of age, who had drunk ayahuasca in a ritual context for at least 24 times in the last two years prior to the assessment. They were compared to a comparison group of 40 adolescents who had never drunk ayahuasca matched by sex, age, and educational level. Both groups live in the same communities and share the same environmental influences.

Ayahuasca-consuming adolescents were randomly selected among participants of three distinct UDV churches whereas the comparison group included randomly selected adolescents according to paring criteria. After a twenty-day washout period, ayahuasca adolescents were interviewed together with comparison group and asked to complete a series of scales aiming to screen for psychiatric conditions. Interviews were conducted by a trained psychiatrist in 2001 in two different Brazilian cities. Both adolescents and their parents were asked to sign an informed consent before enrollment in the study.

Instruments

Measurement of psychiatric morbidity in the community and clinical settings in the last decades has been achieved basically by the use of standardized methods of measurement (Cooper 1987; Eastwood 1971). Many tests used in case identification are usually referred to as "screening tests" (Goldberg 1989) and have been developed to be used in a first stage assessment in populational studies to identify probable cases that will later have their "caseness" status confirmed or not in a second stage. Such a test is devised to be easy and quick to administer, usually does not involve rich diagnostic detail, but enables proper measurement of the condition. Tests used here are acceptable scientific tools both in the sense they proved they consistently measure a given phenomenon (reliability) and in the sense that they are actually measuring what they are designed to measure (validity; see Bartko & Carpenter 1976).

Subjects were assessed in terms of mental status by means of the following psychiatric screening instruments: SRQ (Self Report Questionnaire) to assess overall psychic condition(Iacoponi & Mari 1988; Mari & Williams 1986); CES-D (Center for Epidemiological Studies Depression Scale) for depression (Da Silveira & Joge 2002).; Beck Anxiety Inventory and STAI (State-Trait Anxiety Inventory) as a screening for anxiety disorders (Gorenstein & Andrade 1996); DUSI (Drug Use Screening Inventory) to identify drug misuse (De Micheli & Formigoni 2002; Tarter et al. 1996, 1992); Conners' Adolescent Self-Rating subscale to detect Attention Deficit Disorder(Doering-Silveira & Da Silveira In press); and BSQ (Body Shape Questionnaire) to investigate self image related disorders.(Di Pietro & Da Silveira In press).

Data Analysis

Descriptive statistics were followed by comparisons between ayahuasca and control groups. Strength of associations was tested with chi-square for categorical variables, whereas t-test was used for comparing continuous variables.

TABLE 2
Number and Percentages of Subjects Scoring Positively for Psychiatric Diagnoses in Ayahuasca and Comparison Adolescent Groups (N = 80)

Scales	Cut-offs	Ayahuasca (N = 40)		Comparison Group (N = 40)		Statistical Significance P
		n	%	n	%	
Self-Report Questionnaire	7/8	3	7.5	4	10.0	n.s.
CAGE (SRQ subscale)	1/2	1	2.5	0	0	n.s.
DUSI	2/3	1	2.5	0	0	n.s.
Beck Anxiety	15/16	3	7.5	1	2.5	n.s.
STAI-state	21/22	2	5.0	7	17.5	n.s.
STAI-trait	21/22	24	60.0	32	80.0	0.087
CES-D	15/16	12	30.0	11	27.5	n.s.
Body shape questionnaire	110/111	4	10.0	11	27.5	0.083
ADD	2/3	1	2.5	7	17.5	0.057

RESULTS

Demographic Data

In the ayahuasca group, 22 adolescents (55%) were male and 18 (45%) were female. Their mean age was 16.52 ± 1.34 years. Education level ranged from the first year in high school to first year in college. Ethnic breakdown showed 30 (75% of the sample) were White and 10 (25%) classified themselves as of mixed ethnic origin. Ninety-five percent of the subjects were single and most of them (92.5%) lived with their parents.

In the comparison group, 22 adolescents (55%) were male and 18 (45%) were female. Their mean age was 16.62 ± 1.0 years. They were mostly White (82.5%) and their educational level ranged from first year in high school to third year in high school (first year in high school = 7; second year = 15; third year = 18). Thirty-seven adolescents (92.5%) were single and most of them (97.5%) lived with their parents (see Table 1).

Pattern of Ayahuasca Consumption

Twenty-five adolescents (63%) started drinking ayahuasca systematically during childhood (before the age of 13) while 15 of them (37%) began to drink when adolescents (after they were 13 years old). The time span of systematic (at least once a month) ayahuasca use was 4.05 ± 2.28 years. At the time of assessment, the adolescents abstained from drinking ayahuasca for at least 20 days, with a mean abstinence period of 41.16 ± 15.55 days. Only one adolescent (2.5 %) reported having drunk ayahuasca outside of a religious context and 39 (97.5%) reported that the experience had a profoundly positive influence on their lives.

Psychiatric Assessment

In the ayahuasca group three adolescents (7.5%) had high scores on psychiatric symptoms. In terms of substance use disorders, one adolescent (2.5%) scored positively for problem drinking and another one (2.5%) for substance misuse. Concerning anxiety symptoms, 24 (60.0%) were anxious at the time of assessment but only three adolescents (7.5%) presented high scores on Beck Anxiety Scale and two adolescents (5.0%) on STAI-State scale. Twelve teenagers (30%) presented with depressive symptoms. In the assessment for other psychiatric disorders, four adolescents (10.0%) screened positively for body dysmorphic disorder and one (2.5%) fulfilled DSM IV criteria (three or more items) for attention deficit disorder-inattentive type. Except for the high proportion of depressive subjects in this sample, overall percentages are comparable with general population rates.

In the comparison group, four adolescents (10.0%) presented high scores on psychiatric symptoms. None of the adolescents scored positively either for alcohol related problems or for substance abuse or dependence [see above comment]. Although 32 (80.0%) adolescents reported anxiety symptoms at the time of assessment, only one (2.5%) presented high scores on the Beck Anxiety Scale, but seven adolescents (17.5%) scored high on STAI-State scale. Eleven of them (27.5%) were probably depressive. In the assessment for other psychiatric disorders, 11 adolescents (27.5%) screened positively for body dysmorphic disorder and seven (17.5%) fulfilled DSM IV criteria for Attention Deficit Disorder-Inattentive type.

Comparing both groups (see Table 2), adolescents of the comparison group demonstrated a trend to have more problems than adolescents from the ayahuasca group with anxiety symptoms ($p = 0.087$), self image ($p = 0.083$), and inattentiveness ($p = 0.057$).

After stratification by gender (see Table 3), differences among the ayahuasca and the comparison group were more expressive among women; the exception was for attention problems, where six boys from the control group and only one from ayahuasca group fulfilled diagnostic criteria for ADD. Eleven girls from the ayahuasca group presented high scores for anxiety (STAI-Trait) whereas 17 girls from the

TABLE 3
Number and Percentages of Subjects Scoring Positively for Psychiatric Diagnoses in Ayahuasca and Comparison Adolescent Groups, Stratified by Sex (N = 80)

	Ayahuasca Group (N = 40)		Comparison Group (N = 40)	
Scales	**N**	**%**	**N**	**%**
Men (N = 22)				
STAI-Trait	13	59.1	15	**68.2**
Body shape questionnaire	1	4.1	1	4.5
DSM IV ADD	1	4.1	6	27.3
Women (N = 18)				
STAI-Trait	11	61.1	17	94.4
Body shape questionnaire	3	16.7	10	55.6
DSM IV ADD	0	0	1	5.6

comparison group scored high for the condition. Concerning the body shape questionnaire, only one male adolescent from each group scored high on the instrument, whereas 13 female adolescents presented high scores, with three of them being from the ayahuasca group and 10 from the comparison group.

DISCUSSION

In the preliminary pilot investigation of adult long-term ayahuasca users held in Brazil named the Hoasca Project (Grob et al. 1996), diagnostic interviews identified considerable past psychiatric histories preceding their entry into the ayahuasca church. Interestingly, psychopathology remitted following their regular attendance at ayahuasca ceremonies. It is still unclear if the reported changes can be attributed to the effect of the substance itself or to the religious affiliating process. Besides ayahuasca ingestion, set and setting may have also played a considerable role in this favorable outcome. Members of the syncretic church stressed, as do many other religious groups, the importance of a protective and supportive community (Grob 1999).

In the present study adolescents drinking ayahuasca within a religious context were overall comparable to controls in terms of psychopathological profile. Nevertheless slight differences could be observed in favor of the ayahuasca group in terms of less anxiety symptoms, less body image dysmorphia, and fewer attention deficit disorders. Only trends could be observed between groups, but the small sample size may be responsible for differences not reaching statistical significance.

Church members often report that the more they engage in ayahuasca rituals, the more they "learn" how to focus their attention. This may be reflected in the lower frequency of probable attention deficit cases among them. It is not possible yet to determine if this is the due to a direct effect of ayahuasca in the brain or to the possibility of better training of attentional skills in this particular environment.

The Hoasca project also identified significant personality differences between ayahuasca using and nonusing groups (Grob et al. 1996). Ayahuasca using subjects were considered to be more confident, optimistic, outgoing, energetic, persistent, reflective, and scored higher than controls in measures of social desirability and emotional maturity (Grob 1999). This phenomenon, probably reflecting the strong sense of belonging to a well-structured religious community, can also eventually explain the smaller proportion of ayahuasca using adolescents reporting anxiety symptoms and concerns over body image.

This cross-sectional study made it possible to establish the lower frequencies of psychiatric symptoms in the ayahuasca-consuming adolescents in comparison with nonusing ones. However, it is not possible to know if psychopathologically less affected adolescents are more prone to adhere to the religious group or if the affiliation to such a community exerts a "protective" effect on these adolescents, whatever mechanisms involved may be.

REFERENCES

Bartko, J.J. & Carpenter, W.T. 1976. On the methods and theory of reliability. *Journal of Mental and Nervous Diseases* 163: 307-17.

Callaway, J.C.; McKenna, D.J.; Grob, C.S.; Brito, G.S.; Raymon, L.P.; Poland, R.E.; Andrade, E.N. & Mash, D.C. 1999. Pharmacokinetics of hoasca alkaloids in healthy humans. *Journal of Ethnopharmacology* 65: 243-56.

Callaway, J.C.; Raymon, L.P.; Hearn, W.L.; McKenna, D.J.; Grob, C.S. & Brito, G.S. 1996. Quantitation of N,N-dimethyltryptamine and

harmala alkaloids in human plasma after oral dosing with ayahuasca. *Journal of Analytical Toxicology* 20: 492-97.

Callaway, J.C.; Airaksinen, M.M.; McKenna, D.J.; Grob, C.S. & Brito, G.S. 1994. Platelet serotonin uptake sites increased in drinkers of ayahuasca. *Psychopharmacology* 116: 385-87.

Cooper, B. 1987. *Psychiatric Epidemiology—Progress and Prospects.* Kent, U.K.: Cross Helm.

Da Silveira, D.X. & Jorge, M.R. 2002. Reliability and factor structure of the Brazilian version of the Center For Epidemiologic Studies-Depression. *Psychological Reports* 91: 865-74.

De Micheli, D. & Formigoni, M.L. 2002. Psychometric properties of the Brazilian version of the Drug Use Screening Inventory. *Alcohol: Clinical and Experimental Research* 26 (10):1523-8.

Di Pietro, M. & Da Silveira, D.X. In review. Reliability and dimensionality of the Brazilian version of the Body Shape Questionnaire.

Doering-Silveira, E. & Da Silveira, D.X. In review. Using Conners' Adolescent Self-Rating Sub-scale to detect attention deficit disorder.

Eastwood, M.R. 1971. Screening for psychiatric disorder. *Psychological Medicine* 1: 197-208.

Goldberg, D.P. 1989. Screening for psychiatric disorders. In: P. Williams; G. Wilkinson & K. Rawnsley (Eds.) *The Scope of Epidemiological Psychiatry.* London: Routledge.

Gorenstein, C. & Andrade, L. 1996. Validation of a Portuguese Version of the Beck Depression Inventory and the State-Trait Anxiety Inventory in Brazilian subjects. *British Journal of Medical and Biological Research* 29: 453-57.

Grob, C.S. 1999. The psychology of ayahuasca. In: R. Metzner (Ed.) *Ayahuasca: Hallucinogens, Consciousness, and the Spirit of Nature.* New York: Thunder's Mouth Press.

Grob, C.S.; McKenna, D.J.; Callaway, J.C.; Brito, G.S.; Neves, E.S.; Oberlander, G.; Saide, O.L.; Iacoponi, E. & Mari, J.J 1988. Reliability and factor structure of the Portuguese version of Self-Reporting Questionnaire. *International Journal of Social Psychiatry* 35 (3): 213-22.

Labigalini, E.; Tacla, C.; Miranda, C.T.; Strassman, R.J. & Boone, K.B. 1996. Human psychopharmacology of Hoasca, a plant hallucinogen used in ritual context in Brazil. *Journal of Nervous and Mental Disorders* 184 (2): 86-94.

McKenna, D.J.; Callaway, J.C. & Grob, C.S. l998. The scientific investigation of ayahuasca. A review of past and current research. *Heffter Review of Psychedelic Research* 1: 65-77.

Mari, J.J. & Williams, P. 1986. A validity study of Psychiatric Screening Questionnaire (SRQ-20) in primary care in the city of São Paulo. *British Journal of Psychiatry* 148: 23-6.

Tarter, R.E.; Kirisci, L. & Mezzich, A. 1996. The DUSI: School adjustment correlates of substance abuse. *Measurement and Evaluation in Counseling and Development* 29: 25-34.

Tarter, R.E.; Laird, S.B.; Bukstein, O. & Kaminer, Y. 1992. Validation of the Drug Use Screening Inventory: Preliminary findings. *Psychology of Addictive Behaviors* 6: 233-36.

This article was originally published in the Journal of Psychoactive Drugs in an issue entitled "Ayahuasca Use in Cross Cultural Perspective."
The issue is available from Haight Ashbury Publications, 856 Stanyan Street, San Francisco CA 94117 USA.
Phone 415-752-7601 or visit their website at www.drdave.org/journal.

Ayahuasca in Adolescence: A Neuropsychological Assessment†

Evelyn Doering-Silveira, M.Sc.*; Enrique Lopez, Psy.D.**; Charles S. Grob, M.D.***;
Marlene Dobkin de Rios, Ph.D.****; Luisa K. Alonso, Ph.D.*****;
Cristiane Tacla,Psy.******; Itiro Shirakawa, M.D., Ph.D.*******;
Paulo H. Bertolucci, M.D., Ph.D.******** & Dartiu X. Da Silveira,.M.D.*********

Abstract—The purpose of the study was to evaluate neuropsychologically adolescents who use ayahuasca in a religious context. A battery of neuropsychological tests was administered to adolescents who use ayahuasca. These subjects were compared to a matched control group of adolescents who did not use ayahuasca. The controls were matched with regards to sex, age, and education. The neuropsychological battery included tests of speeded attention, visual search, sequencing, psychomotor speed, verbal and visual abilities, memory, and mental flexibility. The statistical results for subjects from matched controls on neuropsychological measures were computed using independent t-tests. Overall, statistical findings suggested that there was no significant difference between the two groups on neuropsychological measures. Even though, the data overall supports that there was not a difference between ayahuasca users and matched controls on neuropsychological measures, further studies are necessary to support these findings.

Keywords—adolescence, ayahuasca, cognition, hallucinogen, neuropsychology, religion

Ayahuasca is a hallucinogenic beverage made essentially of two Amazonian plants. It is prepared by boiling the stems of a vine named *Banisteriopsis caapi* and the leaves of *Psychotria viridis*, although other plants are often mixed in as well. This psychedelic tea has been used for centuries by native Indian and mestizo shamans in Peru, Colombia, and Ecuador for healing and divination. In the eighteenth century ayahuasca was taken up by the colonists as a result of their proximity to tribal peoples during the Colonial period. The mixing of native contexts with nonnative settings resulted in the incorporation of ayahuasca as a psychoactive ritual sacrament in ceremonies by several

†This research was funded in part with a grant from the Heffter Research Institute, Santa Fe, New Mexico and from the Fundação de Amparo a Pesquisa do Estado de Sao Paulo, Brazil. Special thanks are due to Dr. Glacus Da Souza Brito and Dr. Otavio Castillo Campos Derrera, who served as liaison between the União do Vegetal Church and the Research Team.

*Neuropsychologist, Addiction Unit (PROAD), Department of Psychiatry, Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

**Clincial Assistant Professor, Department of Psychiatry and Behavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, California.

***Director, Division of Child and Adolescent Psychiatry, Harbor/UCLA Medical Center, Torrance, California.

****Associate Clinical Professor, Department of Psychiatry and Human Behavior, University of California, Irvine.

*****Professor, Brasilia Catholic University Graduate School, Brazil.

******Psychologist, Department of Psychiatry, Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

*******Professor, Department of Psychiatry, Federal University of Sao Paulo (UNIFESP), Brazil.

********Professor, Department of Neurology, School of Medicine, Federal University of Sao Paulo (UNIFESP), Brazil.

*********Professor, Department of Psychiatry, School of Medicine, and Director, Addiction Unit (PROAD), Department of Psychiatry, Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

Please address correspondence and reprint requests to E. Doering-Silveira, Rua Florida 320, 04565-000, São Paulo, Brazil; email:dartiu@psiquiatria.epm.br

different religious movements. In Brazil, ayahuasca is used as sacrament within the context of religious practice by the syncretic churches União do Vegetal (UDV) and Santo Daime, among others; this practice was legally approved in 1987. Churches using ayahuasca in Brazil differ somewhat from one another as to their principles, rituals, and composition of the tea.

According to the laws of the UDV, the use of ayahuasca is restricted to religious ceremonies where multigenerational families meet twice a month for approximately four hours. In sound accordance with the principles of this church (UDV), adolescents are encouraged to voluntarily join their parents and drink the ayahuasca tea during the ritual ceremonies. Adherents commonly believe that ayahuasca is harmless and potentially beneficial for adolescents (e.g., prophylaxis against drug abuse) as long as it is imbibed in a religious context. To date, however, this assumption has never been confirmed by means of controlled studies on the effects of periodic ritual use of ayahuasca by adolescents.

In 1993, a multinational research team composed of American and Brazilian physicians, psychologists and social scientists conducted a comprehensive study with UDV adult members in Manaus, the large capital city of the state of Amazon located in the heart of the Brazilian tropical rainforest. This was the first investigation of what is called the Hoasca Project. Phase I evaluations of pharmacokinetics, neuroendocrine assays, and serotonin function were carried out as well as psychiatric, medical health, and baseline neuropsychological screenings. (Callaway et al. 1999, 1996, 1994; McKenna et al. 1998; Grob et al. 1996). Contrasting the findings from 15 men who had been UDV members for at least 10 years (subjects) with demographically-matched controls who did not belong to the UDV and had never consumed ayahuasca, this pilot investigation concluded that there was no evidence of any injurious effect which could have been induced or caused by or be related to the ritualistic use of ayahuasca. On the contrary, these long-term UDV members reported a marked decline in severe psychiatric disorders, including discontinuation of cigarette, alcohol, and recreational drug use following their entry into this sect. Dramatic improvements in their personal values, behavioral compliance, and sense of purpose were described as well. Neuropsychological testing of long-term adult UDV members and matched controls found the UDV members to have statistically significant superior concentration and short-term memory on some measures, though overall both groups scored well.

Currently, in Brazil, adolescent membership of the UDV is estimated at over 1,200. A thorough investigation of UDV adolescents' cognitive profile is definitely warranted when one considers the slow but ever-growing population that consumes ayahuasca worldwide on a regular basis, and the significant proportion of younger people who are among them.

OBJECTIVE

The primary objective of this study is to assess the effects of long-term use of ayahuasca on adolescent cognitive functioning.

SUBJECTS AND METHOD

Sample

Eighty-four adolescents from three cities in Brazil (São Paulo, Campinas, and Brasilia) voluntarily participated in this study. Ayahuasca-consuming adolescents were randomly selected among participants of three distinct UDV churches, whereas the comparison group included randomly selected adolescents according to pairing criteria. Interviews were conducted by a trained psychiatrist in 2001 in two different Brazilian cities. Four of the adolescents (one subject and three controls) were not paired and as such were automatically excluded from the statistical analysis. As a result two groups of 40 adolescents (N = 80) between the ages of 15 and 19, from both sexes, were considered in this study. The first group, hereafter designated as the *subjects*, was composed of 40 adolescents from the Brazilian syncretic church UDV (União do Vegetal) who had drunk ayahuasca within a ritual context at least 24 times during the last two years prior to the neuropsychological assessment. They were 22 males and 18 females, with a mean age of 16.52 years and a standard deviation of 1.34. Most of them were White (78.9%) and their educational level ranged from first year in high school to first year in college. The second group, hereafter designated as the *control group*, was a comparison group composed of 40 adolescents who had never drunk ayahuasca (22 males and 18 females) matched on sex, age, race, and educational level to the subject group, with a mean age of 16.62 years and a standard deviation of 1.00. They were mostly White (82.1%) and their educational level ranged from first year in high school to third year in high school. Both groups had similar social and economic profiles, belonged to the same community, and shared the same environmental influences (although they did not attend the same schools). Subjects were recruited from various public and private schools whereas the comparison group of adolescents was selected from two private schools only.

Procedure

Data collection was accomplished in settings purposefully aimed at enhancing both subjects' and control's maximal motivation and collaborative attitudes as well as safeguarding the neuropsychological assessment against undesirable interferences. Thus, 20 subjects and 20 controls from Brasilia were taken on a four-day stay to a hotel located in a farm near Brasilia. Subjects and controls from São Paulo and Campinas were taken on a two-day stay to a hotel located on a quiet beach near São Paulo during two

consecutive weekends (10 subjects and 10 controls at a time). Having both subjects and controls exposed to the same environmental and psychological conditions allowed for a closer monitoring of possible confounding variables such as cigarette smoking, alcohol and drug use, poor sleeping hours, caffeine ingestion, and use of medicine, among others. Both subject and control tobacco smokers had refrained from cigarette smoking at least one hour before the assessment. Caffeine ingestion was available exclusively during breakfast time. No alcoholic beverages and other drugs were consumed 24 hours before the neuropsychological assessment. Sleeping times ranged from six to eight hours during the nights spent either at the farm hotel or at the beach hotel. Subjects had kept a minimal 20-day interval since last ingestion of ayahuasca on occasion of the neuropsychological assessment. Comfortable, quiet and well lit assessment rooms were provided. Researchers involved in data collection remained "blind" to the identity of participants throughout the study.

All adolescents and their respective parents and/or legal guardians were asked to sign an informed consent before enrollment in the study.

Neuropsychological Assessment

A comprehensive battery of neuropsychological tests was devised to assess the overall level of cognitive functioning of the adolescents. All 40 experimental adolescent subjects and 40 controls were administered a neuropsychological battery. Neuropsychological tests that assessed attention, concentration, intelligence, language, memory, executive functioning, processing speed, visuomotor skills and visuoconstructional abilities were administered. The following measures were administered: Trailmaking Test, Stroop-Victoria version, Rey-Osterrieth Complex Figure Test (ROCFT), the Conners' Continuous Performance Test-II (CPT-II), and the World Health Organization/University of California at Los Angeles Auditory Verbal Learning Test (WHO/UCLA Auditory Verbal Learning Test). Additionally, subtests of the Wechsler Adult Scale of Intelligence-III (WAIS-III) were used. The following subtests were administered from the WAIS-III: Digit Span, Digit Symbol, Symbol Search, and Object Assembly. It is important to note that Portuguese versions were administered for verbal measures. For nonverbal measures, instructions were given in Portuguese.

The Trailmaking Test, which measures sequencing, visual attention and scanning, psychomotor speed, and mental flexibility, requires that a connection be made between 25 encircled numbers randomly arranged on a page in proper order using a pencil for Part A and the connection of 25 encircled numbers and letters in alternating order for Part B (Lezak 2004).

The Digit Span subtest is used as one of the measures to calculate Verbal Intelligence and the Working Memory Index of the WAIS-III. It is used to assess verbal attention. The Digit Span subtest consists of two parts. In the first part, individuals are required to repeat sequences of three to nine digits long (Digit Forward); in the second part, examinees are asked to repeat backwards sequences of two to eight digits long (Digit Backwards). The Digit Symbol and Symbol Search are subtests from the WAIS-III that are used to assess psychomotor speed. They both are used to calculate the Processing Speed Index. The Digit Symbol subtest consists of a series of numbers, each of which is paired with its own corresponding symbol. Using a key, the individual writes the symbol corresponding to its number (Wechsler 1997). In the Symbol Search subtest, individuals scan two groups of symbols and indicate whether the target symbol appears in the search group. In the Object Assembly subtest, examinees are required to assemble puzzles as quickly as possible. It measures visual-spatial constructional abilities.

The Stroop-Victoria version is a test that measures selective attention and cognitive flexibility; it has three separate conditions (Spreen & Strauss 1998). In the first part, individuals are asked to read randomized color blocks (red, green, yellow, and blue). In the second part, the examinees are required to read words printed in different ink colors. In the last condition, individuals are given a card where color names are printed in different colored ink (the printed word never corresponds to the color name); this requires the subjects to name the color of the ink while disregarding the printed word. The Rey-Osterrieth Complex Figure Test is used to measure visuomotor skills, visual-spatial constructional ability, and visual memory (Mitrushina et al. 2005). On this measure, individuals are asked to copy a complex figure and then reproduce the design from memory 30 minutes later.

The Conners' Continuous Performance Test—Second Edition (CPT-II) is a computerized task used to assess abilities such as sustained attention, vigilance, reaction time, and impulsivity (Conners 2005). In this task, individuals are asked to press the space bar for all letters that are displayed on the computer screen except the letter X. There are six blocks, each displaying 60 letters at different interstimulus intervals. The test lasts approximately 14 minutes. A computerized report is generated at the end that includes the total number of omitted letters (omission), number of Xs pressed (commissions), and a variety of other measures related to visual attention (Hit Rate, Hit Rate SE, Variability of SE, D-prime, Beta, Perseverations, Hit RT Block Change, Hit RT SE Block Change, Hit RT ISI Change, and Hit RT SE ISI Change).

The WHO/UCLA Auditory Verbal Learning Test is a verbal memory-list test that assesses verbal learning and memory (Mitrushina et al. 2005). In this measure, examinees are read out loud a list of 15 items (list A) for five consecutive trials, each followed by a free recall test. After the fifth trial, individuals are presented with an interference trial (list B) of 15 items (Trial VI). Immediately

TABLE 1
Neuropsychological Performance of Adolescents Who Drink Ayahuasca Within a Religious Context (N = 40) Compared to a Matched Control Group (N = 40)

	Ayahuasca		Comparison Group		Statistics	
	Mean	SD	Mean	SD	*t*	*p*
Trailmaking Test						
Trail A	29.20	8.86	27.25	8.26	1.02	0.31
Trail B	61.38	25.10	56.00	15.82	1.15	0.26
WAIS-III						
Digit Span Forward	9.38	2.32	9.03	2.69	0.62	0.53
Digit Span Backward	6.83	2.26	6.48	1.97	0.74	0.46
Digit Span Total	16.45	4.27	16.00	3.46	0.52	0.61
Digit Symbol (Coding)	77.80	10.07	81.58	18.73	-1.12	0.27
Symbol Search	37.20	6.24	37.83	6.83	-0.43	0.67
Object Assembly	32.35	9.26	34.17	6.18	-1.04	0.30
Stroop-Victoria version						
Stroop I	13.03	2.27	12.60	2.22	0.85	0.40
Stroop II	16.20	3.89	14.75	3.34	1.79	0.08
Stroop III	24.95	7.14	25.05	8.00	-0.06	0.95
Rey-Osterrieth Test						
Rey Figure Copy	34.64	1.46	34.08	2.58	1.20	0.23
Rey Figure Recall	21.89	5.00	21.69	6.79	0.15	0.88
Continuous Performance Test						
CPT Omissions	2.64	4.99	1.65	1.90	1.17	0.24
CPT Commissions	10.37	6.80	10.80	5.97	-2.97	0.77
Hit Rate	406.93	77.43	400.25	73.70	0.40	0.69
Hit Rate SE	6.06	2.72	5.78	2.09	0.50	0.62
Variability of SE	7.49	5.22	7.40	5.05	0.08	0.94
D-prime	0.86	0.40	0.86	0.48	0.00	1.00
Beta	0.84	0.98	0.80	1.03	0.17	0.86
Perseverations	0.62	1.27	0.52	0.93	0.40	0.69
WHO/UCLA AVLT Trial I	6.73	2.34	7.20	2.05	-9.64	0.34
Trial II	10.05	2.17	11.55	1.66	-3.47	0.00*
Trial III	11.70	2.09	12.40	1.93	-1.56	0.12
Trial IV	12.48	1.82	13.38	1.23	-2.58	0.01*
Trial V	12.90	1.45	13.23	1.72	-0.92	0.36
Total I-V	53.85	7.5	57.75	5.51	-2.65	0.01*
Interference List (Trial VI)	6.58	1.83	6.83	1.81	-6.13	0.54
Short-delay (Trial VII)	12.15	1.90	12.83	1.58	-1.73	0.09
Long-delay (Trial VIII)	12.40	1.93	12.93	1.55	-1.34	0.18
Recognition (Trial IX)	14.55	0.82	14.78	0.62	-1.39	0.17

*Statistically significant ($p < 0.05$).

following the interference trial, individuals are asked to recall the first list without further presentation (Short Delay Recall, Trial VII). After about a 30-minute delay, individuals are again asked to recall the words from list A (Long Delay Recall, Trial VIII). Finally, a recognition trial (Trial IX) is administered where individuals are to identify words recognized from list A.

Data Analysis

Descriptive statistics were followed by the comparison between ayahuasca-using subjects and control groups. Strength of associations was tested with chi-square for categorical variables, whereas t-test was used for comparing continuous variables.

RESULTS

Overall, no significant differences in performance were found between adolescent ayahuasca users and matched controls on most of the neuropsychological measures. Results of the neuropsychological measures between both ayahuasca users and controls are available in Table 1.

On a neuropsychological measure of visual search, sequencing, visual attention, psychomotor speed, and mental flexibility, there was no difference between the two groups (Trail Making Test A and B, $p < 0.31$ and $p < 0.26$, respectively). On a measure of verbal attention, both subjects and controls did not score significantly different in their total Digit Span scores ($p < 0.61$). Additionally, no difference

was found on Digit Span Forward and Digit Span Backward ($p < 0.53$ and $p < 0.46$, respectively). Ayahuasca users and match controls did not score significantly different on both subtests that measure processing speed (Digit Symbol, $p < 0.27$; Symbol Search, $p < 0.67$). Again, there was no difference between both groups on the Object Assembly subtest ($p < 0.30$). On all conditions of the Stroop test, both groups did not score significantly differently (Stroop I, II, and III; $p < 0.40$, $p < 0.08$, and $p < 0.95$, respectively). Ayahuasca users and matched controls did not differ in performance on the ROCFT in both the copy and memory conditions ($p < 0.23$, $p < 0.88$, respectively). On all measures of the Continuous Performance Test, the subjects and controls did not differ significantly (p values on all CPT measures ranged from $p < 0.24$ to $p < 1.00$).

Subjects and matched controls scored similarly on most trials of the WHO/UCLA Auditory Verbal Learning Test. However, they seemed to differ on two of the initial trials (Trial II, $p < 0.00$, and Trial IV, $p < 0.01$). Trial I, Trial III, Trial V were not significantly different ($p < 0.34$, $p < 0.12$, and $p < 0.36$, respectively). The total score of all initial trials (Total I-V) was also statistically significant ($p < 0.01$). As expected, performances on Trial VI (interference list) were comparable among groups. Short-delay memory recall (Trial VII), long-delay memory recall (Trial VIII), and recognition recall (Trial IX) were not significantly different ($p < 0.09$, $p < 0.18$, and $p < 0.17$, respectively). Table 1 displays scores on neuropsychological measures for the adolescent ayahuasca users and matched controls.

DISCUSSION

This is the first study to focus on cognition of long-term ayahuasca-using adolescents. To date there is no scientific information on the consequences of this activity, whether beneficial or deleterious, although the Hoasca Project (referred to in the Introduction to this issue of the *Journal of Psychoactive Drugs*) details some of the consequences for adult participants. The primary finding of the present study is that no overall differences in neuropsychological performance were found between the group of ayahuasca-consuming adolescents and the group of adolescents who had never used the substance. Current scientific knowledge places great emphasis on education acting as a protector against brain insults. Since good performance on neuropsychological tests is greatly influenced by educational level the authors considered good academic achievement a central aspect in this study. Both the experimental subjects and controls had maintained good academic levels, which may have contributed to their good performance.

However, it is important to note that both groups performed well and presented similar results in most neuropsychological measures except for two trials from a verbal learning memory list test, with lower scores for the ayahuasca-consuming adolescents. Although two of the initial five trials (Trial II and IV) did show a statistically significant difference between groups on the WHO/UCLA Auditory Verbal Learning Test, the groups differed only on initial trials, which are not truly indicative of memory differences between both groups. The initial trials are more likely to be assessing learning and encoding abilities and strategies (Mitrushina et al. 2005). On memory trials, later trials (Trial VI, Trial VII, and Trial IX), both adolescent groups did not statistically differ.

It is also important to note that the mean raw scores of the initial trials (Trial II and IV) of both subjects and controls were in the average range in regards to normative data among similar aged adolescents on a similar memory list task (Geffen et al. 1990). This indicates that the mean raw scores of both groups did not significantly differ when compared to adolescent normative data.

Even though this data overall supports the theory that there was not a difference between ayahuasca users and matched controls on neuropsychological measures, one can argue that the difference in scores may suggest that ayahuasca users might differ on subtle cognitive abilities (i.e., learning and encoding). Therefore, it is important that further studies elucidate these findings. Additionally, results are limited because of the small sample size and because long-term effects were not addressed. Results may also not generalize among other populations. Again, further studies are necessary.

While additional investigations are recommended, in this pilot research study, the authors found no evidence of injurious effects of ayahuasca on adolescents who participated with their families in ceremonial rituals using psychoactive substances. In Western society, it is indeed a unique phenomenon when young people are permitted to ingest a powerful hallucinogen. We have been allowed access to study the effects and have found, at least in this pilot preliminary investigation, that ayahuasca did not have a toxic or deleterious effect on adolescent neurocognitive functioning. The question arises that since the UDV adolescents use much less alcohol, marijuana and other intoxicants according to data published in this same issue (Doering-Silveira et al. 2005) all of which are known to have negative effects on cognition when used excessively, whether ayahuasca may protect the UDV adolescents from further harm.

REFERENCES

Callaway, J.C.; McKenna, D.J.; Grob, C.S.; Brito, G.S.; Raymon, L.P.; Poland, R.E.; Andrade, E.N. & Mash, D.C. 1999. Pharmacokinetics of hoasca alkaloids in healthy humans. *Journal of Ethnopharmacology* 65: 243-56.

Callaway, J.C.; Raymon, L.P.; Hearn, W.L.; McKenna, D.J.; Grob, C.S. & Brito, G.S. 1996. Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *Journal of Analytical Toxicology* 20: 492-97.

Callaway, J.C.; Airaksinen, M.M.; McKenna, D.J.; Grob, C.S. & Brito, G.S. 1994. Platelet serotonin uptake sites increased in drinkers of ayahuasca. *Psychopharmacology* 116: 385-87.

Conners, C.K. 2005. *Conners' Continuous Performance Test-II (CPT-II).* Toronto: Multi-Health Systems Inc.

Doering-Silveira, E.; Grob, C.S.; De Rios, M.D.; Lopez, E.; Alonso, L.K.; Tacla, C.; Brito, G.S. & Da Silveira, D.X. 2005. Report on psychoactive drug use among adolescents using ayahuasca within a religious context. *Journal of Psychoactive Drugs* 37 (2).

Geffen, G.; Moar, K.J.; O'Hanlon, A.P.; Clark, C.R. & Geffen, L.B. 1990. Performance measures of 16- to 86-year-old males and females on the Auditory Verbal Learning Test. *Clinical Neuropsychologist* 4 (l): 45-63.

Grob, C.S.; McKenna, D.J.; Callaway, J.C.; Brito, G.S.; Neves, E.S.; Oberlander, G.; Saide, O.L.; Labigalini, E.; Tacla, C.; Miranda, C.T.; Strassman, R.J. & Boone, K.B. 1996. Human psychopharmacology of Hoasca, a plant hallucinogen used in ritual context in Brazil. *Journal of Nervous and Mental Disorders* 184 (2): 86-94.

Lezak, M.D. 2004. *Neuropsychological Assessment—Fourth Edition.* New York: Oxford University Press.

McKenna, D.J.; Callaway, J.C. & Grob, C.S. l998. The scientific investigation of ayahuasca. A review of past and current research. *Heffter Review of Psychedelic Research* 1: 65-77.

Mitrushina, M.N.; Boone, K.B.; Razani, J. & D'Elia, L.F. 2005. *Handbook of Normative Data for Neuropsychological Assessment—Second Edition.* New York: Oxford University Press.

Spreen, O. & Strauss, E. 1998. *A Compendium of Neuropsychological Tests—Second Edition.* New York: Oxford University Press.

Wechsler, D. 1997. *Manual for the Wechsler Adult Scale of Intelligence—Third Edition (WAIS-III).* San Antonio, Texas: The Psychological Corporation.

This article was originally published in the Journal of Psychoactive Drugs in an issue entitled "Ayahuasca Use in Cross Cultural Perspective." The issue is available from Haight Ashbury Publications, 856 Stanyan Street, San Francisco CA 94117 USA. Phone 415-752-7601 or visit their website at www.drdave.org/journal.

© Med Sci Monit, 2008; 14(8): SR15-22
PMID: 18668010

www.MEDSCIMONIT.COM
Special Report

Received: 2008.06.01
Accepted: 2008.07.09
Published: 2008.08.01

Evidence of health and safety in American members of a religion who use a hallucinogenic sacrament

John H. Halpern[1ABCDEFG], Andrea R. Sherwood[2ACDE], Torsten Passie[3CDEF], Kimberly C. Blackwell[4CDE], A. James Ruttenber[5AFG]

[1] Biological Psychiatry Laboratory, Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, Belmont, MA, U.S.A.
[2] Department of Psychology, University of New Mexico, Center for Neuropsychological Services, University of New Mexico, Albuquerque, NM, U.S.A.
[3] Department for Psychiatry, Social Psychiatry and Psychotherapy, Hannover Medical School, Hannover, Germany
[4] Department of Psychology, Program for Prevention Research, Arizona State University, Tempe, AZ, U.S.A.
[5] Department of Preventative Medicine and Biometrics, University of Colorado Health Science Center, Denver, CO, U.S.A.

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Source of support: Primary funding was borne by the investigators who provided significant time without payment. Additional funding was provided by the Church of the Holy Light of the Queen (CHLQ), Ashland, OR for travel expenses and to defray, in part, other research-related costs.

Summary

Background: Ayahuasca is a South American hallucinogenic tea used as a sacrament by the Santo Daime Church, other religions, and traditional peoples. A recent U.S. Supreme Court decision indicates religious ayahuasca use is protected, but little is known about health consequences for Americans.

Material/Methods: 32 (out of 40) American members of one branch of the Santo Daime Church were interviewed providing demographic information, physical exam, drug use timeline, a variety of psychological measures, and data about childhood conduct disorder. Subjects were asked about extent of Church participation, what is liked least and most about ayahuasca, and what health benefits or harms they attribute to ayahuasca.

Results: Members usually attend services weekly (lifetime 269±314.7 ceremonies; range 20–1300). Physical exam and test scores revealed healthy subjects. Members claimed psychological and physical benefits from ayahuasca. 19 subjects met lifetime criteria for a psychiatric disorder, with 6 in partial remission, 13 in full remission, and 8 reporting induction of remission through Church participation. 24 subjects had drug or alcohol abuse or dependence histories with 22 in full remission, and all 5 with prior alcohol dependence describing Church participation as the turning point in their recovery.

Conclusions: Conclusions should not be extrapolated to hallucinogen abusers of the general public. For those who have religious need for ingesting ayahuasca, from a psychiatric and medical perspective, these pilot results substantiate some claims of benefit, especially if subjects interviewed fully reflect general membership. Further research is warranted with blinded raters, matched comparison groups, and other measures to overcome present study limitations.

key words: ayahuasca • hallucinogens • religious use • Santo Daime • assessment

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=865802

Word count: 3984
Tables: 5
Figures: –
References: 39

Author's address: John H. Halpern, M.D., Biological Psychiatry Laboratory, Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA, U.S.A., e-mail: john_halpern@hms.harvard.edu

Background

Traditionally the most important medicine and religious sacrament of Native peoples across South America is "ayahuasca" (also known as "hoasca" or "oasca", "Daime", "yajé" or "yage", "caapi" or "kahpí", "cipó", "natema" or "natem", "dapa", "mihi", and "vegetal") and evidence exists for its ritualistic use extending back into pre-history [1]. In the Quechua language, spoken by over 10 million people, "ayawaska" means "vine of the dead" or "of the ancestors" or "of souls". It is prepared as a "tea" by boiling an abundance of shredded *Banisteriopsis caapi* vine (containing reversible monoamine oxidase inhibitors (MAOI)) with the leaves of *Psychotria viridis* (containing N,N-5,5-dimethyltryptamine (DMT)) as well as sometimes an admixture of other plant products with nicotinic and muscarinic constituents [2–5]. DMT is not orally-active, but a sufficient amount, in combination with an MAOI, will be hallucinogenic for approximately 1–2 hours [6].

The United States lists DMT as a Schedule I hallucinogen in the Controlled Substances Act (CSA). Primarily in the later 1960s, illicit DMT typically was synthetic and smoked for a brief, very intense intoxication of 15 to 20 minutes. DMT has never been considered a major part of the illicit trafficking of drugs of abuse: the U.S. Drug Enforcement Administration's Office of Diversion Control [DEAODC] reports that it is only "sporadically" encountered in the illicit market [7]. DEAODC also reports that from 1996 to 2006 there were only 71 drug samples of DMT from 31 cases noted in the System to Retrieve Information from Drug Evidence (STRIDE), a federal database cataloging seized drug samples analyzed by DEA forensic laboratories, and that, from 1999 to 2006, there were only 65 state and local cases involving 82 DMT samples listed in the National Forensic Laboratory Information System (NFLIS) [7]. For comparison, consider that during just one year (2006) there were 21,783 samples of cocaine listed in STRIDE and 371,602 cocaine samples listed in NFLIS [8].

Over the past 20 years especially, some American, European, and other world travelers have trekked to the Amazon Basin to experience ayahuasca for its spiritual/deeply mystical properties [9–12]. Hallucinogens are also now called "entheogens" ("manifesting God within") by some who ingest these compounds with religious and/or spiritual intent [13]. Indeed, Native peoples, as mentioned, have for hundreds if not thousands of years found important religious significance through the sacramental ingestion of ayahuasca (South America), mescaline-containing *Echinopsis* (*Trichocereus*) cacti (Peru), mescaline-containing *Lophophora williamsii* (peyote) (North America), and ibogaine-containing *Tabernanthe iboga* (West Africa). In Brazil, several religions exist that combine elements of traditional Native belief with Christianity, and over the years these syncretic religions have grown to include members from many countries around the world. The largest Brazilian ayahuasca religions are the Santo Daime, the União do Vegetal (UDV), and the Barquinha [14], with the Santo Daime legal also in Spain and the Netherlands. American members also returned to the United States quietly continuing their religious practice until 1999 when agents of the Drug Enforcement Administration (DEA) confiscated sacramental ayahuasca imported by representatives of the UDV and Santo Daime.

In 2000, the UDV entered a federal lawsuit seeking protection from further religious persecution, return of the seized ayahuasca, and negotiation with the DEA for enacting legal measures for the continued importation and distribution of their sacrament for their church. A pre-trial ruling essentially granted this relief to the UDV based on religious freedom grounds and the requirement that the government must exercise the least restrictive means of control of religious expression as required by the Religious Freedom Restoration Act of 1993. The federal government's response was to repeatedly appeal this ruling until it was finally accepted by the U.S. Supreme Court, which unanimously (8-0) decided in favor of the UDV in 2006. It should be noted, as well, that the Oregon State Board of Pharmacy granted in 2000 a religious exemption from their State's controlled substance laws for the Santo Daime Church's sacramental use of Daime ("Daime" is the Santo Daime's term for ayahuasca which means "give me" in Portuguese), concluding that it was a "non-drug" use and therefore not subject to regulation by the State's drug enforcement agencies.

Research into the acute and long-term effects of religious use of ayahuasca is limited, but its basic clinical pharmacology is known [6,15]. Psychoactive effects usually start 20–30 minutes after oral ingestion, lasting for 1 to 2 hours, as noted above [16]. Visual pseudohallucinations, intensification of affectivity up to ecstatic experiences, significant alterations of time/space perceptions and of body image are commonly experienced [17]. Somatic effects include mild increases of heart rate (5–15 bpm) and blood pressure (approximately 10 mm Hg) as well as elevations of cortisol, growth hormone, and prolactin [18]. DMT produces its effects mainly by influencing two types of serotonin receptors, namely the 5-HT1$_A$ and 5-HT2$_A$ receptors [19]. DMT is quickly reabsorbed in plasma and the tissues of brain, liver, kidneys, lung, and intestines. The maximum plasma levels (after oral ingestion) are reached at approximately 110 minutes. DMT is metabolized mainly by de-amination and N-oxidation as catalyzed by the MAO-enzymes. It is eliminated renally in 3–4 hours.

Several small studies have reported on the relative safety of ayahuasca when ingested in controlled religious settings [18], the absence of harm to cognitive functioning [18,20], the relative health and avoidance of drugs of addiction in adolescent church members [21], and examples of sometimes profound physical and mental healing [22], including one double-blind study noting acute amelioration of anxiety and panic in ceremonies of the Santo Daime [23]. Study populations reported in these peer-reviewed publications are most commonly Brazilian as the largest number of members of these Brazilian ayahuasca religions remains in this country of origin. With the legal status of religious use in the United States still not fully clear and with ongoing government claims of public health and safety concerns, assessment of American members of these religions is of public health importance. Yet the government has never undertaken to conduct or fund any such study. The Santo Daime Church in Oregon (Church of the Holy Light of the Queen) contacted the investigators and requested that they undertake a study of the investigators' own design to assess short and long term health effects of the sacramental ingestion of the Daime tea. We conducted an initial study to complete semi-structured psychiatric interviews of their active members who wished to volunteer meeting with us. This

Table 1. Demographics.

# Male	15		
# Female	17		
Married	47%		
Mean ±Standard Deviation		(Range)	Median
Age	49.6±9.3	(32–67)	49.0
Years of education	16.4±2.4	(11–21)	16.0
Years Santo Daime member	6.5±4.4	(0.5–19)	6.75
Age @ 1st Ceremony	41.3±11.2	(13–63)	40.5
# Ceremonies	269±314.7	(20–1300)	177.0
Attendance/month	4.8±2.9	(0.2–8)	4.0

study was approved by the Colorado Multiple Institutional Review Board as part of a larger project evaluating cognitive competence and drug use in this population that was terminated due to the untimely death of the co-principal investigator (AJR).

Material and Methods

The entire current membership of the Santo Daime Church in Oregon (approximately 40 people) were informed about this study and encouraged by Church leadership to participate. In July, 2006, over the course of one week, 34 members of the Santo Daime Church were interviewed by a research psychiatrist (JHH) with the assistance of a post-doctoral fellow psychiatrist. After informed consent was secured, a subject interview lasted for approximately 2 hours. In addition to obtaining basic demographic data, we completed a careful timeline-based survey of lifetime drug use [24], the Structured Clinical Interview for DSM-IV Disorders (SCID) [25], the 14-item Hamilton Anxiety Rating Scale (HAM-A) [26], the 21-item Hamilton Depression Rating Scale (HAM-D) [27,28], the Symptom Check List 90 Revised (SCL90R) [29], the Uplifts, Hassles, Stresses, and Cognitive Failures questionnaire (UHSCF) [30], the Wender Utah Rating Scale (WURS) [31] for attention-deficit hyperactivity disorder, and we interviewed subjects regarding childhood conduct disorder using questions that closely resembled those used on the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II) [32]. A neurology-focused physical exam was also performed. Finally, we asked detailed questions about extent of participation in Church services, what subjects like least and most about ingestion of their sacrament, and what benefits or detriments to their health, if any, that they attribute to their sacrament. Demographic information on Church members who did not participate in the study was not collected.

Results

Demographics

Of the 34 Santo Daime Church members interviewed, two were relatively new members reporting little participation in Church services as of yet (5 ceremonies and the other only one ceremony), and, as such, were excluded, leaving data on 32 subjects for analysis. Demographic information reveals a mature membership (Table 1) who usually joined in their early 40's and attend at least one Church prayer service a week. Almost half the subjects were married, all were gainfully employed except for one person currently unemployed, and two were retired. Most subjects have a college degree. The physical examination revealed physically healthy individuals.

Table 2. Claimed benefits from church participation/use of ayahuasca*.

Benefits	# of Subjects (out of N=32)
Improved general physical health	12
Increased mental clarity	11
Improved relationships	9
Improved outlook on life	7
Increased sense of purpose in life	6
More spiritual in life	6
Happier/sense of wellbeing	5
More self-confidence	5
Calmer	5
More compassion/empathy	4
Increased energy	4
Smarter/improved concentration	3
Improved anger control/less angry	3
Feel centered	3
More humble	2
Quicker healing time	2

*Subjects were asked in an open-ended, non-structured fashion to "Describe what you like most about the Daime [ayahuasca]" and "What benefits, if any, have you found from participation in Church ceremonies?".

Perception/evaluation of ayahuasca and Church attendance

All subjects reported at some point in their interview that Church participation improved introspection and most often referred to their sacrament as a "good teacher" or "guide". Inquiring about specific perceived benefits, the most frequent replies are listed in Table 2 with additional information about improved mental health discussed elsewhere further below. Improvements of character, including confidence to be "more direct", were described by those subjects finding healthier, more satisfying interpersonal relationships since joining the Church. Two subjects offered detailed descriptions of how prayer and introspection in ceremony helped avert divorce. Some subjects attribute improvements to their chronic medical conditions because of sacramental ayahuasca, including reduction or cessation of migraine (N=3), overweight individuals losing 20 or more pounds (N=4), resolution of asthma (N=1), abatement of

Table 3. Claimed side-effects from ayahuasca*.

Side-Effects	# of Subjects (out of N=32)
Nausea	11
Vomiting	9
Exhaustion for 1–2 days afterwards	9
No side-effects experienced	8
Insomnia only on the following night afterwards	4
Decreased memory for 1 day afterwards	2
Muscle spasm/stiffness	2
Visual changes	2
Headache	1
Hypoglycemia	1
Dry mouth	1
Tachycardia	1

* Subjects were asked in an open-ended, non-structured fashion "What side-effects, if any, have you experienced from ingestion of Daime [ayahuasca]?".

Table 4. What is liked least about Church participation/use of ayahuasca*.

Liked Least	# of Subjects (out of N = 32)
Feel ceremony is arduous/too demanding	12
Taste of ayahuasca	11
Exhaustion for 1–2 days afterwards	8
Nausea/vomiting	5
Nothing is unliked	4
Concern about legality	3
Church "politics"	2

*Subjects were asked in an open-ended, non-structured fashion "Describe what you like least about the Daime [ayahuasca] and/or from participation in Church ceremonies".

seizure disorder (N=1), and 1 individual with chronic fatigue syndrome went from typically being bedridden to obtaining retraining and returning to work part-time as a Registered Nurse after 5 years of full disability.

All responses from our questions about side-effects from ingestion of ayahuasca and what is least liked about practicing their faith are listed in Tables 3 and 4, respectively. Nausea and vomiting typify ayahuasca ingestion, so it is unsurprising that complaints of nausea, vomiting, and bad taste were frequently noted. No one described benefits being outweighed by complaints/problems. Of "What is liked least?", the most frequent response was that the ceremony was "arduous/too demanding", which refers to the rigors of actively participating in multi-hour all-night prayer vigils.

Psychiatric symptoms and drug abuse histories

Psychological measures suggest the members evaluated are mentally healthy (Table 5). No scores on the HAM-A indicate current clinical levels of anxiety and the same is true for the depression scores on the HAM-D except for one individual scoring a 19. That individual on SCID was diagnosed with bipolar I disorder, partial remission, and is currently on mood stabilizing medications without any report of disregulation of mood stability from Church participation or of drug-drug complications. Indeed, this individual reports making "better and less impulsive decisions" and no psychiatric hospitalizations since joining the Church six years ago. Data from the WURS did not identify any individuals with presumed childhood ADHD, and the Conduct Disorder checklist similarly did not reveal any individuals with childhood disorders of conduct. The UHSCF questionnaire results also indicate well-adjusted lives: uplifts scored near the maximum possible score of 15 and mean scores for hassles, stresses, and cognitive failures were low. Normative values for the UHSCF have not been established, but these scores appear similar to or superior to UHSCF scores reported for controls in a separate study comparing ecstasy (MDMA) users with non-users [33] and in the initial UHSCF study of tobacco smokers, non-smokers, and abstaining smokers [33].

For the nine symptom dimensions of the SCL90R, single sample t-tests were used to evaluate whether participants experienced rates of symptomatology comparable to those experienced by the general population (Table 5). With the exception of two dimensions, all scores were significantly lower than the population normative values, indicating that participants in the current sample exhibited lower rates of symptomatology than the general population. On both the Interpersonal Sensitivity dimension (t_{31}=–1.242, p=0.224) and Obsessive Compulsive dimension (t_{31}=–2.02, p=0.052), scores did not differ significantly from those in the general population. The SCL90R also includes three measures indicating overall symptomatology across the nine dimensions. The Positive Symptom Total (PST) reveals the overall number of symptoms endorsed. Scores on this measure were significantly lower than the population normative average (t_{31}=–3.641, p=0.0005), indicating that participants have fewer overall complaints than the general population. The Positive Symptom Distress Index (PSDI) is reflective of the intensity with which symptoms are experienced. Scores on this measure were significantly lower than the population normative average (t_{31}=–3.159, p=0.0018), indicating that participants report experiencing complaints with less intensity than the general population. Finally, the Global Severity Index (GSI) is the most sensitive single indicator of psychological status on the SCL90R. Scores on this index were significantly lower than the population normative average (t_{31}=–4.277, p<0.0001), indicating that participants report lower levels of overall severity than the general population.

The SCID revealed 19 individuals reporting past or present disturbances of mental health (some have more than one diagnosis). One individual still meets current criteria for a panic disorder and another meets criteria for

Table 5. Psychological measures.

Tests administered		Mean ± SD	(Range)	Median		
HAM-A		3.0±3.6	(0–15)	2.0		
HAM-D		2.8±3.7	(0–19)	2.0		
Conduct D/O*		1.1±1.5	(0–6)	1.0	t–value *df*=31	p value#
SCL90R**	Somatization scaled score	45.8±7.5	(35–59)	46.0	–3.168	0.0017
	Obsessive-compulsive standardized score	47.5±7.0	(37–62)	50.0	–2.020	0.0260
	Interpersonal sensitivity standardized score	48.2±8.2	(39–69)	49.0	–1.242	0.1118
	Depression standardized score	45.3±9.8	(34–71)	45.0	–2.713	0.0054
	Anxiety standardized score	44.2±6.0	(37–57)	42.5	–5.468	<0.0001
	Hostility standardized score	46.2±6.9	(39–59)	41.0	–3.115	0.0020
	Phobic standardized scaled score	47.0±5.2	(38–61)	47.0	–3.264	0.0013
	Paranoid ideation standardized score	45.7±7.0	(41–65)	41.0	–3.475	0.0008
	Psychoticism standardized score	45.5±5.4	(30–60)	44.0	–4.714	<0.0001
	Global Severity Index (GSI) standardized score	43.8±8.2	(30–61)	44.5	–4.277	<0.0001
	Positive Symptom Total (PST) standardized score	44.4±8.7	(24–61)	44.5	–3.641	0.0005
	Positive Symptom Distress Index (PSDI) standardized score	45.7±7.7	(30–62)	46.25	–3.159	0.0018
UHSCF	Uplifts	13±1.8	(10–15)	13.0		
	Hassles	4.9±3.1	(0–13)	5.0		
	Stresses	5.6±3.5	(1–13)	5.0		
	Cognitive failures	3.5±3.6	(0–13)	2.5		
WURS***		15.9±12.0	(1–40)	10.5		

* 12 questions were asked about behaviors prior to the age of 18 consistent with conduct disorder with 0 = to no problems of conduct and 12 = to problems of conduct on each question asked.

** Following instructions from the SCL90R manual (Derogatis 1994), raw scores were converted to standard (normalized) area T scores using provided normative data for male and female nonpatients and psychiatric outpatients. The T score is characterized by a distribution with a mean of 50 and a standard deviation of 10, with scores lower than 50 therefore signifying less symptomatology than the population average and scores greater than 50 signifying more.

*** A score greater than 46 is consistent with childhood ADHD.

Two-tailed T-test, evaluating observed sample mean against population normative average of 50.

bipolar I disorder (as mentioned), but both individuals describe seeing outpatient psychiatrists, feel that their participation in Church ceremonies has helped improve their mental health, and cannot identify any harms to management of their ongoing psychiatric issues. Five met criteria for a single prior major depressive episode that pre-dated their Santo Daime membership. Six met criteria for recurrent major depressive disorders with four in remission and two in partial remission. Four met criteria for simple phobia with two in remission and two in partial remission. Three detailed histories of bulimia nervosa in remission. Six met criteria for posttraumatic stress disorder or panic disorder, and all were in full remission. Eight individuals report induction of remission of their psychiatric condition through Church participation.

Drug and alcohol histories were quite varied as evaluated from the SCID and the even more detailed timeline-based history of use. Eight individuals report minimal to no exposures to drugs and very few intoxications from alcohol ever in their life. The other 24 individuals report histories of trying many different drugs of abuse but not a single individual described activation or re-activation of pathological drug use or worsening of use since joining the Santo Daime. Drug/alcohol diagnoses across lifetime for these 24 individuals are as follows (all are in full sustained remission, except for one individual reporting marijuana dependence in partial remission and one individual reporting ongoing marijuana abuse): 8 met criteria for alcohol abuse, 5 for alcohol dependence, 4 for marijuana abuse, 3 for marijuana dependence, 3 for hallucinogen abuse, 1 for sedative-hypnotic dependence, 1 for cocaine abuse, and 1 for stimulant abuse. The subjects with histories of hallucinogen abuse had all ended such problematic use years prior to joining the Church. The one subject meeting criteria for marijuana dependence in partial remission also claimed improve-

ment since joining the Church. All 5 subjects who met criteria for past alcohol dependence and also one subject with a history of alcohol abuse describe Church participation as the key turning point in their recovery.

Discussion

This is the first study to evaluate the health status of American members of the almost 80-year-old Santo Daime faith that originated in Brazil. Like many other religious Americans, devout members attend prayer services about once a week, but, unlike most other religions, those of the Santo Daime do ingest a hallucinogenic sacrament. Subjects reported improved health and relationships resulting from Church membership. They reported improved mental clarity and sense of life purpose, while also reporting nausea, vomiting, and a day or two of feeling tired after ingesting ayahuasca. Ten of the 32 subjects described physical health improvements since joining the Church. Nineteen subjects were diagnosed with psychiatric disorders in their lifetime, but all subjects were in good mental health, with only two members reporting an active psychiatric disorder.

From a psychiatric and medical perspective, the results substantiate some of the claims of benefit already reported in the literature (as noted above) and well known by the Santo Daime Church. Taken together, it appears that Santo Daime Church members are mentally healthy and experience benefits from their participation. The low scores for anxiety on the HAM-A and SCL90R suggest that the acute reductions in anxiety during Santo Daime prayer ceremonies noted by Santos and colleagues [23] may in fact be longer lasting. Despite 24 of the 32 subjects having in their lifetime periods of drug and alcohol abuse and dependence, 91.6% (22 of 24) of these problems are by history only, and none had any reactivation of problematic use since joining the Church. That all 5 subjects with past alcohol dependence and 1 for alcohol abuse describe achieving recovery and abstinence through the Church suggests that participation in the Diame ceremonies may well be worth studying in greater depth as an important treatment modality for alcoholism. Other investigators have already reported sustained abstinence from alcohol in former alcoholics who became members of the UDV [18,34]. There are also other surveys that have presented evidence of improved physical and mental health [35] as well as general safety of religious use [36–38]. There simply is no evidence from within the data collected to assert that there are concerning harms from the full practice of Santo Daime. Most side-effects, as detailed, from ayahuasca were temporal to ingestion, manageable, and rarely persisted beyond a day or two. If ingestion of ayahuasca is sometimes transiently stressful or emotionally problematic for Church members, it is striking that none of those interviewed described this in our questions about "what is liked least?," as listed in Table 4. It is also possible that the structured nature of the prayer services and follow-up meetings provide a reliable path for positive integration and utility after the acute effects of sacramental ayahuasca end.

The federal government has never demanded that Native Americans prove the safety of peyote in the prayer services of the Native American Church (NAC), yet in the current climate of federal resistance to accepting religious protections for the members of Santo Daime and UDV, this is exactly what is being demanded by the government of these non-Native groups. The ayahuasca in a typical serving does contain enough DMT and MAOI to induce a hallucinogenic experience, but in this religious context, as with the NAC and peyote, the ingestion of this ayahuasca appears to meet the same legal standard of a "non-drug, sacramental use". The intent is to commune with God rather than directly seeking a "drug high". As detailed by our subjects (Tables 3 and 4), the demands of the Santo Daime faith are arduous, as many prayer ceremonies continue through the night and the brewed sacrament itself can acutely induce nausea and vomiting after ingestion. Religious individuals of other faiths will recognize much in common with the Santo Daime in their sincerity of expressions of faith and self-improvement through prayer and fellowship.

There are several important limitations to this study. Though all members of this American community were invited to participate, 80% did so. It is possible that the other 20% of members might present quite differently than those interviewed, and, of course, no members of other Santo Daime communities residing in the United States or elsewhere were interviewed, and so our findings for these reasons as well might still not reflect general membership.

Of 32 established members interviewed, almost 60% had psychiatric histories. It is possible, then, that these participants were more familiar with speaking with psychiatrists and therefore more willing to be interviewed than the 6 members who did not volunteer. Yet we would expect our results to be skewed towards more unhealthy evaluations within our sample by being populated by more people with mental health histories. Instead, our results still revealed mentally healthy individuals, and as such, suggest participation in the Santo Daime Church is not proving harmful even to those members most susceptible to mental health problems. It is also possible that a type of self-selection bias occurred that precluded interviews with those who experienced harm: members who derived the most benefit remained in the Church and may have readily volunteered whereas those who have not benefited avoided participation. This Santo Daime community has approximately 110 former members. Former members of many religions describe their opposition to certain religious practices and duties, and this may be quite useful to evaluate in future studies of the Santo Daime, but the attitudes and wellbeing of active members should not be ignored because former Church members were not similarly evaluated. Almost all of the active members did volunteer, and these participants also asserted that their stories of healing and wellbeing and personal growth are common among members of Santo Daime.

Other study limitations include lack of comparison group and not administering measures blinded. We were however doing psychiatric assessments: our comparators are based on training, prior clinical experience, and use of the SCID, which is a reliable semi-structured interview for psychiatric diagnosis. The other test measures were either self-rated forms for the participants to complete or have clear protocols for physician administration. We also did not interview members before and after joining the Santo Daime, which would offer prospective data on claims of change. With available funds, we believe it could prove valuable to track individuals who are about to participate in the Santo Daime Church and then

continue to follow for several years those who remain members and those who don't. Future research could address some of these issues by use of a matched comparison group of non-members who are similarly religious and by use of raters blinded to group assignment. Expansion of assessment also may capture problems not yet identified; a careful battery of neuropsychological testing, for example, may reveal impaired performance on some measures. However, evidence already exists that long-term use of ritual-based hallucinogens does not lead to decreases in neurocognition [39].

Conclusions

If the Santo Daime continues to look favorably upon research of their membership, such data may prove helpful not just to a skeptical federal government but to key decision makers and stakeholders at the community level as well as to those specifically who wish to pray with the Santo Daime and/or explore how belonging to this faith may directly benefit them or not. DMT, the hallucinogenic substance found in ayahuasca, is, as mentioned, listed as a Schedule I drug in the CSA. Placement into Schedule I was not based on any specific negative research finding about DMT but, rather, was based on concerns for harmful consequences for Americans to ingest powerful mind-altering drugs of no known safety or utility. Yet America does have a long and positive experience of finding room for Native Americans who express their faith through the Peyote Way (and indeed the NAC is the largest Native religion in the U.S. with some 300,000 members). Much like with the NAC, the Santo Daime prayer ceremonies do provide membership a structured setting of known safety with the clear utility of religious meaning. Perhaps it will be through these types of religions that we will learn more clearly about pharmacologic benefits from these drugs where the safe environment resides in the church rather than the lab.

Acknowledgements

In memoriam to A. James Ruttenber, Ph.D, M.D. We appreciate research assistants Vince Eckert and Kevin J. Franciotti for their help with data entry and management and postdoctoral fellow R. Andrew Sewell, M.D. for assistance with subject interviews. We also wish to extend our gratitude to the leaders and membership of the Church of the Holy Light of the Queen, Ashland, OR for entrusting us with access to them and for providing financial support. Special thanks are also extended to Mr. Jonathan Goldman and Ms. Miriam Ramsey for making office space available and coordinating interview appointments, respectively.

Conflict of interest

Dr. Halpern reports having received fees from CHLQ for advising them on health related issues and will continue to provide such advice. Drs. Halpern, Ruttenber, and Sherwood have received some research funding from CHLQ for this study. Dr. Passie and Ms. Blackwell report no conflicts of interest.

References:

1. Schultes RE, Hoffman A, Rätsch C: Plants of the gods: their sacred, healing, and hallucinogenic powers. 2nd ed. Rochester (VT): Healing Arts Press, 2001
2. Callaway JC: Various alkaloid profiles in decoctions of Banisteriopsis caapi. J Psychoactive Drugs, 2005; 37: 151–55
3. Casale JF, Koles JE: Analysis of ayahuasca ('Santo Daime'). Microgram, 1995; 28: 296–99
4. Freedland CS, Mansbach RS: Behavioral profile of constituents in ayahuasca, an Amazonian psychoactive plant mixture. Drug Alcohol Depend, 1999; 54: 183–94
5. McKenna DJ, Towers GH, Abbott F: Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of ayahuasca. J Ethnopharmacol, 1984; 10: 195–223
6. Riba J, Rodriguez-Fornells A, Urbano G et al: Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. Psychopharmacol (Berl), 2001; 154: 85–95
7. Office of Diversion Control, Drug Enforcement Administration, U.S. Department of Justice. *http://www.deadiversion.usdoj.gov/drugs_concern/dmt/dmt.htm*. Accessed 12/30/2007
8. Office of Diversion Control, Drug Enforcement Administration, U.S. Department of Justice. *http://www.deadiversion.usdoj.gov/drugs_concern/cocaine/cocaine.htm*. Accessed 12/30/2007
9. de Rios MD: Drug tourism in the Amazon. Anthropol Conscious, 1994; 5: 16–19
10. de Rios MD: Mea culpa: drug tourism and the anthropologist's responsibility. Anthropol News, 2006; 47: 20
11. Halpern JH, Pope HG Jr: Hallucinogens on the Internet: a vast new source of underground drug information. Am J Psychiatry, 2001; 158: 481–83
12. Winkelman M: Drug tourism or spiritual healing? Ayahuasca seekers in Amazonia. J Psychoactive Drugs, 2005; 37: 209–18
13. Ruck CAP, Bigwood J, Staples R et al: Entheogens. J Psychedelic Drugs, 1979; 11: 145–46
14. Labate BC, Araujo WS (eds.): O uso ritual da ayahuasca. 2nd ed. Campinas, Brazil: Mercado de Letras, 2004
15. Riba J, Valle M, Urbano G et al: Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metaboline excretion, and pharmacokinetics. J Pharmacol Exp Ther, 2003; 306: 73–83
16. Callaway JC, McKenna DJ, Grob CS et al: Pharmacokinetics of Hoasca alkaloids in healthy humans. J Ethnopharmacol, 1999: 65; 243–56
17. Shanon B: The antipodes of the mind: charting the phenomenology of the ayahuasca experience. Oxford: Oxford University Press, 2002
18. Grob CS, McKenna DJ, Callaway JC et al: Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. J Nerv Ment Dis, 1996; 184: 86–94
19. Nichols DE: Hallucinogens. Pharmacol Therapeutics, 2004; 101: 131–81
20. Doering-Silveira E, Grob CS, de Rios MD et al: Ayahuasca in adolescence: a neuropsychological assessment. J Psychoactive Drugs, 2005; 37: 123–28
21. Doering-Silveira E, Lopez E, Grob CS et al: Report on psychoactive drug use among adolescents using ayahuasca within a religious context. J Psychoactive Drugs, 2005; 37: 141–44
22. Groisman A, Sell AB: Healing power: neurophenomenology, culture, and therapy of Santo Daime. In: Winkelman M, Andritzky W (eds.): Yearbook of cross-cultural medicine and psychotherapy, vol 4. Berlin: VWM – Verlag für Wissenschaft und Bildung, 1996; 279–87
23. Santos RG, Landeira-Fernandez J, Strassman RJ et al: Effects of ayahuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members. J Ethnopharmacol, 2007; 112: 507–13
24. Yin M, Wade TD, Lawler-Heavner J, Ruttenber AJ: Computer-generated time lines for visualizing and editing epidemiological and biomedical data. Comput Methods Programs Biomed, 1998; 56: 23–29
25. First M, Spitzer R, Gibbon M, Williams J: Structured clinical interview for DSM-IV-TR Axis I disorders, research version, non-patient edition (SCID-I/NP). New York: Biometrics Research, New York State Psychiatric Institute, 2001
26. Hamilton M: The assessment of anxiety states by rating. Br J Med Psychol, 1959; 32: 50–55
27. Hamilton M: A rating scale for depression. J Neurol Neurosurg Psychiatry, 1960; 23: 56–61
28. Hamilton M: Development of a rating scale; for primary depressive illness. Br J Soc Clin Psychol, 1967; 6: 278–96
29. Derogatis LR: SCL-90-R: administration, scoring and procedures manual. Minneapolis: National Computer Systems, Inc., 1994
30. Parrott AC, Kaye F: Uplifts, hassles and cognitive failures in cigarette smokers and non-smokers. Behav Pharmacol, 1999; 10: 639–46

31. Ward M, Wender P, Reimherr F: The Wender Utah rating scale: an aid in the retrospective diagnosis of childhood attention deficit hyperactivity disorder. Am J Psychiatry, 1993; 150: 885–89
32. First M, Gibbon M, Spitzer R et al: Structured clinical interview for DSM-IV Axis II disorders (SCID-II). Washington DC: American Psychiatric Press, 1997
33. Parrott AC, Sisk E, Turner JJD: Psychobiological problems in heavy 'ecstasy' (MDMA) polydrug users. Drug Alcohol Depend, 2000; 60: 105–10
34. Miranda CT, Labigalini E, Tacla C: Alternative religion and outcome of alcohol dependence in Brazil. Addiction, 1995; 90: 847
35. Barbosa PC, Giglio JS, Dalgalarrondo P: Altered states of consciousness and short-term psychological after-effects induced by the first time ritual use of ayahuasca in an urban context in Brazil. J Psychoactive Drugs, 2005; 37: 193–201
36. de Rios MD, Grob CS, Lopez E et al: Ayahuasca in adolescence: qualitative results. J Psychoactive Drugs, 2005; 37: 135–39
37. da Silveira DX, Grob CS, de Rios MD et al: Ayahuasca in adolescence: a preliminary psychiatric assessment. J Psychoactive Drugs, 2005; 37: 129–33
38. Gable RS: Risk assessment of ritual use of oral Dimethyltryptamine (DMT) and Harmala alkaloids. Addiction, 2007; 102: 24–34
39. Halpern JH, Sherwood AR, Hudson JI et al: Psychological and cognitive effects of long-term peyote use among Native Americans. Biol Psychiatry, 2005; 58: 624–31

Neuroimaging Studies

Literature research conducted by José Carlos Bouso for
The International Center for Ethnobotanical Education, Research & Service

Psychopharmacology
DOI 10.1007/s00213-006-0358-7

ORIGINAL INVESTIGATION

Jordi Riba · Sergio Romero · Eva Grasa · Esther Mena · Ignasi Carrió · Manel J. Barbanoj

Increased frontal and paralimbic activation following *ayahuasca*, the pan-amazonian inebriant

Received: 14 November 2005 / Accepted: 13 February 2006

Abstract *Rationale:* Ayahuasca is a South American psychoactive plant tea which contains the serotonergic psychedelic *N*,*N*-dimethyltryptamine (DMT) and monoamine-oxidase inhibitors that render DMT orally active. Previous investigations with ayahuasca have highlighted a psychotropic effect profile characterized by enhanced introspective attention, with individuals reporting altered somatic perceptions and intense emotional modifications, frequently accompanied by visual imagery. Despite recent advances in the study of ayahuasca pharmacology, the neural correlates of acute ayahuasca intoxication remain largely unknown. *Objectives:* To investigate the effects of ayahuasca administration on regional cerebral blood flow. *Methods:* Fifteen male volunteers with prior experience in the use of psychedelics received a single oral dose of encapsulated freeze-dried *ayahuasca* equivalent to 1.0 mg DMT/kg body weight and a placebo in a randomized double-blind clinical trial. Regional cerebral blood flow was measured 100–110 min after drug administration by means of single photon emission tomography (SPECT). *Results:* Ayahuasca administration led to significant activation of frontal and paralimbic brain regions. Increased blood perfusion was observed bilaterally in the anterior insula, with greater intensity in the right hemisphere, and in the anterior cingulate/frontomedial cortex of the right hemisphere, areas previously implicated in somatic awareness, subjective feeling states, and emotional arousal. Additional increases were observed in the left amygdala/parahippocampal gyrus, a structure also involved in emotional arousal. *Conclusions:* The present results suggest that ayahuasca interacts with neural systems that are central to interoception and emotional processing and point to a modulatory role of serotonergic neurotransmission in these processes.

Keywords Ayahuasca · Dimethyltryptamine · Psychedelics · SPECT · Regional cerebral blood flow · Human

J. Riba · S. Romero · E. Grasa · M. J. Barbanoj
Centre d'Investigació de Medicaments,
Institut de Recerca, Servei de Farmacologia Clínica
Hospital de la Santa Creu i Sant Pau,
St. Antoni Maria Claret, 167,
Barcelona 08025, Spain

J. Riba · S. Romero · E. Grasa · M. J. Barbanoj
Departament de Farmacologia i Terapèutica,
Universitat Autònoma de Barcelona,
Barcelona, Spain

E. Mena · I. Carrió
Servei de Medicina Nuclear
Hospital de la Santa Creu i Sant Pau,
Barcelona, Spain

S. Romero
Departament ESAII,
Centre de Recerca en Enginyeria Biomèdica
Universitat Politècnica de Catalunya (UPC),
Barcelona, Spain

Present address:
J. Riba (✉)
Department of Neuropsychology
Otto-von-Guericke University,
Magdeburg, Germany
e-mail: jriba@santpau.es
Tel.: +34-93-2919019
Fax: +34-93-4352408

Introduction

The psychotropic ayahuasca tea, a central element of Amazonian shamanism (Schultes and Hofmann 1987), is commonly obtained by infusing together the stems of the *Banisteriopsis caapi* liana and the leaves of the *Psychotria viridis* bush, two plants endemic to the region (Schultes and Hofmann 1980, 1987). In the past, ayahuasca use among the indigenous inhabitants of the Amazon was traditionally restricted to medicine men and was only open to lay members of the group during certain communal celebrations and rites of passage into adulthood (Reichel-Dolmatoff 1990). However, in recent years, it has given way to more

widespread use. "Ayahuasca tourism" is on the rise in the region (Halpern 2004; McKenna 2004; Salak 2004), and several Brazilian-based religious groups who use ayahuasca in their rituals are seeking legal authorization to practice their religion in the United States and Europe (Greenhouse 2005; Halpern 2004; McKenna 2004).

From the early 19th century accounts written by explorers (Spruce 1908; Villavicencio 1858) to present day clinical trials (Riba et al. 2001b), reports on the subjective effects elicited by ayahuasca have described a remarkable modified state of awareness in which introspective attention is typically enhanced. Subjects report experiencing a highly emotional state in which bodily sensations are modified or intensified and in which visual imagery frequently emerges, often laden with a marked personal content (Riba et al. 2001b). Within the context of traditional use, this modified state of awareness is reportedly used by shamans to diagnose and treat the psychological and physical afflictions of their patients (Dobkin de Rios 1984), whereas among the Brazilian ayahuasca churches, the tea is considered a sacrament that allows contact with the divine (Labate and Araújo 2002). From a medical perspective, the spread of ayahuasca use to Europe and North America in the last decade has raised concern among public health authorities (Anonymous 2000). However, in a wave of renewed psychiatric interest in psychedelics as therapeutic agents (Check 2004; Kotler 2005; Melton 2004), there have been claims of its potential use as a treatment for substance-related and other psychiatric disorders (McKenna 2004).

Chemical analyses and pharmacological studies conducted with ayahuasca have shown that the tea combines in a single preparation the orally labile serotonergic psychedelic *N*,*N*-dimethyltryptamine (DMT) from *P. viridis* with monoamine oxidase (MAO)-inhibiting β-carboline alkaloids (Buckholtz and Boggan 1977) from *B. caapi* (McKenna et al. 1984). Remarkably, these β-carbolines block the metabolic breakdown of DMT by the visceral MAO, allowing its access to systemic circulation (Riba et al. 2003). In the central nervous system, DMT binds to the serotonin-2A receptor, where it acts as a partial agonist (Rabin et al. 2002). Neuroendocrine measures after acute ayahuasca administration (Callaway et al. 1999) and peripheral serotonin transporter levels in long-term users (Callaway et al. 1994) further support an interaction between ayahuasca and serotonergic neurotransmission.

Although various aspects of the pharmacology of ayahuasca in humans have been described in recent years (Callaway et al. 1999; Riba et al. 2001b, 2003), the biological substrates underlying the psychological modifications it elicits remain largely unknown. To examine the neural correlates of acute ayahuasca effects, we conducted a blood perfusion single photon emission tomography (SPECT) study in a group of fifteen male volunteers who had prior experience in the use of psychedelics. SPECT scans conducted after administration of a single dose of a freeze-dried and encapsulated formulation of ayahuasca were compared with scans obtained after a placebo.

Materials and methods

Volunteers

Fifteen male volunteers experienced in the use of psychedelics, i.e., minimum use on at least ten occasions, were recruited. Volunteers were in good physical health, confirmed by medical history, laboratory tests, ECG and urinalysis, and psychological health (structured psychiatric interview for the DSM-IV). Exclusion criteria were as in previous studies (Riba et al. 2001b, 2003) and included current or previous history of psychiatric disorders and alcohol or other substance dependence. Participants had a mean age of 28 years (range 20–38), mean weight 66.8 kg (range 60.1–85), and mean height 176.2 cm (range 163–196). Participants had used psychedelics from ten to hundreds of times. The most commonly used psychedelics were psilocybian mushrooms (15/15) followed by LSD (14/15) and ketamine (9/15). Some volunteers had experienced using peyote (5/15) and mescaline (2/15) and only one had taken ayahuasca before his participation in the study. Besides psychedelics, volunteers had consumed cannabis (15/15), cocaine (15/15), MDMA (13/15), and amphetamines (13/15). The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans, and was approved by the hospital's ethics committee and the Spanish Ministry of Health. All volunteers gave their written informed consent to participate.

Drug

An encapsulated freeze-dried formulation of ayahuasca was obtained as described in previous studies (Riba et al. 2001b, 2003). The ayahuasca batch employed contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg THH per gram of freeze-dried material. The dose administered in the present investigation was of 1 mg DMT/kg body weight, and was chosen based on an earlier work in which it had been proven to elicit full-blown psychotropic effects (Riba et al. 2001b). Placebo capsules contained 0.75 g lactose.

Study design

In a double-blind randomized fashion, each volunteer received either a single oral dose of encapsulated freeze-dried ayahuasca or a placebo in two experimental sessions at least 1 week apart. Volunteers were requested to abstain from any medication or illicit drug use 2 weeks before the beginning of the experimental sessions and until the completion of the study. Volunteers also abstained from alcohol, tobacco, and caffeinated drinks 24 h before each experimental day. Urinalysis for illicit drug use was performed for each experimental session. After arrival at 9:00 a.m. under fasting conditions, volunteers had a light breakfast and a cannula was inserted in an arm vein for

radiotracer administration. Capsules with either the drug or placebo were administered at 12:00 noon with 250 ml tap water. Throughout the experimental session, the volunteers remained seated on a comfortable reclining chair in a quiet, dimly lit room until SPECT image acquisition. All volunteers remained overnight in the laboratory and were discharged at 12:00 noon of the following day.

Study methods

SPECT imaging

At 100–110 min after drug administration, an injection of 30 mCi technetium-99m-labeled ethylcysteinate dimer was administered through the previously placed intravenous cannula. The volunteers, sitting on a comfortable reclining chair in the same room since drug administration, were instructed 5 min before bolus injection to close their eyes and remain as relaxed as possible during the injection procedure and to remain with eyes closed for an additional 10 min after completion of the bolus. After this time, they were allowed to open their eyes again and they remained in the room until image acquisition, which was conducted 1 h later, at 3 h after drug administration.

SPECT imaging of the brain was performed with the volunteer's head supported by a headrest using a two-headed HELIX gamma camera (General Electric Medical Systems) equipped with fan-beam collimators. Data were acquired using a 128×128 image matrix in three degree steps. The total acquisition procedure lasted for around 50 min. Images were reconstructed by filtered back-projection using a Metz filter and the Chang method for attenuation correction using a factor of 0.075.

Statistical analyses were conducted on a voxel-by-voxel basis using the SPM2 software (The Wellcome Department of Imaging Neuroscience, http://www.fil.ion.ucl.ac.uk/spm/spm2.html) on a Matlab platform (Mathworks). Images were converted to the analyze format and were spatially normalized to the Montreal Neurological Institute (MNI) standard anatomical space. Standardized images were then smoothed using a 16-mm gaussian kernel. The statistical analysis used a "multiple subjects, conditions, and covariates" model, with the gray matter threshold set at 0.8 and normalization of global cerebral blood flow to 50 with proportional scaling. Contrasts were used to search for voxels of relative change between ayahuasca and placebo. Results are presented at p-value <0.002, uncorrected for multiple comparisons, corresponding to a t-value of 3.44. Only activations involving clusters with more than 50 voxels are reported. Maximum t-values within an activation cluster are reported in MNI coordinates.

Subjective effect measures

Self-rated subjective effects were measured by administering Spanish versions of the Hallucinogen Rating Scale or HRS (Riba et al. 2001a) and the Addiction Research Center Inventory or ARCI (Lamas et al. 1994). The HRS (Strassman et al. 1994) measures psychedelic-induced subjective effects and includes six scales: *Somesthesia*, reflecting somatic effects; *Affect*, sensitive to emotional and affective responses; *Volition*, indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; *Cognition*, describing modifications in thought processes or content; *Perception*, measuring visual, auditory, gustatory, and olfactory experiences; and finally *Intensity*, which reflects the strength of the overall experience. The range of scores for all HRS scales is 0 to 4. The ARCI (Martin et al. 1971) consists of five scales or groups: MBG, morphine–benzedrine group, measuring euphoria and positive mood; PCAG, pentobarbital–chlorpromazine–alcohol group, measuring sedation; LSD, lysergic acid diethylamide scale, measuring somatic–dysphoric effects; BG, the benzedrine group, measuring intellectual energy and efficiency, and the A scale, an empirically derived scale measuring amphetamine-like effects. The range of scores is 0 to 16 for MBG, −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG, and 0 to 11 for A. Volunteers answered the ARCI immediately before drug administration, and 4 h after drug intake, whereas the HRS was only answered at 4 h postadministration.

Before statistical analysis, ARCI scores were transformed to differences from preadministration values. The transformed ARCI scores and raw HRS scores were analyzed by means of t tests with drug (placebo, *ayahuasca*) as factor. In all tests performed, differences were considered statistically significant for p-values lower than 0.05.

Results

SPECT

The results of the statistical parametric mapping analysis between ayahuasca and placebo scans are shown in Fig. 1. Ayahuasca administration led to bilateral activation of the anterior insula/inferior frontal gyrus, with greater intensity in the right hemisphere. Additional areas of increased blood perfusion were observed in the frontomedian wall of the right hemisphere. At this level, the largest cluster of suprathreshold voxels was located in the anterior cingulate/medial frontal gyrus. A smaller cluster was found in the ventral anterior cingulate/subcallosal gyrus. In the left hemisphere, activation was also observed in an area corresponding to the amygdala/ parahippocampal gyrus. No significant decreases in regional cerebral blood flow were observed anywhere in the brain.

Subjective effects

Subjective effect results are shown in Table 1.

Ayahuasca administration induced significant increases in all six HRS scales. The ARCI questionnaire showed statistically significant increases in the A scale measuring

stimulatory effects, the MBG scale measuring positive mood and euphoria, and the LSD scale measuring somatic symptoms. Scores on the BG scale measuring intellectual efficiency and the PCAG scale measuring sedation were not significantly different from placebo.

Discussion

At the dose administered in the present study, ayahuasca induced manifest psychotropic effects as evidenced by the significant increases in various subscales of the measurement instruments administered. Effects included characteristic changes in somatic sensations (HRS–Somesthesia, ARCI–LSD), modifications in thought content and increased arousal (HRS–Cognition, ARCI–A), modifications in visual perception and visions with eyes open and closed (HRS–Perception), and mood modifications (HRS–Affect, ARCI–MBG). The general pattern of these effects was analogous to that found and described in previous studies and placed ayahuasca among the psychedelics (Riba et al. 2001b, 2003).

Table 1 Means (SD) of the scores obtained for the HRS and ARCI questionnaires subscales (n=15), and results of the statistical analysis performed. Statistically significant differences with placebo are indicated by asterisks

	Placebo	Ayahuasca
HRS		
Somaesthesia	0.62 (0.18)	1.32 (0.80)***
Affect	0.31 (0.12)	1.41 (0.72)***
Perception	0.02 (0.08)	1.65 (1.04)***
Cognition	0.02 (0.07)	1.54 (1.13)***
Volition	0.85 (0.68)	1.77 (0.67)***
Intensity	0.03 (0.13)	2.20 (1.07)***
ARCI		
A	0.20 (0.77)	3.20 (2.21)***
BG	0.27 (0.96)	−0.8 (2.81)
MBG	−0.33 (1.11)	2.87 (4.31)**
PCAG	−0.60 (4.15)	0.53 (4.72)
LSD	0.33 (1.63)	4.40 (2.64)***

**P<0.01, ***P<0.001

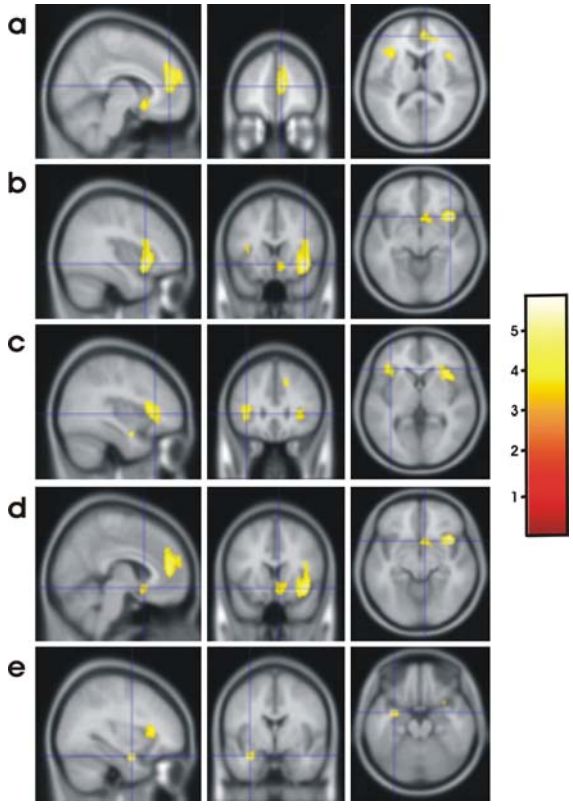


Fig. 1 Statistical parametric maps of increases in regional cerebral blood flow in each of the five clusters showing suprathreshold voxels. Each cluster is shown in the three orthogonal views (*left*, sagittal; *middle*, coronal; *right*, transverse) through the voxel with the maximum t-value. MNI coordinates (x, y, and z) and the number of voxels in each cluster are provided. **a** Right anterior cingulate/right medial frontal gyrus (8, 46, 12; n=594, t=5.39); **b** right insula/right inferior frontal gyrus (38, 16, −8; n=674, t=5.79); **c** left insula/left inferior frontal gyrus (−36, 28, 2; n=347, t=4.78); **d** ventral anterior cingulate/subcallosal gyrus (6, 14, −10; n=119, t=3.97); **e** amygdala/parahippocampal gyrus (−32, −2, −20; n=74, t=5.71). Results are shown at a p value <0.002 uncorrected, superimposed on an SPM T1 NMR template corresponding to the average of 152 subjects

Regarding the intensity of the reported subjective effects, HRS scores in the present study were higher than in a previous study by our group in which 0.6 and 0.85 mg DMT/kg doses were administered (Riba et al. 2003). They were also higher than those reported by Grob et al. (1996) after the administration of an ayahuasca dose containing approximately 0.5 mg DMT/kg body weight. Compared with intravenous DMT as described by Strassman et al. (1994), HRS scores after the present ayahuasca dose fell between those reported after 0.2 and 0.4 mg/kg IV DMT.

The above subjective effects were accompanied by increased regional cerebral blood flow in paralimbic and neocortical areas of the forebrain, with the highest significance values in the statistical comparison attained in the right anterior insula, the left amygdala/parahippocampal gyrus, and the right anterior cingulate/medial frontal gyrus. Consistent with the deeply introspective experience induced by ayahuasca, these structures, especially the right anterior insula, have recently been proposed to be key structures of a neural system supporting interoception (Craig 2002, 2003). In this respect, thalamocortical projections relay to the anterior insula cutaneous and visceral homeostatic information thought to represent the physiological condition of the entire body (Craig 2002, 2003). Recent research has shown bilateral insular and cingulate activation in tasks requiring interoceptive attention, with the right anterior insula specifically subserving explicit awareness of bodily processes (Critchley et al. 2004). This neural system supporting interoceptive awareness has been proposed to provide the basis for subjective feeling states and self-awareness (Craig 2002). A recent magnetic resonance imaging study with experienced meditators found increased cortical thickness in the right anterior insula. The long-term meditators enrolled in the study had practiced a form of meditation whose main goal

was to focus attention on internal states. The authors interpreted the observed cortical thickness increases as an increased awareness of interoceptive stimuli such as breathing sensations (Lazar et al. 2005).

Damasio (2003) and Craig (2002, 2003) have postulated that access to representations of bodily states supported by the right anterior insula plays a key role in the generation of subjective feelings. In fact, activation of the right anterior insula has been observed in many studies of recall-generated emotions (Damasio et al. 2000; Phan et al. 2002; Reiman et al. 1997). Similar to our present results, these experiments have frequently revealed a concomitant activation of the anterior insula and the medial prefrontal/anterior cingulate gyrus—a brain area associated with the motivational aspects of emotion (Devinsky et al. 1995; Paus 2001), and also of the amygdala, which has been associated with negative emotional valence and more recently with general emotional arousal (Hamann 2003; Phan et al. 2002).

It is worth mentioning that similar patterns of brain activation have been observed in previous SPECT and PET studies using other classical psychedelics. These studies have typically found increased activation of frontal regions. Hermle et al. (1992) conducted a SPECT study after mescaline (a psychedelic phenylethylamine) administration and found a "hyperfrontality pattern". A PET investigation of psilocybin (a psychedelic tryptamine) found increased fluorodeoxyglucose uptake in the frontomedial and frontolateral cortices, the ACC, and the temporomedial cortex (Vollenweider et al. 1997). In another PET study of psilocybin, the highest increases in fluorodeoxyglucose uptake were found in the anterior cingulate cortex, followed by the right frontal operculum (Gouzoulis-Mayfrank et al. 1999).

DMT and the other classical psychedelics mentioned in the previous paragraph have a well-known serotonergic mechanism, binding with high affinity to the 5-HT_{1A} (the indolethylamines) and the 5-HT_{2A} receptor (the phenylethylamines and indolethylamines) where they act as partial agonists (McKenna et al. 1990; Pierce and Peroutka 1989; Titeler et al. 1988). Evidence supporting this serotonergic mechanism has been obtained in a human study that found 5-HT_{2A} antagonists to block the psychotropic effects elicited by these drugs (Vollenweider et al. 1998). Furthermore, their neuroendocrine profile is also compatible with serotonergic activation (Strassman and Qualls 1994), and long-term users of ayahuasca have been found to show an increase in the density of platelet serotonin transporters (Callaway et al. 1994). Considering all this evidence, it can be postulated that the activation of paralimbic and prefrontal structures observed in the present study was mediated by drug-induced changes in serotonergic neurotransmission. This opens the interesting possibility that serotonergic neurotransmission via the 2A receptor may play a modulatory role in neural processes subserved by these brain areas.

In conclusion, the present findings indicate that acute ayahuasca administration is associated with the activation of brain regions recently postulated to play prominent roles in the neurobiology of interoception and emotional processing. An interaction at this level could underlie the characteristic subjective effects elicited by the drug. Furthermore, results point to a potential modulatory role of serotonergic neurotransmission in these processes—a possibility that merits further investigation.

Acknowledgements This work was supported by grant SAF 2002-02746 from the Spanish Ministry of Education and Science and a private donation by Richard Wolfe.

We wish to thank Araceli Cabrero, Sylvie Cotxet, David Martínez, and Llúcia Benito for their help in data collection. The experiment reported in the present article complies with the Spanish law.

References

Anonymous (2000) Ayahuasca: from the Amazon to the urban jungles. In: The world geopolitics of drugs 1998/1999. Observatoire géopolitique des drogues, Paris, pp 103–107

Buckholtz NS, Boggan WO (1977) Monoamine oxidase inhibition in brain and liver produced by beta-carbolines: structure–activity relationships and substrate specificity. Biochem Pharmacol 26:1991–1996

Callaway JC, Airaksinen MM, McKenna DJ, Brito GS, Grob CS (1994) Platelet serotonin uptake sites increased in drinkers of ayahuasca. Psychopharmacology (Berl) 116:385–387

Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, Andrade EN, Andrade EO, Mash DC (1999) Pharmacokinetics of Hoasca alkaloids in healthy humans. J Ethnopharmacol 65:243–256

Check E (2004) Psychedelic drugs: the ups and downs of ecstasy. Nature 429:126–128

Craig AD (2002) How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 3:655–666

Craig AD (2003) Interoception: the sense of the physiological condition of the body. Curr Opin Neurobiol 13:500–505

Critchley HD, Wiens S, Rotshtein P, Ohman A, Dolan RJ (2004) Neural systems supporting interoceptive awareness. Nat Neurosci 7:189–195

Damasio AR (2003) Looking for Spinoza: joy, sorrow, and the feeling brain. Harcourt, Orlando, FL

Damasio AR, Grabowski TJ, Bechara A, Damasio H, Ponto LL, Parvizi J, Hichwa RD (2000) Subcortical and cortical brain activity during the feeling of self-generated emotions. Nat Neurosci 3:1049–1056

Devinsky O, Morrell MJ, Vogt BA (1995) Contributions of anterior cingulate cortex to behaviour. Brain 118:279–306

Dobkin de Rios M (1984) Visionary vine: hallucinogenic healing in the Peruvian Amazon. Waveland, Prospect Heights, IL

Gouzoulis-Mayfrank E, Schreckenberger M, Sabri O, Arning C, Thelen B, Spitzer M, Kovar KA, Hermle L, Büll U, Sass H (1999) Neurometabolic effects of psilocybin, 3,4-methylenedioxyethylamphetamine (MDE) and *d*-methamphetamine in healthy volunteers. A double-blind, placebo-controlled PET study with [^{18}F]FDG. Neuropsychopharmacology 20:565–581

Greenhouse L (2005) Supreme court to hear case of dispute over religious group's use of banned drug. The New York Times (April 19), p 15

Grob CS, McKenna DJ, Callaway JC, Brito GS, Neves ES, Oberlaender G, Saide OL, Labigalini E, Tacla C, Miranda CT, Strassman RJ, Boone KB (1996) Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. J Nerv Ment Dis 184:86–94

Halpern JH (2004) Hallucinogens and dissociative agents naturally growing in the United States. Pharmacol Ther 102:131–138

Hamann S (2003) Nosing in on the emotional brain. Nat Neurosci 6:106–108

Hermle L, Fünfgeld M, Oepen G, Botsch H, Borchardt D, Gouzoulis E, Fehrenbach RA, Spitzer M (1992) Mescaline-induced psychopathological, neuropsychological, and neurometabolic effects in normal subjects: experimental psychosis as a tool for psychiatric research. Biol Psychiatry 32:976–991

Kotler S (2005) Psychedelics in rehab. Psychology Today (Mar/Apr)

Labate B, Araújo W (2002) O Uso Ritual da Ayahuasca. Mercado de Letras, Sao Paulo

Lamas X, Farré M, Llorente M, Camí J (1994) Spanish version of the 49-item short form of the Addiction Research Center Inventory. Drug Alcohol Depend 35:203–209

Lazar SW, Kerr CE, Wasserman RH, Gray JR, Greve DN, Treadway MT, McGarvey M, Quinn BT, Dusek JA, Benson H, Rauch SL, Moore CI, Fischl B (2005) Meditation experience is associated with increased cortical thickness. Neuroreport 16:1893–1897

Martin WR, Sloan JW, Sapira JD, Jasinski DR (1971) Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. Clin Pharmacol Ther 12:245–258

McKenna DJ (2004) Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. Pharmacol Ther 102:111–129

McKenna DJ, Towers GH, Abbott F (1984) Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of ayahuasca. J Ethnopharmacol 10:195–223

McKenna DJ, Repke DB, Lo L, Peroutka SJ (1990) Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. Neuropharmacology 29:193–198

Melton L (2004) Dream drug or demon brew. New Sci 182 (2453):42–43

Paus T (2001) Primate anterior cingulate cortex: where motor control, drive and cognition interface. Nat Rev Neurosci 2:417–424

Phan KL, Wager T, Taylor SF, Liberzon I (2002) Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage 16:331–348

Pierce PA, Peroutka SJ (1989) Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. Psychopharmacology (Berl) 97:118–122

Rabin RA, Regina M, Doat M, Winter JC (2002) 5-HT2A receptor-stimulated phosphoinositide hydrolysis in the stimulus effects of hallucinogens. Pharmacol Biochem Behav 72:29–37

Reichel-Dolmatoff G (1990) The cultural context of an aboriginal hallucinogen: *Banisteriopsis caapi*. In: Furst P (ed) Flesh of the Gods: the ritual use of hallucinogens. Waveland, Prospect Heights, IL, pp 84–113

Reiman EM, Lane RD, Ahern GL, Schwartz GE, Davidson RJ, Friston KJ, Yun LS, Chen K (1997) Neuroanatomical correlates of externally and internally generated human emotion. Am J Psychiatry 154:918–925

Riba J, Rodriguez-Fornells A, Strassman RJ, Barbanoj MJ (2001a) Psychometric assessment of the hallucinogen rating scale. Drug Alcohol Depend 62:215–223

Riba J, Rodriguez-Fornells A, Urbano G, Morte A, Antonijoan R, Montero M, Callaway JC, Barbanoj MJ (2001b) Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. Psychopharmacology (Berl) 154:85–95

Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanoj MJ (2003) Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J Pharmacol Exp Ther 306:73–83

Salak K (2004) The vision seekers. The New York Times (September 12)

Schultes RE, Hofmann A (1980) The botany and chemistry of hallucinogens. Thomas, Springfield, IL

Schultes RE, Hofmann A (1987) Plants of the gods: origins of hallucinogenic use. A. van der Marck Editions, New York

Spruce R (1908) Notes of a botanist on the Amazon and Andes. Macmillan, London

Strassman RJ, Qualls CR (1994) Dose–response study of N, N-dimethyltryptamine in humans. I. Neuroendocrine, autonomic and cardiovascular effects. Arch Gen Psychiatry 51:85–97

Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R (1994) Dose–response study of N,N-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch Gen Psychiatry 51:98–108

Titeler M, Lyon RA, Glennon RA (1988) Radioligand binding evidence implicates the brain 5-HT2 receptor as a site of action for LSD and phenylisopropylamine hallucinogens. Psychopharmacology (Berl) 94:213–216

Villavicencio M (1858) Geografía de la República del Ecudador. Craighead, New York

Vollenweider FX, Leenders KL, Øye I, Hell D, Angst J (1997) Differential psychopathology and patterns of cerebral glucose utilisation produced by (S)- and (R)-ketamine in healthy volunteers using positron emission tomography (PET). Eur Neuropsychopharmacol 7:25–38

Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D (1998) Psilocybin induces schizophrenia-like psychosis in humans via serotonin-2 agonist action. Neuroreport 9:3897–3902

Seeing With the Eyes Shut: Neural Basis of Enhanced Imagery Following Ayahuasca Ingestion

Draulio B. de Araujo,[1,2,3]* Sidarta Ribeiro,[1,4] Guillermo A. Cecchi,[5] Fabiana M. Carvalho,[3] Tiago A. Sanchez,[6] Joel P. Pinto,[3] Bruno S. de Martinis,[3] Jose A. Crippa,[3] Jaime E.C. Hallak,[3] and Antonio C. Santos[3]

[1]*Brain Institute, Federal University of Rio Grande do Norte (UFRN), Natal, RN, Brazil*
[2]*Division of Radiology, Onofre Lopes University Hospital (UFRN), Natal, RN, Brazil*
[3]*Ribeirao Preto School of Medicine and National Institute for Tanslational Medicine (INCT-TM, CNPq), University of Sao Paulo (USP), Ribeirao Preto, SP, Brazil*
[4]*Edmond and Lily Safra International Institute of Neuroscience of Natal (ELS-IINN), Natal, RN, Brazil*
[5]*Computational Biology Center, T.J. Watson IBM Research Center, Yorktown Heights, NY, USA*
[6]*Department of Radiology, Faculty of Medicine, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil*

Abstract: The hallucinogenic brew Ayahuasca, a rich source of serotonergic agonists and reuptake inhibitors, has been used for ages by Amazonian populations during religious ceremonies. Among all perceptual changes induced by Ayahuasca, the most remarkable are vivid "seeings." During such seeings, users report potent imagery. Using functional magnetic resonance imaging during a closed-eyes imagery task, we found that Ayahuasca produces a robust increase in the activation of several occipital, temporal, and frontal areas. In the primary visual area, the effect was comparable in magnitude to the activation levels of natural image with the eyes open. Importantly, this effect was specifically correlated with the occurrence of individual perceptual changes measured by psychiatric scales. The activity of cortical areas BA30 and BA37, known to be involved with episodic memory and the processing of contextual associations, was also potentiated by Ayahuasca intake during imagery. Finally, we detected a positive modulation by Ayahuasca of BA 10, a frontal area involved with intentional prospective imagination, working memory and the processing of information from internal sources. Therefore, our results indicate that Ayahuasca seeings stem from the activation of an extensive network generally involved with vision, memory, and intention. By boosting the intensity of recalled images to the same level of natural image, Ayahuasca lends a status of reality to inner experiences. It is therefore understandable why Ayahuasca was culturally selected over many centuries by rain forest shamans to facilitate mystical revelations of visual nature. *Hum Brain Mapp 00:000–000, 2011.* © 2011 Wiley-Liss, Inc.

Key words: Ayahuasca; mental imagery; functional magnetic resonance imaging; psychedelic substance; N,N-dimethyltryptamine; monoamine oxidase inhibitors

Additional Supporting Information may be found in the online version of this article.

Contract grant sponsor: The Brazilian Federal Agencies: CNPq, CAPES; FINEP; The Sao Paulo State financial agency (FAPESP).

*Correspondence to: Draulio B. de Araujo, Brain Institute (UFRN), R. Nascimento Castro, 2155, 59056-450, Natal, RN, Brazil.
E-mail: draulio @neuro.ufrn.br

Received for publication 6 December 2010; Revised 2 May 2011; Accepted 18 May 2011

DOI: 10.1002/hbm.21381

Published online in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

Ayahuasca, a central pillar of Amerindian traditional medicine, has been traditionally used by rain forest populations during religious ceremonies. Since the 1980s, Ayahuasca-based religions have been spreading to urban centers in South as well as in North America [Santos et al., 2007]. In 1992, the Brazilian government approved a resolution legalizing the use of Ayahuasca within a religious context, and similar action was taken by the United States Supreme Court in 2006.

Ayahuasca is served as a tea prepared as a decoction of a bush (*Psychotria viridis*) and a liana (*Banisteriopsis caapi*). *Psychotria viridis* is a rich source of the psychedelic substance *N,N*-dimethyltryptamine (DMT), whereas *Banisteriopsis caapi* contains β-carbolines such as harmine, harmaline, and tetrahydroharmine, which are potent monoamine oxidase inhibitors (MAOi). The synergistic interaction of these alkaloids determines the psychotropic action of Ayahuasca [Buckholtz and Boggan 1977]. DMT is a serotonergic agonist that acts mainly on 5-HT_{2A} and 5-HT_{2C} receptors [Smith et al., 1998], but in itself it is not orally active, since it is inactivated by MAO. However, the inhibition of MAO by β-carbolines allows DMT to be psychoactive when ingested. MAOi also contribute directly to the neuropharmacological effects of Ayahuasca by increasing extracellular levels of 5-HT.

The effects of Ayahuasca begin ∼ 30–40 min after oral intake, and last up to 4 hours. Autonomic responses include increases in cardiac and respiratory rates, blood pressure, temperature, and pupil diameter [Riba et al., 2003]. Ayahuasca users report many psychological effects, which include changes in self-perception, spatio-temporal scaling [Shanon 2003], and sensory hallucination. Among all perceptual changes induced by Ayahuasca, the most remarkable one are vivid visual hallucinations called *mirações* (seeings) [Riba et al., 2001; Shanon 2002]. Individuals report a variety of scenarios, as exuberant as a colored dream. Such seeings appear in different forms and context, ranging from simple to complex situations, from the vision of an animal to a long conversation with somebody unknown. However, despite their visual nature, Ayahuasca seeings are all internally generated, without the need of external stimuli [Shanon 2002]. Although enhanced imagery occupies center stage in Ayahuasca-based rituals, very little is known about the neural mechanisms underlying such seeings.

The ability to generate visual mental imagery has long been a subject of research [Denis 1991; Richardson 1969]. Apart from the debate that this theme has provoked among psychologists and philosophers [Kosslyn et al., 2001; Pylyshyn 1981], we will herein consider mental imagery as the action of mentally summoning a visual representation of the world. Previous studies using functional Magnetic Resonance Imaging (fMRI) have investigated the neural basis of imagery (reviewed in Kosslyn et al., 2001; Mellet et al., 1998] and demonstrated a substantial overlap among brain regions involved with imagery and perception [Ganis et al., 2004]. The involvement of associative visual areas during imagery has been consistently reported in the literature (DEsposito et al., 1997], but the participation of the primary visual area (V1; Brodmann area 17, BA17) remains controversial [Ishai and Sagi 1995; Ishai et al., 2000; Kosslyn and Thompson 2003].

To investigate the neural basis of imagery induced by Ayahuasca, we used BOLD (Blood Oxygenation Level Dependent) fMRI during three sequential conditions: natural image of pictures, imagery of the same pictures, and natural image of scrambled versions of the same pictures. The same paradigm was applied before and after the oral intake of Ayahuasca.

MATERIALS AND METHODS

Subjects

Ten frequent Ayahuasca users participated in the study (mean age: 29 years, from 24 to 48 years, 5 female), after informed consent was obtained from all subjects in accordance with the guidelines approved (No 14672/2006) by the Human Research Committee and the Ethics Committee of the University of Sao Paulo. One subject was excluded from analyses due to uncorrectable amounts of head movement, leaving 9 participants (5 women) in the final dataset.

Ayahuasca Dosage

Each subject drank 120–200 mL of Ayahuasca (2.2 mL/kg of body weight). The Ayahuasca batch used in the experiment contained 0.8 mg/mL of DMT and 0.21 mg/mL of harmine. No harmaline was present in the batch, at the chromatography detection threshold of 0.02 mg/mL. To quantify these amounts, a 1 mL sample of the Ayahuasca batch taken by the subjects was homogenized with sodium acetate buffer solution pH = 9, extracted with 5 mL of diethyl ether in a shaker (20 min), and centrifuged at 3,000 rpm for 15 min. The organic phase was collected and evaporated under a nitrogen stream. The residue was dissolved in 1 mL of methanol and 1 μL was analyzed by gas chromatography/mass spectrometry (GC/MS). GC/MS analyses were performed using a Varian CP3800 gas chromatograph coupled to a Varian Saturn 2000 ion trap mass spectrometer (Varian Inc.). A capillary column (DB-5MS 30 m × 0.25 mm i.d. × 0.25 μm film thickness (Agilent) was used. The chromatographic conditions were as follows: injector temperature was 250°C in the splitless mode, oven temperature program was 80°C for 1 min ramped at 5°C/min to 220°C and held for 10 min, and then to 300°C for 5 min. Helium at a flow rate of 0.8 mL/min was used as carrier gas.

MR Image Acquisition

All images were acquired in a 1.5T scanner (Siemens, Magneton Vision). The functional dataset was acquired using

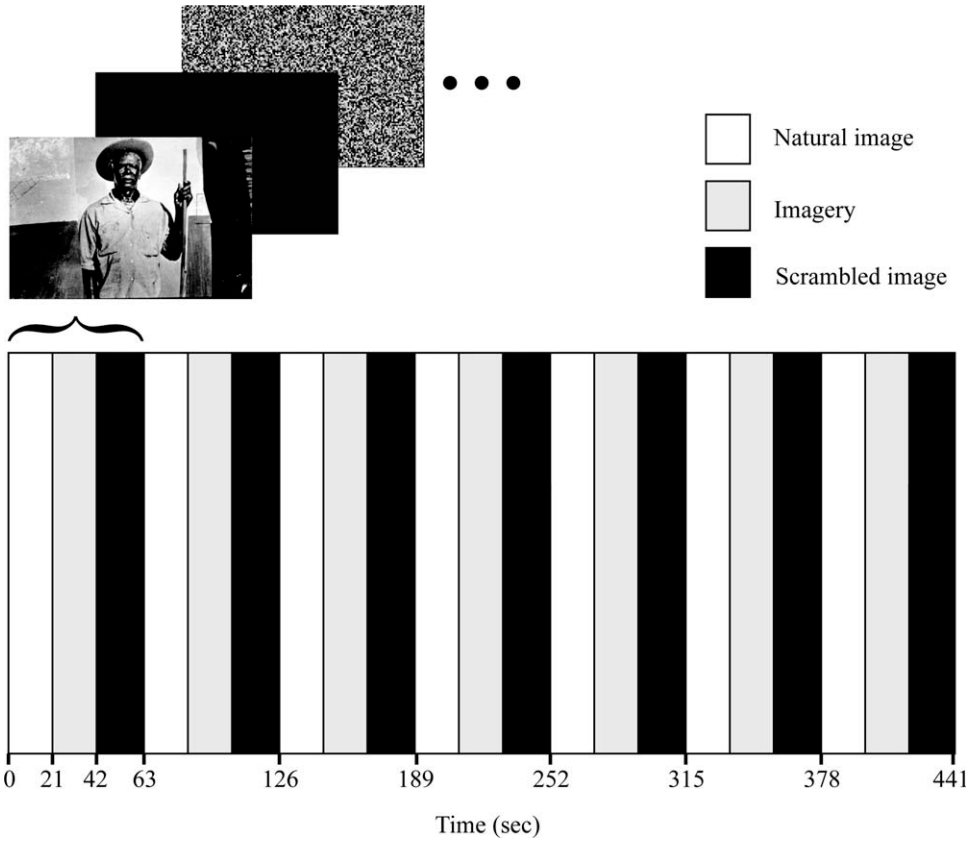


Figure 1.
Experimental design of one fMRI scanning session. Subjects were submitted to three conditions (natural image, imagery with closed eyes, and scrambled image) in a block design (21 sec per condition; 3 conditions per block, 7 blocks per session). Each subject performed two fMRI sessions. In the first session, subjects were scanned before Ayahuasca intake. Immediately after the first scan, subjects drank Ayahuasca. The second fMRI session began 40 min after intake.

EPI-BOLD like sequences, and comprises 147 brain volumes with the following parameters: TR = 3,000 ms; TE = 60 ms; flip angle = 90°, FOV = 220 mm; matrix = 128 × 128, slice thickness = 5 mm, and number of slices = 16. High spatial resolution images were obtained from typical Gradient Recalled Echo sequences, constituted of 156 sagital slices, covering both hemispheres, with a 1 mm^3 of voxel resolution.

Experimental Design

Each subject performed two fMRI sessions. In the first session, subjects were scanned before Ayahuasca intake. Immediately after the first scan, subjects drank the tea. Psychiatric scales were applied at intervals of 0, 40, 80, and 200 min after Ayahuasca intake. The second fMRI session began 40 min after intake, when subjects were engaged in the same set of tasks of the first session. Figure 1 shows a diagram of the experimental design (one session). In each fMRI session, subjects were submitted to three conditions in a block design (21 sec per condition; 3 conditions per block, 7 blocks per session). In the first condition (natural image), subjects passively viewed images of people, animals or trees (one different image per block, no repetition). The second condition (imagery) consisted of an imagery task, during which subjects were asked to close their eyes and mentally generate the same image they had just seen. During the last condition (scrambled image) subjects passively viewed a scrambled version of the image previously presented in the natural image condition. The scrambled image served as baseline for the analysis. Each scanning session lasted 441 sec (7 blocks × 3 conditions × 21 sec). The stimulation protocol was designed in Presentation® software (Version 0.60, Neurobehavioral Systems). Images were projected onto a translucent screen and reached the eyes of the subjects by reflection on a mirror system adapted to the head coil.

Retinotopic Mapping

We used a flickering white and black checkerboard formed into a ray-shaped configuration subtending 22.5°

(1/8) of polar angle. The disk segment started at the right horizontal meridian and slowly rotated anticlockwise for a full cycle of 360°. Each mapping session consisted of thirteen repetitions of a full rotation, which lasts 67 sec. Retinotopy of polar angle was revealed with cross-correlation analysis. The BOLD signal was correlated with an ideal response function that assumes the first 1/8 of a stimulation cycle as being the reference section (corrected for a hemodynamic delay). Areas activated at particular polar angles were revealed through selecting the lag value that resulted in the highest cross-correlation value for a particular voxel[Linden et al., 1999]. Lag values were color-coded and used to compute the cross-correlation maps, to find the retinotopic boundaries of early visual areas V1, V2, and V3.

Image Processing

Images were processed in Brain Voyager QX 1.9 (Brain Innovation, Maastricht, The Netherlands). Data analysis consisted of preprocessing steps, which included, 3D head motion correction (sinc interpolation), slice scan time correction (sinc interpolation), spatial smoothing (4.0 mm FWHM, 3D Gaussian filter), a high pass filtered at 0.01 Hz, and linear trend removal. The contrasts, in each condition, were evaluated using a general linear model (GLM) taking into account the hemodynamic response function (modeled by a two-gamma function) and baseline state. Group differences were analyzed using a fixed effect GLM with separate subject predictors, and statistical threshold was set taking into account a correction for multiple comparison based on the false discovery rate (FDR). Within and between multi-subjects corrections were made by setting q(FDR) <0.05. After transformation into Talairach space, the group analysis model included three orthogonal contrasts: mental imagery before intake versus baseline, mental imagery before intake versus mental imagery after intake and real imagery before intake versus real imagery after intake. Clusters of activation were then segregated into regions of at least 50 mm^3.

Image Analyses

The anatomical MRI of all subjects were investigated by an experienced neuroradiologist, and all the reports were classified as normal. The analysis of fMRI data was based on the GLM. The hemodynamic response function was modeled based on the conventional difference between two gamma functions. Unless otherwise specified, the GLM analysis was based on a fixed effect (FFX) assumption. Statistical maps were corrected for multiple comparison based on the FDR, and a threshold of q(FDR <0.05) was set for all group-level contrasts. Contrast maps were overlaid onto a coregistered anatomical image. The individual beta values used in the co-variance analyses were extracted from all statistically significant voxels in the ROI. Moreover, BOLD signal averages were computed for imagery as well as natural image conditions, both before and after potion intake. The strategy used is similar to computing EEG evoked potential averages along a series of trials. To generate BOLD signal time course averages Brain Voyager QX 1.9 (Brain Innovation, Maastricht, The Netherlands) was used. In brief, the averaged signal was computed for the selected ROI based on the average of all trials of particular conditions of interest (imagery before and after, natural image before and after), encompassing a baseline period (scrambles image). These time courses were then averaged across subjects.

Functional Connectivity Analysis

This analysis was used to capture temporal correlations between areas, as a simplified snapshot of the dynamical state of the system. The correlations are represented by a graph, with nodes corresponding to areas, and links to functional connections. The time traces fed into the algorithm were, for each area, the fold activation over noise. Area-to-area correlations were computed as $c_{ij}(\tau) = \langle (y_i(t) - \langle y_i \rangle)(y_j(t+\tau) - \langle yj \rangle) \rangle \sigma_i^{-1} \sigma_j^{-1}$, where $\sigma_n^2 = \langle y_n(t) - \langle y_i \rangle \rangle$. The maximum correlation, and the delayed at which it was met, were computed as $C_{ij} = \max_\tau |c_{ij}(\tau)|$ and $T_{ij} = \arg\max_\tau |c_{ij}(\tau)|$. A link was considered to be present whenever $|C_{ij}| > 0.5$. A link was considered to be present if the P-value of the correlation survived FDR correction at 0.05; with only one exception, all the links were found to be significant. If the delay at which this threshold was met exceeded 5 TR's (i.e., 15 sec), then the link was considered to be directed, with the direction pointing from the "leader" to the "follower"; otherwise, the link was considered as undirected, that is, no temporal precedence relationship could be determined. The choice of 5 TR's was based on the auto-correlation of the activations, which on average drop significantly for this time delay (see Supporting Information for details on this analysis).

RESULTS

Psychological Effects

To estimate the time course of the psychological changes associated with Ayahuasca intake, we applied two psychiatric scales at intervals of 0, 40, 80, and 200 min after Ayahuasca intake: the brief psychiatric ratings scale (BPRS) to detect psychotic symptoms [Crippa et al., 2001], and the Young Mania Rating Scale (YMRS), to measure mania symptoms [Vilela et al., 2005]. Figure 2 shows that all subjects experienced increases in the two psychiatric scales following Ayahuasca ingestion. The effects were significant at 40 and 80 min post-intake ($P = 0.036$ for BPRS and $P = 0.036$ YMRS), Wilcoxon corrected for multiple comparisons of 4 time points), with respect to baseline ($T = 0$

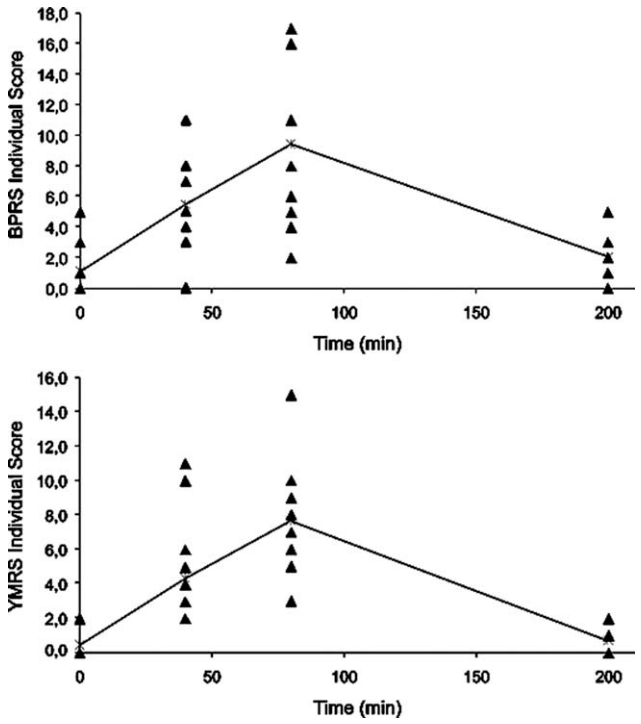


Figure 2.

Individual scores reached on the psychiatric scales BPRS and YMRS at 0, 40, 80, and 200 min for all subjects. Ayahuasca intake took place at $T = 0$ min. The psychological effects with respect to baseline ($T = 0$ min) reached significance at 40 min ($P = 0.05$ for BPRS, $P < 0.036$ for YMRS, Wilcoxon corrected for multiple comparisons), immediately before scanning, and peaked at 80 min ($P = 0.036$ for BPRS and $P = 0.036$ for YMRS, Wilcoxon corrected for multiple comparisons). Line connects mean values at different time points. Note that the variability is very low at 0 and 200 min, when most subjects presented identical scores.

min), in agreement with the time course of psychological [Riba et al., 2003], electroencephalographic changes induced by Ayahuasca [Riba et al., 2002] and single photon emission computed tomography [Riba et al., 2006]. Importantly, all subjects reported a marked increase in the ability to perform the imagery task after tea intake, corroborating that imagined scenes become much more vivid and detailed after Ayahuasca [Shanon 2002].

BOLD Signal Modulation by Ayahuasca

To test for the modulation of brain activity during imagery by Ayahuasca, fMRI data from individual subjects were analyzed using the following additional contrast: imagery after intake (IA) > imagery before intake (IB). Statistically significant areas of increased BOLD signal (q(FDR) < 0.05) comprised bilateral precuneus (BA7, 18, 19, 31), cuneus (BA 17, 18, 19, 30), lingual gyrus (BA 17, 18, 19, 30), fusiform gyrus (BA 19 and 37), middle occipital gyrus (BA 18, 19, 37), parahippocampal gyrus (BA 30), posterior cingulate gyrus (BA 23, 29, 30, 31), superior temporal gyrus (BA22 and 42), superior and middle frontal gyrus (BA 8, 9, 10, 47), and inferior frontal gyrus (BA 47).

To identify which areas were specifically modulated by Ayahuasca during the imagery task, and not by a generalized, nonspecific effect of Ayahuasca across all conditions, a further contrast was built in which the main effect of the imagery task (IA > IB) was subtracted from the main effect of natural image task (natural image after intake (NIA) > natural image before intake (NIB)). To determine the extent and borders of the early visual areas (V1, V2, and V3), a phase-encoded retinotopic mapping was performed in an additional fMRI session [Linden et al., 1999]. As shown in Figure 3, BOLD signal increase was most prominent in the occipital cortex, extending into inferior

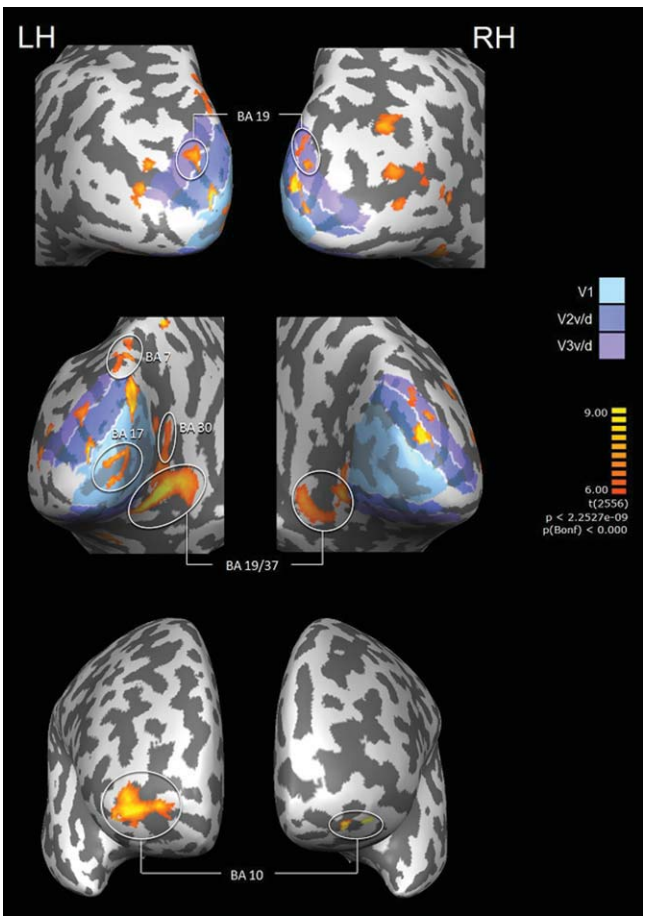


Figure 3.

Main effect of the imagery task (q(FDR) < 0.05). The contrast corresponds to (imagery after > imagery before)-(natural image after > natural image before). Retinotopic mapping appears in light blue, blue and purple corresponding to areas V1, V2, and V3, respectively.

TABLE I. Areas modulated by Ayahuasca during the imagery task

Region	Hem	BA	% Change	Cluster Vol.	x	y	z	σ_x	σ_y	σ_z
Middle Occipital Gyrus	Left	18, 19	1.18%	138 mm^3	−28	−88	4	1	1	1
	Right	19, 37	1.02%	30 mm^3	43	−66	5	1	2	2
Fusiform Gyrus	Left	19	1.52%	30 mm^3	−22	−54	−8	1	2	1
	Right	37	0.51%	264 mm^3	43	−56	−16	1	2	1
Middle Frontal Gyrus	Right	10	−0.47%	519 mm^3	33	51	1	2	1	1
Superior Frontal Gyrus	Right	10	−0.47%	216 mm^3	27	50	1	3	2	1
Lingual Gyrus	Left	17, 18, 19	1.52%	597 mm^3	−16	−57	1	5	15	2
	Right	17, 18, 19	1.35%	417 mm^3	12	−54	1	1	8	1
Parahippocampal Gyrus	Left	30	1.37%	837 mm^3	−14	−46	1	3	8	3
	Right	30	1.19%	237 mm^3	13	−41	−2	2	5	3
Cuneus	Left	17, 18	1.53%	1578 mm^3	−6	−90	5	3	6	6
	Right	17, 18	1.21%	294 mm^3	13	−94	4	2	2	1
Precuneus	Left	7	1.29%	174 mm^3	−3	−67	39	1	2	1
Middle Temporal Gyrus	Right	37	0.51%	108 mm^3	45	−63	4	1	1	1

Hem = hemisphere; Cluster volume = volume of the cluster in mm^3; % Change = ROI maximum percent BOLD signal change related to the same contrast used to generate the current table; x, y, and z = coordinates of the center of the cluster; (σ_x,σ_y,σ_z) = respective standard deviation.

The contrast subtracts the main effect of the imagery task (IA > IB) from the main effect of natural image (NIA > NIB). The Talairach coordinates correspond to the center of the cluster of activity with its respective standard deviation.

and mesial temporal lobe, and to portions of the frontal lobe. Statistically significant voxels (q(FDR) < 0.05) were found bilaterally in the occipital cortex (BA 17, 18, 19), comprising the three early visual areas with their respective retinotopic representations. Statistically significant modulation was also detected bilaterally in the parahippocampal gyrus (BA 30), middle temporal cortex (BA 37) and frontal cortex (BA 10; Fig. 3; Table I).

BOLD Signals Averages Before and After Ayahuasca Intake

To further inspect the modulation elicited by Ayahuasca, we computed the averaged percent BOLD signal changes for the same ROI that were specifically modulated by Ayahuasca during the imagery task (BA 10, 17, 18, 19, 30, and 37). Figure 4 shows the extracted mean values and standard deviations were based on all trials. These included a baseline interval (5 sec–scrambled image, presented in Fig. 4 as a shaded gray area) and the whole periods for the following conditions: imagery before (Fig. 4, blue line), imagery after (Fig. 4, red line), natural image before (Fig. 4, light gray line), natural image after (Fig. 4, green line). As can be observed, the averaged BOLD responses for scrambled image (Fig. 4, shaded gray area) were similar to the ones from the natural image conditions (before intake–Fig. 4, light gray line), in all ROI from the visual cortex (BA 17, 18, and 19). This was expected since the BOLD signal in visual cortex should not be different in the scrambled condition when compared to the natural image condition. The same pattern was preserved when observing the averaged signals after Ayahuasca intake. However, the average BOLD response during the imagery condition shows a remarkable increase after Ayahuasca intake. This can be observed by comparing the traces of the imagery before (blue lines) with imagery after intake (red lines). It is important to note that the averaged BOLD signal amplitude on visual areas during the imagery task before Ayahuasca intake (blue line) is expected to be smaller than during the scrambled period, when the visual system is actually being stimulated. After Ayahuasca intake, however, the averaged signal reaches amplitudes compatible to the ones obtained during the scrambled conditions. The same pattern of positive modulation was observed in all ROI, in the occipital (BA17, BA19, and BA7), temporal (BA30 and BA37) and frontal areas (BA10). The effect of Ayahuasca in occipital areas was particularly noteworthy, because the signal amplitude after intake increased markedly during imagery, but not during natural image (see Fig. 4). Supported by retinotopic mapping (see Fig. 3), the specific BA17 location modulated by Ayahuasca corresponds to the cuneus and lingual gyrus, which are related to the peripheral visual field [Yoshor et al., 2007]. Also worth mentioning is the modulation by Ayahuasca of the parahipocampal cortex and the retrosplenial cortex (BA30 and BA37) during the imagery task. These structures are important for the retrieval of episodic memories [de Araujo et al., 2002], and have recently been implicated in the processing and representation of contextual associations [Bar 2004]. As shown in Figure 4, in addition to occipital and temporal areas, Ayahuasca also potentiated parts of the frontopolar cortex (BA10) known to be involved in imagery [Kosslyn et al., 1999]. Among all regions modulated by Ayahuasca, BA10 was the only one that showed a positive BOLD signal during the imagery task even before Ayahuasca intake (see Fig. 4), and it was potentiated after tea ingestion during the imagery task (see Fig. 4).

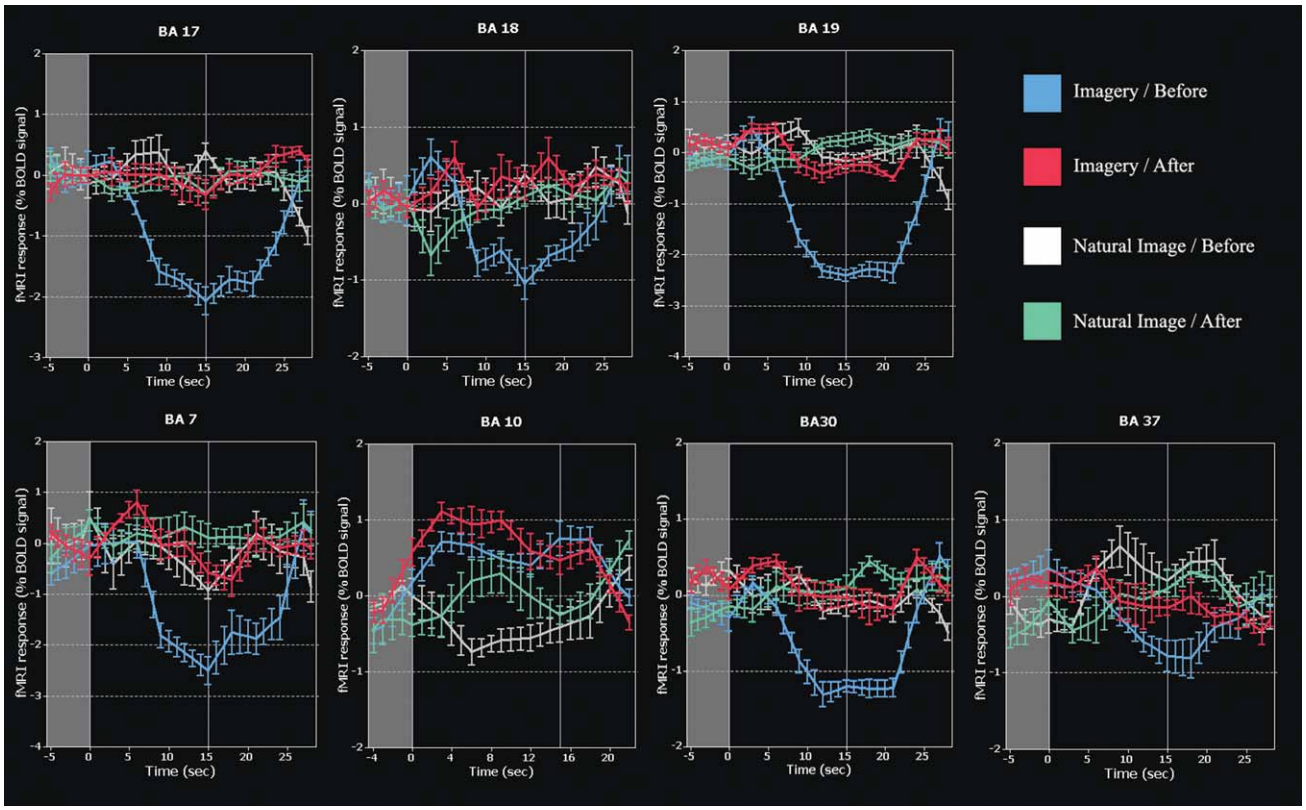


Figure 4.

Average time courses of BOLD responses before and after Ayahuasca intake (blue for imagery before intake, red for imagery after intake, white for natural image before intake, and green for natural image after intake). Each time point corresponds to an fMRI TR = 3 sec. Note the marked increase in BOLD signal during imagery following Ayahuasca intake. Shaded gray area represents baseline periods (scrambled image condition). Blue line–imagery before intake; red line–imagery after intake; light gray line–natural image before intake; green line–natural image after intake.

Correlation Between BOLD Amplitude and Psychiatric Scales

To investigate the relationship between neural and psychological changes, we calculated correlations between BOLD signal amplitude in different brain areas during post-intake sessions, and the values reached on the two psychiatric scales 40 min after intake, that is, immediately before subjects entered the scanner (see Fig. 5). A significant correlation was observed exclusively between BA17 activation and BPRS data (Spearmann's Rho = 0.78, P = 0.037, two-tailed, corrected for multiple comparisons of 7 brain areas; BA7, BA10, BA17, BA18, BA19, BA30, and BA37). No significant correlations were observed between BPRS individual scores and activation of any other brain region, nor between YMRS scores and BOLD signal modulation in any brain region. This indicates that BA17 activation levels during post-Ayahuasca imagery are specifically correlated with the occurrence of increased manifestation of perceptual changes, which included visual and auditory, as measured by the psychiatric scales.

Functional Connectivity

Is the Ayahuasca potentiation of intentional imagery accompanied by changes in the coordination of frontal, temporal, and occipital cortical areas? To address this issue, we implemented a functional connectivity analysis based on delayed correlations [Cecchi et al., 2007]. The results are presented in Figure 6. The top row shows the full connectivity in the four conditions: imagery and natural image, pre/post intake. To understand the changes in connectivity, we sorted apart the links for areas BA17, BA10, and BA19, and showed them individually in separate rows. An interesting change observed for imagery is that BA17 becomes a leader of BA7 and BA37 after intake (Fig. 6, second row), while maintaining its leadership with

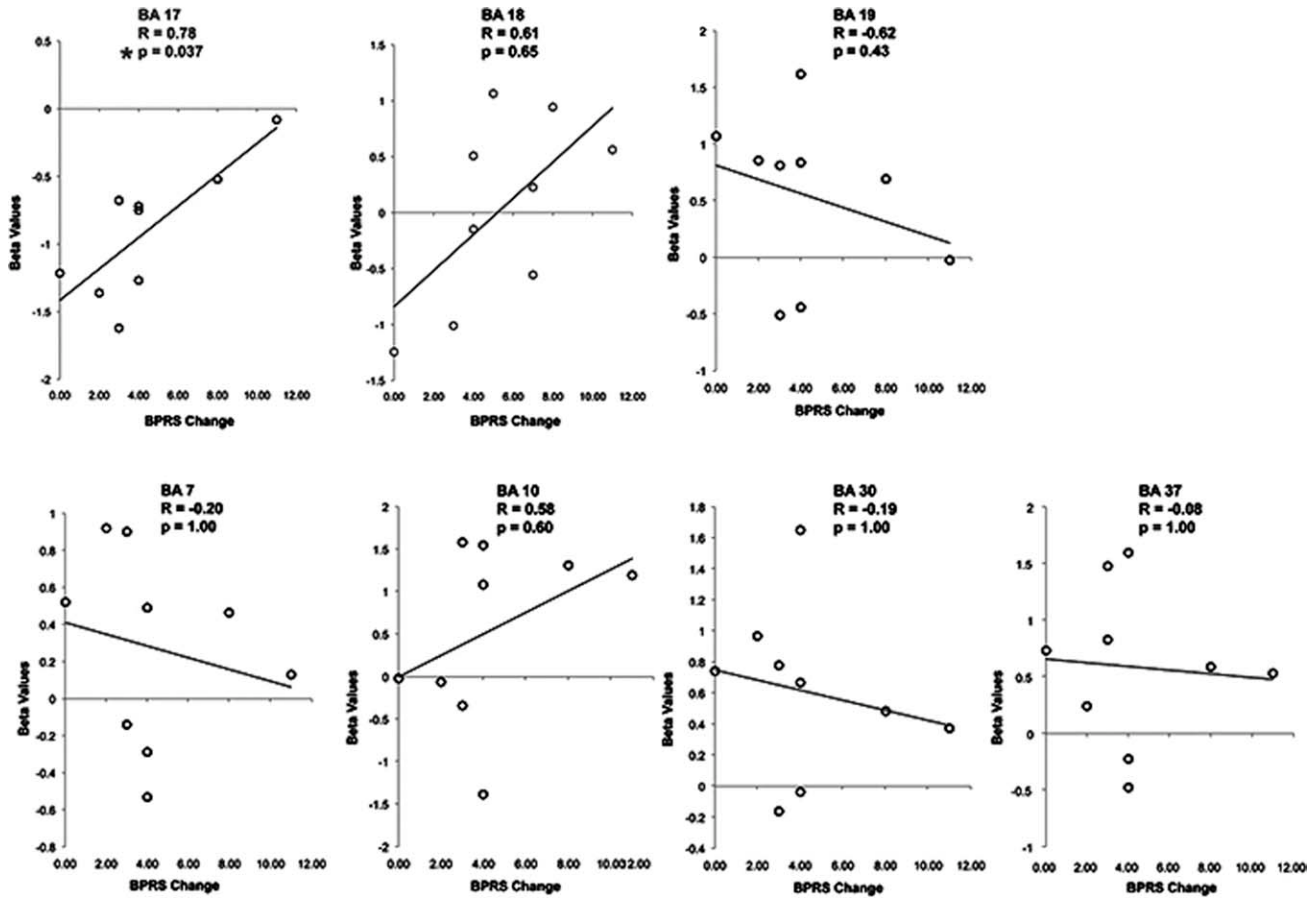


Figure 5.

Scatter plot and trend lines of the beta values against individual BPRS scores at 40 min, with respect to baseline ($T = 0$ min). Statistically significance was found only for BA17 ($P = 0.037$, corrected for multiple comparisons).

respect to BA19 and BA30. Overall, the connectivity pattern centered on BA17 for post-intake imagery seems to be a superposition of connectivity features observed during pre-intake natural image and imagery. Other effect associated with imagery after Ayahuasca intake is the change from "leader" to "follower" for BA10 with respect to BA7 and BA30, by their turn "followers" of BA17. Furthermore, both for natural image and for imagery, BA19 becomes a "follower" area, lagging behind BA10 and BA17 (fourth row from the top).

DISCUSSION

The effects of Ayahuasca reported here are putatively mediated by the activation of serotonergic receptors widely distributed in the brain, including the several cortical areas modulated during post-Ayahuasca imagery [Petit-Taboue et al., 1999]. Interestingly, Ayahuasca did not enhance occipital BOLD signal during the natural image condition in comparison with the scrambled image condition. Perhaps activity in BA17 and other visual areas reaches a ceiling when subjects see with open eyes, irrespective of Ayahuasca ingestion. In contrast, the activation levels of BA17 during imagery were very different depending on whether Ayahuasca had been previously administered or not. While BA17 activation was very low during pre-Ayahuasca imagery, post-intake imagery was concomitant with very high BA17 activity, comparable to BOLD signal amplitude during natural image condition (see Fig. 4). Our study did not control for potential effects related to the order of the sessions with and without Ayahuasca ingestion. Although this question was not addressed in our study, it seems very not parsimonious to attribute the remarkable BOLD signal modulations described here to an order effect. Importantly, the effects in BA17 were positively and quite selectively correlated with the individual scores reached on the BPRS psychiatric scale (Spearman's Rho = 0.78, $P = 0.037$, two-tailed, corrected for multiple comparisons of 7 brain areas), pointing to a tight relationship between

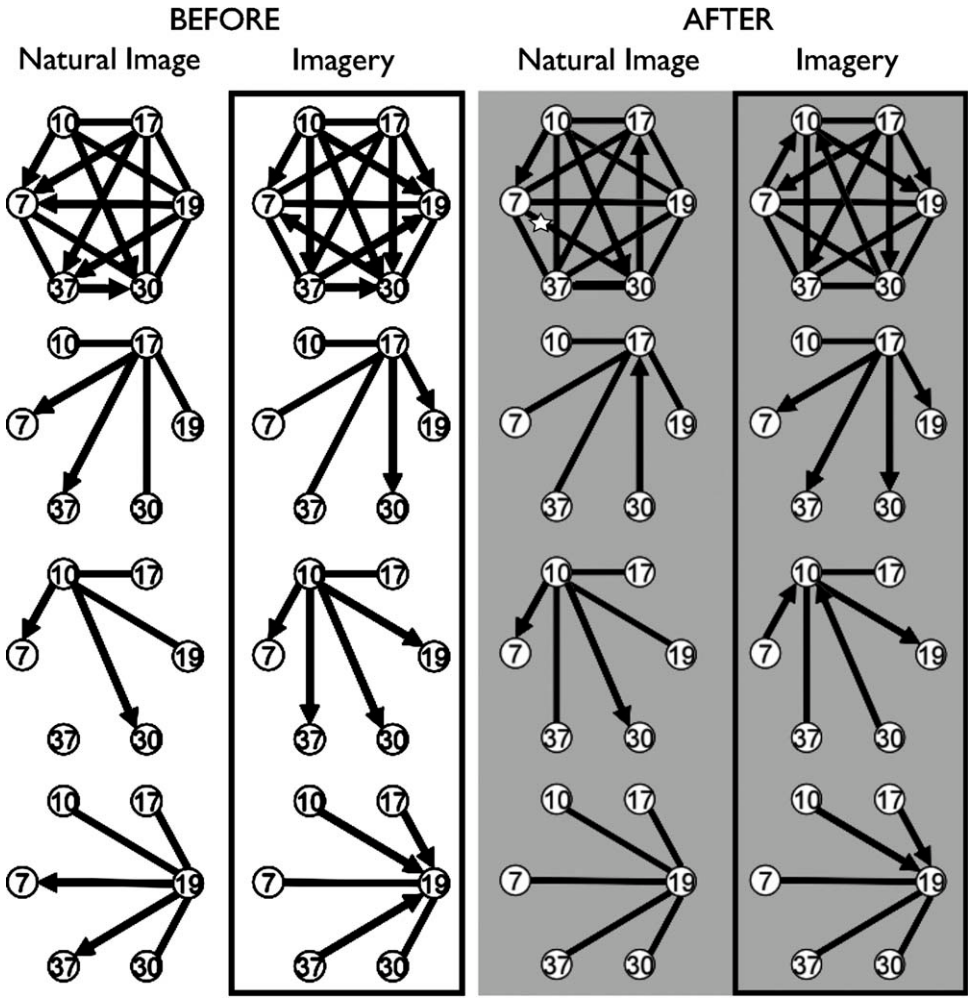


Figure 6.

BOLD signal correlations between brain areas before and after Ayahuasca intake. The labels correspond to the same Brodmann areas defined in Figure 3. Arrow direction indicates that the source precedes the target by at least 5 TR's (15 sec); undirected links indicate that there is no such temporal delay between the areas. The top row shows the full connectivity for the four conditions. The second, third, and fourth rows from the top correspond to links involving only BA17, BA10, and BA19, respectively.

neural changes in BA17 and psychotic effects caused by Ayahuasca intake (see Fig. 5).

In addition to BA17, Ayahuasca had a strong effect in other brain areas related to vision. The nonprimary visual areas strongly modulated by Ayahuasca (BA7, BA18, and BA19) are known to be activated during psychopathological hallucinations [Allen et al., 2008] as well as during normal dreaming, within rapid-eye-movement (REM) sleep [Braun et al., 1998; Wehrle et al., 2007]. Although these areas showed BOLD signal time courses similar to that of BA17 (see Fig. 4), no statistically significant correlation was found between their signal modulation during imagery and the psychiatric scales used here (see Fig. 5).

The activity of cortical areas BA30 and BA37, known to be involved with episodic memory retrieval and the processing of contextual associations, was also potentiated by Ayahuasca intake during imagery. Within this framework, Ayahuasca engages cortical regions necessary for the integration of separate visual elements into a whole scene [Chadwick et al., 2010]. This suggests that the seeings induced by the tea are associated with an endogenous engagement of mnemonic circuits, possibly feeding visual areas with the content of the Ayahuasca seeings.

Activation of BA 10 during mental imagery has already been reported [Kosslyn 1999], but its exact role in mental imagery is yet to be characterized. Previous studies have

shown that BA10 activity correlates with the amount of intentional effort involved in self-awareness and the imagination of future events [Goldberg et al., 2006]. Furthermore, neuroimaging and lesion studies reported that BA10 plays an important role in prospective memory [Burgess et al., 2001]. It has been recently proposed that this region is involved with the temporal direction of an imaginary event [Addis and Schacter 2008], and there is evidence of its engagement when internally generated information needs to be evaluated [Christoff and Gabrieli 2000; Turner et al., 2008]. At present, it is believed that working memory depends on the interaction of BA10 and the dorsolateral prefrontal cortex (DLPFC): While the former processes information from internal sources, the latter is concerned with information generated externally [Vinogradov et al., 2006].

It is noteworthy to mention that although all subjects studied were experienced Ayahuasca users, their hemodynamic responses have a canonical shape. Such observation comes from another experiment conducted on the same subjects when performing a classical verbal fluency task, before and after Ayahuasca ingestion [Prado et al., 2009]. The results show a consistent and expected response in expressive language centers, such as Broca's area (BA 44), with a classical hemodynamic response function.

In this study, subjects were asked to intentionally imagine visual scenes. The broad range of neuroanatomical sites significantly affected by Ayahuasca during intentional imagery underlies the remarkable psychological changes produced by the tea. The findings from the connectivity analysis indicate that Ayahuasca intake strongly alters fronto-occipital relationships, producing marked changes in the temporal ordering of events across several brain regions. In particular, Ayahuasca intake is accompanied by an increased capacity of BA17 to lead other cortical areas during imagery. The functional prevalence and temporal precedence of BA17 during post-Ayahuasca imagery suggest that the seeings caused by Ayahuasca ingestion, robust even with the eyes shut, may in fact be initiated in the primary visual cortex.

Ayahuasca-induced seeings have been traditionally used within religious contexts to give access to a deeply meaningful internal world. Altogether, our results indicate that these seeings stem from the activation, during voluntary imagery, of an extensive network of occipital, temporal, and frontal cortical areas respectively involved with vision, memory, and intention. By boosting the intensity of recalled images to the same level of natural image, Ayahuasca lends a status of reality to inner experiences. It is therefore understandable why Ayahuasca was culturally selected over many centuries by rain forest shamans to facilitate mystical revelations of visual nature.

ACKNOWLEDGMENTS

The authors would like to express our gratitude to the Santo Daime Church, especially to Mestre Irineu, Pelicano, Jacy, and Fernandes, and to all the subjects who participated in the study. They thank Prof. Ronald T. Wakai for important discussions.

REFERENCES

Addis DR, Schacter DL (2008): Constructive episodic simulation: Temporal distance and detail of past and future events modulate hippocampal engagement. Hippocampus 18:227–237.

Allen P, Laroi F, McGuire PK, Aleman A (2008): The hallucinating brain: A review of structural and functional neuroimaging studies of hallucinations. Neurosci Biobehav Rev 32:175–191.

Bar M (2004): Visual objects in context. Nat Rev Neurosci 5:617–629.

Braun AR, Balkin TJ, Wesensten NJ, Gwadry F, Carson RE, Varga M, Baldwin P, Belenky G, Herscovitch P (1998): Dissociated pattern of activity in visual cortices and their projections during human rapid eye movement sleep. Science 279:91–95.

Buckholtz NS, Boggan WO (1977): Monoamine-Oxidase Inhibition in Brain and Liver Produced by Beta-Carbolines-Structure-Activity-Relationships and Substrate-Specificity. Biochem Pharmacol 26:1991–1996.

Burgess PW, Quayle A, Frith CD (2001): Brain regions involved in prospective memory as determined by positron emission tomography. Neuropsychologia 39:545–555.

Cecchi GA, Rao AR, Centeno MV, Baliki M, Apkarian AV, Chialvo DR. 2007. Identifying directed links in large scale functional networks: application to brain fMRI. BMC Cell Biol 8:S5.

Chadwick MJ, Hassabis D, Weiskopf N, Maguire EA (2010): Decoding individual episodic memory traces in the human hippocampus. Curr Biol 20:544–547.

Christoff K, Gabrieli JDE (2000): The frontopolar cortex and human cognition: Evidence for a rostrocaudal hierarchical organization within the human prefrontal cortex. Psychobiology 28:168–186.

Crippa JAS, Sanches RF, Hallak JEC, Loureiro SR, Zuardi AW (2001): A structured interview guide increases Brief Psychiatric Rating Scale reliability in raters with low clinical experience. Acta Psychiatr Scand 103:465–470.

de Araujo DB, Baffa O, Wakai RT (2002): Theta oscillations and human navigation: A magnetoencephalography study. J Cogn Neurosci 14:70–78.

Denis M.1991. Image and Cognition. Greenbaum C, translator. New York: Prentice Hall/Harvester Wheatsheaf.

DEsposito M, Detre JA, Aguirre GK, Stallcup M, Alsop DC, Tippet LJ, Farah MJ (1997): A functional MRI study of mental image generation. Neuropsychologia 35:725–730.

Ganis G, Thompson WL, Kosslyn SM (2004): Brain areas underlying visual mental imagery and visual perception: an fMRI study. Brain Res Cogn Brain Res 20:226–241.

Goldberg II, Harel M, Malach R (2006): When the brain loses its self: Prefrontal inactivation during sensorimotor processing. Neuron 50:329–339.

Ishai A, Sagi D (1995): Common mechanisms of visual-imagery and perception. Science 268:1772–1774.

Ishai A, Ungerleider LG, Haxby JV (2000): Distributed neural systems for the generation of visual images. Neuron 28:979–990.

Kosslyn SM (1999): The role of Area 17 in visual imagery: Convergent evidence from PET and rTMS. Science 284:917–917.

Kosslyn SM, Thompson WL (2003): When is early visual cortex activated during visual mental imagery?Psychol Bull 129:723–746.

Kosslyn SM, Pascual-Leone A, Felician O, Camposano S, Keenan JP, Thompson WL, Ganis G, Sukel KE, Alpert NM (1999): The role of Area 17 in visual imagery: Convergent evidence from PET and rTMS. Science 284:167–170.

Kosslyn SM, Ganis G, Thompson WL (2001): Neural foundations of imagery. Nat Rev Neurosci 2:635–642.

Linden DEJ, Kallenbach U, Heinecke A, Singer W, Goebel R (1999): The myth of upright vision. A psychophysical and functional imaging study of adaptation to inverting spectacles. Perception 28:469–481.

Mellet E, Petit L, Mazoyer B, Denis M, Tzourio N (1998): Reopening the mental imagery debate: Lessons from functional anatomy. Neuroimage 8:129–139.

Petit-Taboue MC, Landeau B, Barre L, Onfroy MC, Noel MH, Baron JC (1999): Parametric PET imaging of 5HT(2A) receptor distribution with F-18-setoperone in the normal human neocortex. J Nucl Med 40:25–32.

Prado DA, Pinto J, Crippa J, Santos A, Ribeiro S, Araujo D, Zuardi A, Chaves C, Hallak J (2009): Effects of the Amazonian psychoactive plant beverage ayahuasca on prefrontal and limbic regions during a language task: A fMRI study. Eur Neuropsychopharmacol 19:S314–S315.

Pylyshyn ZW (1981): Psychological explanations and knowledge-dependent processes. Cognition 10:267–274.

Riba J, Rodriguez-Fornells A, Urbano G, Morte A, Antonijoan R, Montero M, Callaway JC, Barbanoj MJ (2001): Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. Psychopharmacology 154:85–95.

Riba J, Anderer P, Morte A, Urbano G, Jane F, Saletu B, Barbanoj MJ (2002): Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage ayahuasca in healthy volunteers. Br J Clin Pharmacol 53:613–628.

Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanoj MJ (2003): Human pharmacology of ayahuasca: Subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J Pharmacol Exp Ther 306:73–83.

Riba J, Romero S, Grasa E, Mena E, Carrio I, Barbanoj MJ (2006): Increased frontal and paralimbic activation following ayahuasca, the pan-amazonian inebriant. Psychopharmacology 186:93–98.

Richardson A.1969. Mental Imagery. London: Routledge & Kegan Paul PLC. p192.

Santos RG, Landeira-Fernandez J, Strassman RJ, Motta V, Cruz APM (2007): Effects of ayahuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members. J Ethnopharmacol 112:507–513.

Shanon B (2002): Ayahuasca visualizations-A structural typology. J Conscious Stud 9:3–30.

Shanon B (2003): Altered states and the study of consciousness-The case of ayahuasca. J Mind Behav 24:125–153.

Smith RL, Canton H, Barrett RJ, Sanders-Bush E (1998): Agonist properties of N,N-Dimethyltryptamine at serotonin 5-HT2A and 5-HT2C receptors. Pharmacol Biochem Behav 61: 323–330.

Turner MS, Simons JS, Gilbert SJ, Frith CD, Burgess PW (2008): Distinct roles for lateral and medial rostral prefrontal cortex in source monitoring of perceived and imagined events. Neuropsychologia 46:1442–1453.

Vilela JAA, Crippa JAS, Del-Ben CM, Loureiro SR (2005): Reliability and validity of a Portuguese version of the Young Mania Rating Scale. Braz J Med Biol Res 38:1429–1439.

Vinogradov S, Luks TL, Simpson GV, Schulman BJ, Glenn S, Wong AE (2006): Brain activation patterns during memory of cognitive agency. Neuroimage 31:896–905.

Wehrle R, Kaufmann C, Wetter TC, Holsboer F, Auer DP, Pollmacher T, Czisch M (2007): Functional microstates within human REM sleep: First evidence from fMRI of a thalamocortical network specific for phasic REM periods. Eur J Neurosci 25:863–871.

Yoshor D, Bosking WH, Ghose GM, Maunsell JHR (2007): Receptive fields in human visual cortex mapped with surface electrodes. Cereb Cortex 17:2293–2302.

Abuse Potential

Literature research conducted by José Carlos Bouso for
The International Center for Ethnobotanical Education, Research & Service

Drug and Alcohol Dependence 111 (2010) 257–261

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Short communication

Assessment of addiction severity among ritual users of ayahuasca☆

Josep Maria Fábregas[a], Débora González[a], Sabela Fondevila[b], Marta Cutchet[a], Xavier Fernández[c], Paulo César Ribeiro Barbosa[d], Miguel Ángel Alcázar-Córcoles[e], Manel J. Barbanoj[g,h], Jordi Riba[f,g,h], José Carlos Bouso[f,g,*]

[a] Instituto de Etnopsicología Amazónica Aplicada (IDEAA), 08037 Barcelona, Spain
[b] Centro UCM-ISCIII para la Evolución del Cerebro y Comportamiento Humanos, Sección de Neurociencia Cognitiva, 5, 28029 Madrid, Spain
[c] Independent Researcher, 08005 Barcelona, Spain
[d] Departamento de Filosofia e Ciências Humanas, Universidade Estadual de Santa Cruz (UESC), Ilhéus, 45662 Bahia, Brazil
[e] Departamento de Psicología Biológica y de la Salud, Facultad de Psicología, Universidad Autónoma de Madrid (UAM), 28049 Madrid, Spain
[f] Human Experimental Neuropsychopharmacology, IIB Sant Pau, 08025 Barcelona, Spain
[g] Centre d'Investigació de Medicaments, (CIM-Sant Pau), IIB-Sant Pau, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau, 08025 Barcelona, Spain
[h] Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Barcelona, Spain

ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form 31 March 2010
Accepted 31 March 2010
Available online 15 June 2010

Keywords:
Ayahuasca
Hallucinogens
Addiction Severity Index (ASI)

ABSTRACT

Ayahuasca is a psychoactive beverage used for magico-religious purposes in the Amazon. Recently, Brazilian syncretic churches have helped spread the ritual use of ayahuasca abroad. This trend has raised concerns that regular use of this N,N-dimethyltryptamine-containing tea may lead to the medical and psychosocial problems typically associated with drugs of abuse. Here we assess potential drug abuse-related problems in regular ayahuasca users. Addiction severity was assessed using the Addiction Severity Index (ASI), and history of alcohol and illicit drug use was recorded. In Study 1, jungle-based ayahuasca users ($n = 56$) were compared vs. rural controls ($n = 56$). In Study 2, urban-based ayahuasca users ($n = 71$) were compared vs. urban controls ($n = 59$). Follow-up studies were conducted 1 year later. In both studies, ayahuasca users showed significantly lower scores than controls on the ASI Alcohol Use, and Psychiatric Status subscales. The jungle-based ayahuasca users showed a significantly higher frequency of previous illicit drug use but this had ceased at the time of examination, except for cannabis. At follow-up, abstinence from illicit drug use was maintained in both groups except for cannabis in Study 1. However, differences on ASI scores were still significant in the jungle-based group but not in the urban group. Despite continuing ayahuasca use, a time-dependent worsening was only observed in one subscale (Family/Social relationships) in Study 2. Overall, the ritual use of ayahuasca, as assessed with the ASI in currently active users, does not appear to be associated with the deleterious psychosocial effects typically caused by other drugs of abuse.

1. Introduction

Ayahuasca is a psychoactive plant tea originally used by Amazonian indigenous groups for medicinal and magico-religious purposes (Schultes and Hofmann, 1979). The tea is typically obtained from *Banisteriopsis caapi* and *Psychotria viridis* (Schultes and Hofmann, 1979). *B. caapi* contains beta-carboline alkaloids with MAOI (monoamine oxidase inhibitor) action; whereas *P. viridis* contains the hallucinogen N,N-dimethyltryptamine (DMT) (McKenna, 2004). DMT is not active orally because it is enzymatically destroyed, but its combination with the MAOIs from *B. caapi* renders it orally active (Riba, 2003; Riba et al., 2003).

In recent years, Brazilian churches, such as the Santo Daime and the União do Vegetal, which use ayahuasca in their rituals (MacRae, 1998), have expanded to Europe and North America (Tupper, 2008). This has led to legal processes against several of these churches due to the controlled substance status of DMT (Bullis, 2008; Tupper, 2008; US Supreme Court, 2006).

However, neurobiological evidence on medical and sociopsychological problems related to addiction raise interesting questions about the abuse potential of ayahuasca. Drugs of abuse typically show dopaminergic effects, activating the striatum and the ventral-tegmental area, within the so-called "neural reward circuit" (Camí and Farré, 2003). Drug-induced functional changes at this level are thought to lead to the adverse consequences caused by these substances (McLellan et al., 2006).

☆ Supplementary data tables are available with the online version of this article. See Appendix.

* Corresponding author at: Human Experimental Neuropsychopharmacology Institut de Recerca, Hospital de la Santa Creu i Sant Pau, C/ Sant Antoni María Claret, 167, 08025 Barcelona, Spain. Tel.: +34 93 291 5596; fax: +34 93 291 9286.
E-mail address: jbouso@santpau.cat (J.C. Bouso).

0376-8716/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.drugalcdep.2010.03.024

DMT, on the other hand, interacts with serotonergic neurotransmission, binding to 5-hydroxytryptamine$_{2A}$ receptors (for a review see Riba, 2003). Though there is evidence that some hallucinogens may act also on the dopaminergic system (Nichols, 2004; Passie et al., 2008; Vollenweider et al., 1999), a recent study using the neuroimaging technique SPECT (Single Photon Emission Computerized Tomography) did not find any changes in reward-related regions such as the striatum or the ventral-tegmental area (Riba et al., 2006)

Ethnographic research (Furst, 1972; Labate and Araújo, 2004) also challenges the classification of ayahuasca as an addictive drug, i.e., a substance capable of inducing pleasant states followed, after continued use, by adaptive changes in the central nervous system leading to tolerance, physical dependence, sensitization, craving and relapse (Camí and Farré, 2003). Furthermore, the therapeutic use of ayahuasca in indigenous traditional medicine is socially sanctioned and politically tolerated in Bolivia, Ecuador, and Colombia. And in Peru, it has recently been declared part of the national cultural heritage (Instituto Nacional de la Cultura, 2008).

Despite the growing use of ayahuasca worldwide, few studies have been conducted to assess the impact of long-term regular use of ayahuasca on mental health (Da Silveira et al., 2005; Doering-Silveira et al., 2005; Grob et al., 1996; Halpern et al., 2008). In this paper we report the results of two studies specifically designed to assess any adverse medical and psychosocial consequences related with continued ayahuasca consumption.

2. Methods

2.1. Participants

Participants belonging to several Brazilian ayahuasca churches were enrolled. Control subjects were recruited to match the age, sex and educational level of ayahuasca users. Participants were distributed as follows.

2.1.1. Study 1(jungle-based community context). Group 1. Ayahuasca users from a community within the Amazon rain forest.

This group was recruited from Céu do Mapià, a community of religious ayahuasca users in the Brazilian State of Amazonas. Céu do Mapià is the headquarters of the Centro Eclético da Fluente Luz Universal Raimundo Irineu Serra (CEFLURIS), an important ayahuasca church within the Santo Daime movement, with branches throughout South-America, the US, Canada, Europe and Japan. The mean frequency of ritual attendance was about six times per month.

Group 2. Céu do Mapià comparison group.

A sample of non-ayahuasca users was recruited from Boca do Acre, the nearest town to the Céu do Mapià community. Boca do Acre is a small Amazonian town of about 7000 inhabitants.

2.1.2. Study 2 (urban context). Group 3. Urban-based ayahuasca users.

This group consisted of members of another ayahuasca religious group called *Barquinha*, located in the city of Río Branco. The city of Río Branco, the capital of the State of Acre, has about 150,000 inhabitants. The frequency with which Barquinha members attended rituals in our sample was about eight times per month.

Group 4. Urban-based comparison group.

Subjects with no history of ayahuasca use were recruited in the city of Río Branco as a comparison group.

The main inclusion criterion for participants in the ayahuasca groups was to have been taking ayahuasca for a minimum of 15 years with a frequency of at least twice a month.

Both studies were conducted in accordance with the Declarations of Helsinki, as amended in Edinburgh 2000, and subsequent updates. All subjects signed an informed consent prior to participation. The study was approved by the human research committee of UNINORTE University (Rio Branco, Acre State, Brazil).

2.2. Study variables

2.2.1. Sociodemographic variables. Age (years), sex (male/female) and years of education were used to match study and control groups. Additional sociodemographic indicators such as employment status (according to Hollingshead's categories), race, marital status and religion were recorded for comparison purposes.

2.2.2. Addiction Severity Index. The Brazilian Portuguese version of the 5th Edition of the Addiction Severity Index (ASI) (McLellan et al., 1992) was administered on two separate occasions with an interval of 8–12 months, according to a longitudinal study approach. The ASI is a semi-structured interview designed to assess the impact of drug use in a multi-dimensional fashion. It assesses the participant's Medical Status, Employment/Support, Drug and Alcohol Use, Legal Status, Family/Social Relationships, and Psychiatric Status. It provides general information on the participant's current condition and his/her level of deterioration. The composite measures range from 0 to 1 (a higher score indicating greater severity) and provide an index of severity of problems in the last 30 days.

2.2.3. History of alcohol and illicit drug use. We recorded use of alcohol and nine different psychotropic drug categories in participants' lifetime and in the last month.

2.3. Statistical analysis

2.3.1. Sociodemographic variables and history of alcohol and illicit drug use. Gender, race, marital status, religion and frequency of alcohol and illicit drug use were compared between ayahuasca users and controls in each study by means of χ^2. Age, years of education, employment status and income were compared between groups within each study by means of unpaired Student's *t*-test.

2.3.2. ASI variables. Individual and group scores were obtained for the seven ASI composite subscales. Group differences within each study were analyzed for each variable using unpaired Student's *t*-tests at both baseline and at 1-year follow-up.

To test for significant differences in time-dependent variations in ASI scores, we performed analyses of variance (ANOVAs) with repeated measures on the different ASI subscale scores at baseline and at 1 year. Thus, a within-subjects factor was defined: *timepoint* (pre vs. post) and two between-subjects factors: *study* (Study 1 vs. Study 2) and *group* (ayahuasca users vs. controls). Interactions of interest were *group* by *timepoint* and *study* by *group* by *timepoint*.

Since both studies were longitudinal, there was an experimental mortality between the first and second assessment. Statistical analyses were performed using the computerized package SPSS 17.0.

3. Results

3.1. Study 1

3.1.1. Demographics. Fifty-six regular ayahuasca users and 56 non-users were assessed at baseline. There were no statistical differences between groups in sex, age, years of education, or income (see Table 1). However, a statistical difference was noted in employment. The comparison group was more qualified according to the Hollingshead categories. Thirty-nine volunteers from the ayahuasca group and 49 from the comparison group were assessed at 1-year follow-up. No statistical differences were found in the above variables. The number of whites was larger in the ayahuasca-use group whereas the control group was mainly composed of mestizos. The predominant marital status was "never married" in the jungle-based community, and "married" in the comparison group.

3.1.2. ASI scores. At baseline, the ayahuasca group scored significantly lower in the Medical Status, Alcohol Use, and Psychiatric Status subscales, and significantly higher in the Drug Use subscale (see Table 2). There were no statistical differences between groups in the Employment/Support Status or Family/Social Relationships subscales. Both groups scored 0 for the Legal Status subscale. One year later the ayahuasca group scored significantly lower than the comparison group on the Alcohol Use and Psychiatric Status subscales, and significantly higher in the Drug Use subscale.

3.1.3. History of alcohol and illicit drug use. Statistically significant differences in prior illicit drug were found for several drug categories. Detailed results are available as supplementary online material.

3.2. Study 2

3.2.1. Demographics. Seventy-one urban ayahuasca users (group 3) and 59 controls (group 4) were assessed at baseline. At the 1 year follow-up 39 ayahuasca users and 19 controls were assessed. No significant differences in demographics were found.

Table 1
Sociodemographic characteristics as means (standard deviation) for age, years of education, employment and income and as frequencies for race, marital status and religion. Asterisks indicate p values for between group (ayahuasca vs. controls) Student's t-tests (age, education, employment and income) and χ^2 tests (gender, race, marital status and religion) at baseline and at follow up for studies 1 and 2. Aya. = ayahuasca-using group; Comp. = comparison group.

	Study 1				Study 2			
	Mapiá Aya. (baseline)	Boca do Acre Comp. (baseline)	Mapiá Aya. (follow-up)	Boca do Acre Comp. (follow-up)	Rio Branco Aya. (baseline)	Rio Branco Comp. (baseline)	Rio Branco Aya. (follow-up)	Rio Branco Comp. (follow-up)
Matching variables								
N (men/women)	56 (29/27)	56 (24/32)	39 (19/20)	49 (19/30)	71 (33/38)	59 (31/28)	39 (21/18)	19 (7/12)
Age	36 (13.46)	33.71 (12.53)	39.21 (12.90)	34.69 (12.25)	37.32 (12.77)	38.15 (12.22)	38.82 (13.06)	40.63 (11.63)
Years education	10.55 (3.45)	10.96 (4.35)	11.08 (3.19)	11.51 (4.40)	10.27 (3.90)	11.08 (3.30)	10.87 (4.16)	12.53 (3.03)
Additional sociodemographic variables								
Employment	6.04 (1.68)	4.91 (2.58)**	5.79 (1.61)	5.08 (2.70)	5.80 (2.63)	5.73 (2.61)	5.82 (2.59)	5.32 (2.43)
Income	329.46 (414.06)	555.61 (1013.85)	519.74 (627.52)	642.96 (647.71)	738.11 (943.86)	1028.93 (1072.83)	713.95 (1001.25)	1065.95 (939.92)
Race								
White	40 (71.42%)	11 (19.64%)†††	30 (76.92%)	10 (20.41%)†††	38 (53.52%)	34 (57.63%)	23 (58.98%)	11 (57.89%)
Mestizos	15 (26.78%)	45 (80.36)	9 (23.07%)	39 (79.59%)	31 (43.66%)	21 (35.59%)	15 (38.46%)	6 (31.59%)
Asian	1 (1.78%)	–	–	–	1 (1.41%)	1 (1.69%)	–	1 (5.26%)
Black	–	–	–	–	1 (1.41%)	3 (5.08%)	1 (2.56%)	1 (5.26%)
Marital status								
Married	13 (23.21%)	33 (58.93%)††	14 (35.90%)	31 (63.26%)†	25 (35.21%)	17 (28.82%)	23 (58.97%)	8 (42.1%)
Remarried	1 (1.79%)	1 (1.79%)	–	1 (2.05%)	2 (2.82%)	1 (1.69%)	1 (2.56%)	–
Separated	7 (12.5%)	2 (3.57%)	7 (17.94%)	5 (10.20%)	10 (14.08%)	9 (15.25%)	4 (10.26%)	5 (26.32%)
Divorced	4 (7.14%)	–	4 (10.26%)	–	6 (8.45%)	5 (8.47%)	–	1 (5.26%)
Never married	31 (55.36%)	20 (35.71%)	14 (35.90%)	12 (24.49%)	28 (39.44%)	27 (45.77%)	11 (28.21%)	5 (26.32%)
Religion								
Daime/Barquinha	56 (100%)	–†††	39 (100%)	–†††	71 (100%)	–†††	39 (100%)	–†††
Catholic	–	35 (62.5%)	–	33 (67.35%)	–	30 (58%)	–	12 (63.16%)
Protestant	–	15 (26.78%)	–	10 (20.41%)	–	17 (28.81%)	–	7 (36.84%)
Other	–	3 (5.36%)	–	3 (61.12%)	–	2 (3.39%)	–	–
None	–	3 (5.36%)	–	3 (6.12%)	–	10 (16.95%)	–	–

** $p < 0.01$ in the Student's t-test.
† $p < 0.05$ in the χ^2 test (comparison includes multiple categories).
†† $p < 0.01$ in the χ^2 test (comparison includes multiple categories).
††† $p < 0.001$ in the χ^2 test (comparison includes multiple categories).

Table 2
ASI composite means (standard deviation). Asterisks indicate p values for between group (ayahuasca vs. controls) Student's t-tests at baseline and at follow up for studies 1 and 2. Aya. = ayahuasca-using group; Comp. = comparison group; Fam/Soc = Family/Social relationships; Psych = Psychiatric Status.

ASI subscale	Study 1				Study 2			
	Mapiá Aya. (baseline) $n=56$	Boca do Acre Comp. (baseline) $n=56$	Mapiá Aya. (follow-up) $n=39$	Boca do Acre Comp. (follow-up) $n=49$	Rio Branco Aya. (baseline) $n=71$	Rio Branco Comp. (baseline) $n=59$	Rio Branco Aya. (follow-up) $n=39$	Rio Branco Comp. (follow-up) $n=19$
Medical	0.11 (0.19)	0.22 (0.27)*	0.11 (0.22)	0.17 (0.21)	0.17 (0.26)	0.27 (0.32)	0.07 (0.14)	0.21 (0.24)*
Employment	0.72 (0.17)	0.65 (0.21)	0.73 (0.15)	0.66 (0.23)	0.54 (0.31)	0.40 (0.31)*	0.55 (0.28)	0.47 (0.28)
Alcohol	0.003 (0.009)	0.014 (0.018)***	0.0007 (0.001)	0.006 (0.014)**	0.001 (0.004)	0.02 (0.08)*	0.0004 (0.001)	0.004 (0.012)
Drug	0.09 (0.03)	0.00 (0.00)***	0.085 (0.029)	0.00 (0.00)***	0.025 (0.012)	0.0003 (0.002)***	0.03 (0.02)	0.00 (0.00)**
Legal	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.002 (0.014)	0.00 (0.00)	0.01 (0.06)	0.00 (0.00)	0.00 (0.00)
Fam/Soc	0.07 (0.09)	0.11 (0.18)	0.063 (0.101)	0.09 (0.14)	0.05 (0.10)	0.16 (0.18)***	0.10 (0.19)	0.08 (0.14)
Psych.	0.03 (0.07)	0.10 (0.15)**	0.02 (0.06)	0.06 (0.10)*	0.01 (0.06)	0.11 (0.15)***	0.063 (0.12)	0.14 (0.19)

* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

3.2.2. ASI scores. At baseline, the ayahuasca group scored significantly lower than controls in Alcohol Use, Family/Social Relationships, and Psychiatric Status subscales, and significantly higher in the Employment/Support Status and in the Drug Use subscales (see Table 2). One year later, the ayahuasca group scored significantly lower in the Medical Status, and higher in the Drug Use subscale than the comparison group.

3.2.3. History of alcohol and illicit drug use. No statistically significant differences in prior alcohol and illicit drug were found. Detailed results are available as supplementary materials with the online version of the article (see Appendix).

3.3. Analysis of time-dependent changes in the two studies combined

A significant *study* by *group* by *timepoint* was observed for the Drug Use subscale [$F(1,142)=4.9$, $p=0.028$]. Scores on this subscale showed a larger decrease (improvement) in the ayahuasca using group than in the control group, but only in Study 1. Another significant *study* by *group* by *timepoint* interaction was observed for the Family/Social Relationships subscale [$F(1,142)=5.4$, $p=0.022$]. Scores on this subscale showed a larger increase (worsening) in the ayahuasca using group than in the control group, but only in Study 2. All other interactions for all seven subscales were non-significant.

4. Discussion

To our knowledge, this is the first research study in which the ASI has been used to assess potential addiction-related problems derived from the regular ritual use of a hallucinogen. Results showed that both ayahuasca-using groups scored significantly lower than their respective controls on the ASI Alcohol Use and Psychiatric Status subscales. At the 1 year follow-up these differences were still significant in the jungle-based group but not in the urban group. Despite maintained ayahuasca use, significant time-dependent increases (worsenings) were only observed in the family/social relationships subscale in Study 2. This effect may not be related with ayahuasca use in itself but rather with the member's involvement with the church, as the worsening was observed in the urban but not in the more isolated jungle group. On the other hand, as shown in the supplementary online material, the ayahuasca jungle-based group did not report current use of illicit drugs despite a history of a significantly higher prior use than the control group.

ASI scores in our samples were in general lower than those obtained for several groups of Brazilian (Brasiliano and Hochgraf, 2006; Mathias et al., 2009; Pechansky et al., 2003) and international drug abusers (Carise, 2005). Although this questionnaire had not been administered to ayahuasca users before, previous studies have not found neuropsychiatric disorders in long-term users (Grob et al., 1996). Two other studies carried out in adolescents also failed to find psychiatric disorders (Da Silveira et al., 2005) and neuropsychological deficits (Doering-Silveira et al., 2005). A recent study of a US group of ritual ayahuasca users did not find evidence of psychopathology when scores where checked against normative data (Halpern et al., 2008).

The above results are in line with the data obtained in our present study for the Medical Status and Psychiatric Status subscales. Our results suggest that ayahuasca has a low abuse potential, as previously concluded by others (Gable, 2007).

In our studies, both ayahuasca groups scored worse than controls in the Drug Use subscale. This is because ayahuasca use was taken into account when computing the score in the Drug Use subscale. Additionally, the Mapiá group (Study 1) uses *Cannabis sativa*. However, if this combined use of ayahuasca and cannabis had been problematic, scores in the other subscales would have been higher (McLellan et al., 2006), which was not the case. Also, the detailed study of prior illicit drug use showed that subjects had ceased to consume barbiturates, sedatives, cocaine and amphetamines (see supplementary online material). The fact that neither group scored in the Legal subscale may also reflect a lack of social problems related to their involvement with an ayahuasca-using church. These results are analogous to those by Grob et al. (1996) who found that previously-existing addiction problems had resolved after participants began ritual use of ayahuasca.

In conclusion, the ritual use of ayahuasca, as assessed with the ASI in currently active users, does not seem to be associated with the psychosocial problems that other drugs of abuse typically cause. Future studies should further address whether this is due to the specific pharmacological characteristics of ayahuasca or to the context in which the drug is taken.

Role of funding source

Funding for this study was provided by IDEAA, Instituto de Etnopsicología Amazónica Aplicada, Barcelona (Spain)/Prato Raso (Brasil).

Contributors

Josep Maria Fabregas: study design, coordination of field work, manuscript writing.

Debora Gonzalez, Sabela Fondevila, Marta Cutchet, and Paulo Cesar Ribeiro Barbosa: data collection. Xavier Fernandez: study design and data collection.

Migual Angel Alcazar-Corcoles: study design and data analysis. Jordi Riba: data analysis and manuscript writing. Manel J. Barbanoj: data analysis and manuscript writing. Jose Carlos Bouso: study design, coordination of researchers, manuscript writing and data analysis.

Conflict of interests

None.

Acknowledgements

The authors thank all volunteers for their participation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.drugalcdep.2010.03.024.

References

Brasiliano, S., Hochgraf, P.B., 2006. A influência da comorbidade com transtornos alimentares na apresentação de mulheres dependentes de substâncias psicoativas. Rev. Psiq. Clín. 33, 134–144.
Bullis, R.K., 2008. The "vine of the soul" vs. the Controlled Substances Act: implications of the hoasca case. J. Psychoactive Drugs 40, 193–199.
Camí, J., Farré, M., 2003. Drug addiction. N. Engl. J. Med. 349, 975–986.
Carise, D., 2005. Clients Presenting for Substance Abuse Treatment—ASI Differences Across Countries. NIDA International Forum: Linking Drug Abuse and HIV/AIDS Research, Orlando, Florida, USA, June 17–20.
Da Silveira, D.X., Grob, C.S., de Rios, M.D., Lopez, E., Alonso, L.K., Tacla, C., Doering-Silveira, E., 2005. Ayahuasca in adolescence: a preliminary psychiatric assessment. J. Psychoactive Drugs 37, 129–133.
Doering-Silveira, E., Lopez, E., Grob, C.S., de Rios, M.D., Alonso, L.K., Tacla, C., Shirakawa, I., Bertolucci, P.H., Da Silveira, D.X., 2005. Ayahuasca in adolescence: a neuropsychological assessment. J. Psychoactive Drugs 37, 123–128.
Furst, P.T. (Ed.), 1972. Flesh of the Gods. The Ritual Use of Hallucinogens. Waveland Press, Inc., Prospect Heights, IL.
Gable, R.S., 2007. Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. Addiction 102, 24–34.
Grob, C.S., McKenna, D.J., Callaway, J.C., Brito, G.S., Neves, E.S., Oberlaender, G., Saide, O.L., Labigalini, E., Tacla, C., Miranda, C.T., Strassman, R.J., Boone, K.B., 1996. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. J. Nerv. Ment. Dis. 184, 86–94.
Halpern, J.H., Sherwood, A.R., Passie, T., Blackwell, K.C., Ruttenber, A.J., 2008. Evidence of health and safety in American members of a religion who use a hallucinogenic sacrament. Med. Sci. Monit. 14, SR15–SR22.
Instituto Nacional de Cultura, Perú, 2008. Declaración Patrimonio Cultural de la nación a los conocimientos y usos tradicionales del Ayahuasca practicados por comunidades nativas amazónicas, Resolución Directoral Nacional n° 836.
Labate, B.C., Araújo, W.S. (Eds.), 2004. O uso ritual da ayahuasca. Campinas, Mercado de Letras.
MacRae, E., 1998. Guiado por la luna. Shamanismo y uso ritual de la ayahuasca en el culto del Santo Daime. Abya-Yala, Quito.
Mathias, A.C.R., Vargens, R.W., Kessler, F.H., Cruz, M.S., 2009. Differences in addiction severity between social and probable pathological gamblers among substance abusers in treatment in Rio de Janeiro. Int. J. Ment. Health Addict. 7, 239–249.
McKenna, D.J., 2004. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. Pharmacol. Ther. 102, 111–129.
McLellan, A.T., Kushner, H., Metzger, D., Peters, R., Smith, I., Grissom, G., Pettinati, H., Argeriou, M., 1992. The Fifth Edition of the Addiction Severity Index. J. Subst. Abuse Treat. 9, 199–213.
McLellan, A.T., Cacciola, J.C., Alterman, A.I., Rikoon, S.H., Carise, D., 2006. The Addiction Severity Index at 25: origins, contributions and transitions. Am. J. Addict. 15, 113–124.
Nichols, D.E, 2004. Hallucinogens. Pharmacol. Ther. 101, 131–181.
Passie, T., Halpern, J.H., Stichtenoth, D.O., Emrich, H.M., Hintzen, A., 2008. The pharmacology of lysergic acid diethylamide: a review. CNS Neurosci. Ther. 14, 295–314.
Pechansky, F., von Diemen, L., Kessler, F., Hirakata, V., Metzger, D., Woody, G.E., 2003. Preliminary estimates of human immunodeficiency virus prevalence and incidence among cocaine abusers of Porto Alegre, Brazil. J. Urban Health 80, 115–126.
Riba, J., 2003. Human pharmacology of ayahuasca. Doctoral Thesis. Barcelona, Universitat Autònoma de Barcelona. http://www.tesisenxarxa.net/TDX-0701104-165104/.
Riba, J., Valle, M., Urbano, G., Yritia, M., Morte, A., Barbanoj, M.J., 2003. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J. Pharmacol. Exp. Ther. 306, 73–83.
Riba, J, Romero, S., Grasa, E., Mena, E., Carrió, I., Barbanoj, M.J., 2006. Increased frontal and paralimbic activation following ayahuasca, the pan-Amazonian inebriant. Psychopharmacology (Berl.) 186, 93–98.
Schultes, R.E., Hofmann, A. (Eds.), 1979. Plants of the Gods: Origins of Hallucinogenic Use. McGraw-Hill, New York.
Tupper, K.W., 2008. The globalization of ayahuasca: harm reduction or benefit maximization? Int. J. Drug Policy 19, 297–303.
US Supreme Court, no. 04-1084. Gonzales, V., decided 21 February 2006. Centro Espirita Beneficente Uniao Do Vegetal 546 US 2006.
Vollenweider, F.X., Vontobel, P., Hell, D., Leenders, K.L., 1999. 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man—a PET study with [11C]raclopride. Neuropsychopharmacology 20, 424–433.

Revision Papers

Literature research conducted by José Carlos Bouso for
The International Center for Ethnobotanical Education, Research & Service

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology & Therapeutics 102 (2004) 111–129

Pharmacology & Therapeutics

www.elsevier.com/locate/pharmthera

Associate editor: B.L. Roth

Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges

Dennis J. McKenna*

Center for Spirituality and Healing, Academic Health Center, University of Minnesota, C592 Mayo Memorial Building, Mayo Mail Code 505, 420 Delaware Street Southeast, Minneapolis, MN 55455, USA

Abstract

Ayahuasca is a hallucinogenic beverage that is prominent in the ethnomedicine and shamanism of indigenous Amazonian tribes. Its unique pharmacology depends on the oral activity of the hallucinogen, *N*,*N*-dimethyltryptamine (DMT), which results from inhibition of monoamine oxidase (MAO) by β-carboline alkaloids. MAO is the enzyme that normally degrades DMT in the liver and gut. Ayahuasca has long been integrated into mestizo folk medicine in the northwest Amazon. In Brazil, it is used as a sacrament by several syncretic churches. Some of these organizations have incorporated in the United States. The recreational and religious use of ayahuasca in the United States, as well as "ayahuasca tourism" in the Amazon, is increasing. The current legal status of ayahuasca or its source plants in the United States is unclear, although DMT is a Schedule I-controlled substance. One ayahuasca church has received favorable rulings in 2 federal courts in response to its petition to the Department of Justice for the right to use ayahuasca under the Religious Freedom Restoration Act. A biomedical study of one of the churches, the Uñiao do Vegetal (UDV), indicated that ayahuasca may have therapeutic applications for the treatment of alcoholism, substance abuse, and possibly other disorders. Clinical studies conducted in Spain have demonstrated that ayahuasca can be used safely in normal healthy adults, but have done little to clarify its potential therapeutic uses. Because of ayahuasca's ill-defined legal status and variable botanical and chemical composition, clinical investigations in the United States, ideally under an approved Investigational New Drug (IND) protocol, are complicated by both regulatory and methodological issues. This article provides an overview of ayahuasca and discusses some of the challenges that must be overcome before it can be clinically investigated in the United States.

Keywords: Ayahuasca; Hoasca; β-carbolines; DMT; Serotonin transporters; IND; Clinical studies; Alcoholism; Substance abuse; Immune modulation

Abbreviations: DMT, *N*,*N*-dimethyltryptamine; 5-HT, 5-hydroxytryptamine; IND, Investigational New Drug (application); IRB, International Review Board; MAO, monoamine oxidase; MAOI, monoamine oxidase inhibitor; THH, tetrahydroharmine; UDV, Uñiao do Vegetal.

Contents

* Tel.: 612-624-0112; fax: 612-626-5280.
E-mail address: mcken031@umn.edu (D.J. McKenna).

0163-7258/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2004.03.002

1. Introduction

Of the numerous plant psychotropics utilized by indigenous populations of the Amazon Basin, perhaps none is as interesting or complex, botanically, chemically, or ethnographically, as the beverage known variously as ayahuasca, caapi, or yage. The beverage is most widely known as *ayahuasca*, a Quechua term meaning "vine of the souls," which is applied both to the beverage itself and to one of the source plants used in its preparation, the malpighiaceous jungle liana, *Banisteriopsis caapi* (Schultes, 1957). In Brazil, transliteration of this Quechua word into Portuguese results in the name, hoasca. Hoasca, or ayahuasca, occupies a central position in mestizo ethnomedicine, and the chemical nature of its active constituents and the manner of its use make its study relevant to contemporary issues in neuropharmacology, neurophysiology, and psychiatry.

2. What is ayahuasca?

In a traditional context, ayahuasca is a beverage prepared by boiling—or soaking—the bark and stems of *B. caapi* together with various admixture plants. The admixture employed most commonly is the Rubiaceous genus *Psychotria*, particularly *Psychotria viridis*. The leaves of *P. viridis* contain alkaloids, which are necessary for the psychoactive effect. Ayahuasca is unique in that its pharmacological activity is dependent on a synergistic interaction between the active alkaloids in the plants. One of the components, the bark of *B. caapi*, contains β-carboline alkaloids, which are potent monoamine oxidase-A (MAO-A) inhibitors; the other component, the leaves of *P. viridis* or related species, contains the potent short-acting hallucinogenic agent *N*,*N*-dimethyltryptamine (DMT). DMT is not orally active when ingested by itself, but can be rendered orally active when ingested in the presence of a peripheral MAO inhibitor, such as the β-carbolines. This interaction is the basis of the psychotropic action of ayahuasca (McKenna et al., 1984). There are also reports (Schultes, 1972) that other *Psychotria* species are similarly utilized in other parts of the Amazon. In the northwest Amazon, particularly in the Colombian Putumayo and Ecuador, the leaves of *Diplopterys cabrerana*, a jungle liana in the same family as *Banisteriopsis*, are added to the brew in lieu of the leaves of *Psychotria*. The alkaloid present in *Diplopterys*, however, is identical to that in the *Psychotria* admixtures, and pharmacologically, the effect is similar. In Peru, various admixtures in addition to *Psychotria* or *Diplopterys* are frequently added, depending on the magical, medical, or religious purposes for which the drug is being consumed. Although a virtual pharmacopoeia of admixtures are occasionally added, the most commonly employed admixtures (other than *Psychotria*, which is a constant component of the preparation) are various Solanaceous genera, including tobacco (*Nicotiana* sp.), *Brugmansia* sp., and *Brunfelsia* sp. (Schultes, 1972; McKenna et al., 1995). These Solanaceous genera are known to contain alkaloids, such as nicotine, scopalamine, and atropine, which have effects on both central and peripheral adrenergic and cholinergic neurotransmission.

3. Prehistorical origin of ayahuasca

The origins of the use of ayahuasca in the Amazon Basin are unknown. It is uncertain where the practice may have originated, and about all that is certain is that it was already widespread among numerous indigenous tribes throughout the Amazon Basin by the time ayahuasca came to the attention of Western ethnographers in the mid-19th century (McKenna, 1999). This fact alone argues for its antiquity; beyond that, little is known. Plutarco Naranjo, the Ecuadorian ethnographer, has summarized what little information is available on the prehistory of ayahuasca (Naranjo, 1979, 1986). There is abundant archeological evidence, in the form of pottery vessels, anthropomorphic figurines,

snuffing trays and tubes, etc. that plant hallucinogen use was well established in the Ecuadorian Amazon by 1500–2000 BC (Naranjo, 1979, 1986). Unfortunately, most of the specific evidence, in the form of vegetable powders, snuff trays, and pipes, is related to the use of psychoactive plants *other* than ayahuasca, such as coca, tobacco, and the hallucinogenic snuff derived from *Anadenanthera* species and known as *vilka* and various other names. There is nothing in the form of iconographic materials or preserved botanical remains that would unequivocally establish the prehistorical use of ayahuasca. It is probable that these pre-Colombian cultures, sophisticated as they were in the use of a variety of psychotropic plants, were also familiar with ayahuasca and its preparation. Recent archaeological evidence has come to light (Heckenberger et al., 2003) indicating the existence of a complex, technologically sophisticated riverine/agrarian civilization in the upper Xingu region of Brazil dating at least to 1200 AD. While these discoveries do not directly address the question of the antiquity of knowledge of ayahuasca, they do support the supposition that a complex civilization capable of impacting and actively managing its forest environment for agricultural purposes, would also be likely to be similarly knowledgeable regarding the uses of medicinal species occurring in the ecosystem. The lack of data is frustrating, however, particularly with respect to a question that has fascinated ethnopharmacologists since the late 1960s when its importance was first brought to light through the work of Schultes and his students.

As mentioned above, ayahuasca is unique among plant hallucinogens in that it is prepared from a combination of 2 plants: the bark or stems of *Banisteriopsis* species, together with the leaves of *Psychotria* species or other DMT-containing admixtures. The beverage depends on this unique combination for its activity. There seems small likelihood of "accidentally" combining the 2 plants to obtain an active preparation when neither is particularly active alone, yet we know that at some point in prehistory, this fortuitous combination was discovered. At that point, ayahuasca was "invented." Just how this discovery was made, and who was responsible, we may never know, though several charming myths address the topic. Mestizo *ayahuasqueros* in Peru will, to this day, say that this knowledge comes directly from the "plant teachers" (Luna, 1984a, 1984b). The mestres of the Brazilian syncretic sect, the Uñiao do Vegetal (UDV), say with equal conviction that the knowledge came from "the first scientist," King Solomon, who imparted the technology to the Inca king during a little publicized visit to the New World in antiquity. In the absence of data, these explanations are all that we have. All that we can say with confidence is that the knowledge of the techniques for preparing ayahuasca, including knowledge of the appropriate admixture plants, had diffused throughout the Amazon Basin by the time the use of ayahuasca came to the attention of any modern researcher (Anonymous, 1855).

4. Traditional and indigenous use of ayahuasca

The use of ayahuasca under a variety of names is a widespread practice among various indigenous aboriginal tribes endemic to the Amazon Basin (Schultes, 1957). Such practices undoubtedly were well established in pre-Columbian times, and in fact, may have been known to the earliest human inhabitants of the region. Considerable genetic intermingling and adoption of local customs followed in the wake of European contact, and ayahuasca, along with a virtual pharmacopoeia of other medicinal plants, gradually became integrated into the ethnomedical traditions of these mixed populations. Today, the drug forms an important element of ethnomedicine and shamanism as it is practiced among indigenous mestizo populations in Peru, Colombia, and Ecuador. The sociology and ethnography of the contemporary use of ayahuasca in mestizo ethnomedicine has been extensively described (Dobkin de Rios, 1972, 1973; Luna, 1984a, 1984b, 1986).

5. Syncretic religious use of ayahuasca

From the perspective of the sociologist or the ethnographer, discussion of the use of ayahuasca or hoasca can conveniently be divided into a consideration of its use among indigenous aboriginal and mestizo populations, and its more recent adoption by contemporary syncretic religious movements, such as the UDV, Barquinia, and Santo Daime sects in Brazil. It is within the context of acculturated groups such as these that questions regarding the psychological, medical, and legal aspects of the use of ayahuasca become most relevant, and also, most accessible to study.

The use of ayahuasca in the context of mestizo folk medicine closely resembles the shamanic uses of the drug as practiced among aboriginal peoples. In both instances, the brew is used for curing, for divination, as a diagnostic tool, and a magical pipeline to the supernatural realm. This traditional mode of use contrasts from the contemporary use of ayahuasca tea within the context of Brazilian syncretic religious movements. (Note: The hoasca religions are "syncretic" in that they represent a fusion of indigenous religious practices with elements of Christianity.) Within these groups, the members consume ayahuasca tea at regular intervals in a ritual manner that more closely resembles the Christian Eucharist than the traditional aboriginal use. The individual groups of the UDV, termed *nucleos*, are similar to a Christian Hutterite sect, in that each group has a limited membership, which then splits to form a new group once the membership expands beyond the set limit. The *nucleo* consists of the congregation, a group leader or mestre, various acolytes undergoing a course of study and training to become mestres, and a temple. These structures, often circular in layout and beautifully decorated, are the sites where the sacrament is prepared and consumed at prescribed times, usually the first and third Saturday of each month.

The membership of these newer syncretic groups spans a broad socioeconomic range and includes many educated, middle-class, urban professionals (including a number of physicians and other health professionals). Some older members have engaged in the practice for 30 or more years without apparent adverse health effects.

The UDV and the Santo Daime sects are the largest and most visible of several syncretic religious movements in Brazil that have incorporated the use of ayahuasca into their ritual practices. Of the 2 larger sects, it is the UDV that possesses the strongest organizational structure as well as the most highly disciplined membership. Of all the ayahuasca churches in Brazil, the UDV has also been the most pivotal in convincing the government to remove ayahuasca from its list of banned drugs. In 1987, the government of Brazil approved the ritual use of hoasca tea[1] in the context of group religious ceremonies. This ruling has potentially significant implications, not only for Brazil, but for global drug policy, as it marks the first time in over 1600 years that a government has granted permission to its nonindigenous citizens to use a psychedelic substance in the context of religious practices.

6. Chemistry of ayahuasca and its source plants

The chemical constituents of ayahuasca and the source plants used in its preparation have been well characterized (Rivier & Lindgren, 1972; McKenna et al., 1984). *B. caapi* contains the β-carboline derivatives harmine, tetrahydroharmine (THH), and harmaline as the major alkaloids (Callaway et al., 1996). Trace amounts of other β-carbolines have also been reported (Rivier & Lindgren, 1972; Hashimoto & Kawanishi, 1975, 1976; McKenna et al., 1984), as well as the pyrrolidine alkaloids shihunine and dihydroshihunine (Kawanishi et al., 1982) (Fig. 1). The admixture plant, *P. viridis*, contains a single major alkaloid, DMT, while *N*-methyl tryptamine and methyl-tetrahydro-β-carboline have been reported as trace constituents (Rivier & Lindgren, 1972; McKenna et al., 1984). The admixture plant *Psychotria carthagenensis* has been reported to contain the same alkaloids (Rivier & Lindgren, 1972) but a subsequent investigation could not confirm the presence of DMT in the single collection examined (McKenna et al., 1984). The concentrations of alkaloids reported in *B. caapi* range from 0.05% dry weight to 1.95% dry weight; in *Psychotria*, the concentration of alkaloids ranged from 0.1% to 0.66% dry weight (Rivier & Lindgren, 1972; McKenna et al., 1984). Similar ranges and values were reported by both groups of investigators.

The concentrations of alkaloids in the ayahuasca beverages are, not surprisingly, several times greater than in the source plants from which they are prepared. Based on a quantitative analysis of the major alkaloids in several samples of ayahuasca collected on the upper Rio Purús, Rivier and Lindgren (1972) calculated that a 200-mL dose of ayahuasca contained an average of 30 mg of harmine, 10 mg THH, and 25 mg DMT. Callaway et al. (1996) determined the following concentrations of alkaloids in the hoasca tea utilized in the biomedical study with the UDV (in mg/mL): DMT, 0.24; THH, 1.07; harmaline, 0.20; and harmine 1.70. A typical 100-mL dose of hoasca thus contains (in mg): DMT, 24; THH, 107; harmaline, 20; and harmine, 170. Interestingly, these concentrations are above the threshold of activity for intravenous administration of DMT (Strassman & Qualls, 1994).

McKenna et al. (1984) reported somewhat higher values for the alkaloid content of several samples of Peruvian ayahuasca. These investigators calculated that a 100-mL dose of these preparations contained a total of 728 mg total alkaloid, of which 467 mg is harmine, 160 mg is THH, 41 mg is harmaline, and 60 mg is DMT. This is well within the range of activity for DMT administered intramuscularly (Szara, 1956) or intravenously (Strassman & Qualls, 1994) and is also well within the range for harmine to act effectively as a monoamine oxidase inhibitor (MAOI). In vitro, these β-carbolines function as MAOI at ~10 nM (e.g., harmine's IC_{50} for MAOI is $\sim 1.25 \times 10^{-8}$ M; cf. Buckholtz & Boggan, 1977; McKenna et al., 1984). In mice, harmaline administered intraperitoneally (5 mg/kg) causes 100% inhibition by 2 hr postinjection, the activity falling off rapidly thereafter (Udenfriend et al., 1958). This dose corresponds to ~375 mg in a 75-kg adult, but, based on the measured concentration of harmine in the liver, it is likely that one-half this dose or less would also be effective. The reasons for the discrepancy in alkaloid concentrations between the samples examined by Rivier and Lindgren (1972) and those examined by McKenna et al. (1984) are readily explained by the differences in the methods of preparation. The method employed in preparing ayahuasca in Pucallpa, Peru, where the samples analyzed by McKenna et al. (1984) were collected, results in a much more concentrated brew than the method employed on the upper Rio Purús, the region which was the source of the samples examined by Rivier and Lindgren. The concentrations and proportions of alkaloids can vary considerably in different batches of ayahuasca, depending on the method of preparation, as well as the amounts and proportions of the source plants.

β-Carbolines, by themselves, may have some psychoactivity and thus may contribute to the overall psychotropic activity of the ayahuasca beverage; however, it is probably inaccurate to characterize the psychotropic properties of β-carbolines as "hallucinogenic" or "psychedelic" (Shulgin et al., 1997). As MAO inhibitors, β-carbolines can increase brain levels of serotonin, and the primarily sedative effects of high doses of β-carbolines are thought to result from their blockade of serotonin deamination. The primary action of β-carbolines in the ayahuasca beverage is their inhibition of

[1] In the parlance of the UDV, the tea is sometimes called *hoasca*, which is a Portuguese transliteration of *ayahuasca*. The term as used here applies specifically to the tea used within the UDV, while *ayahuasca* is used to denote non-UDV sources of the brew.

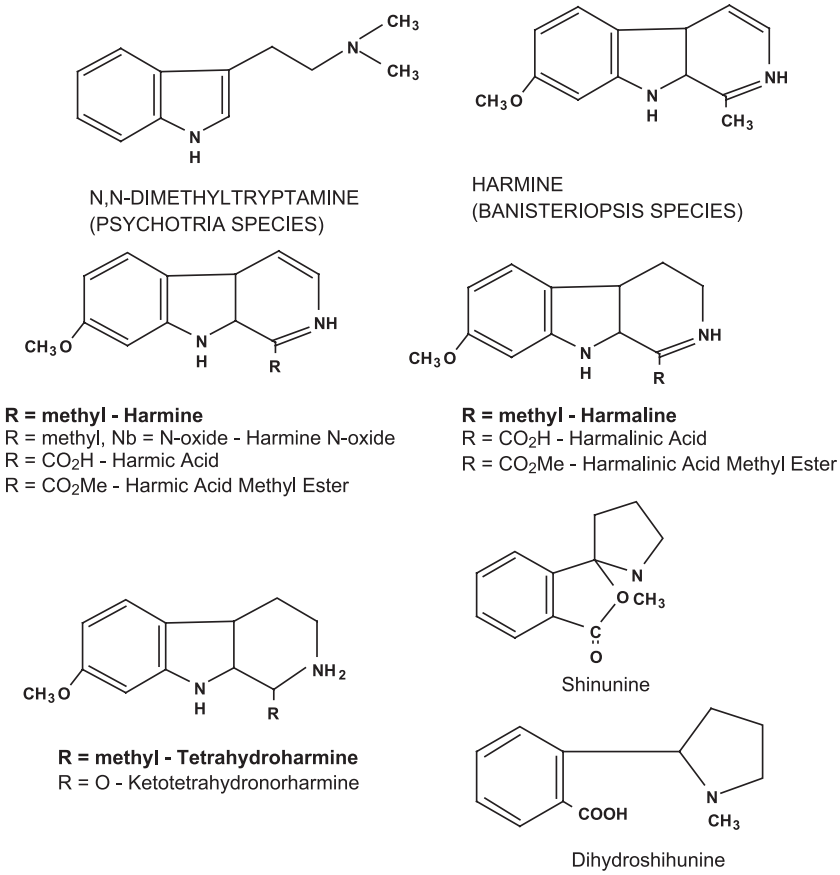


Fig. 1. Structures of ayahuasca alkaloids.

peripheral MAO, which protects the DMT in the brew from peripheral degradation and thus renders it orally active. There is some evidence, however, that THH, the second most abundant β-carboline in the beverage, acts as a weak 5-hydroxytryptamine (5-HT) uptake inhibitor and MAOI. Thus, THH may prolong the half-life of DMT by blocking its intraneuronal uptake, and hence, its inactivation by MAO, localized in mitochondria within the neuron. On the other hand, THH may block serotonin uptake into the neuron, resulting in higher levels of 5-HT in the synaptic cleft; this 5-HT, in turn, may attenuate the subjective effects of orally ingested DMT by competing with it at postsynaptic receptor sites (Callaway et al., 1999).

7. Pharmacological actions of ayahuasca and its active alkaloids

The psychotropic activity of ayahuasca is a function of the inhibition of peripheral MAO by the β-carboline alkaloids in the mixture. This action prevents the peripheral oxidative deamination of the DMT, which is the primary psychotropic component, rendering it orally active and enabling it to reach its site of action in the central nervous system in an intact form (Schultes, 1972; McKenna et al., 1984). DMT alone is inactive following oral administration at doses up to 1000 mg (Shulgin, 1982; Nichols et al., 1991). DMT is active by itself following parenteral administration starting at around 25 mg (Szara, 1956; Strassman & Qualls, 1994). Because of its oral inactivity, users have employed various methods of parenteral administration. For example, synthetic DMT is commonly smoked as the freebase; in this form, the alkaloid volatilizes readily and produces an immediate, intense psychedelic episode of short duration (5–15 min), usually characterized by multicolored, rapidly moving visual patterns behind the closed eyelids (Stafford, 1977). The Yanomamo Indians and other Amazonian tribes prepare a snuff from the sap of various trees in the genus *Virola*, which contain large amounts of DMT and the related compound, 5-methoxy-DMT, which is also orally inactive (Schultes & Hofmann, 1980; McKenna & Towers, 1985). The effects of the botanical snuffs containing DMT, while not as intense as smoking DMT freebase, are similarly rapid in onset and of limited duration. The ayahuasca beverage is unique in that it is the only traditionally used psychedelic where the enzyme-inhibiting principles in one plant (β-carbolines) are used to facilitate the oral activity of the psychoactive principles in another plant (DMT). The psychedelic experience that follows ingestion of ayahuasca differs markedly from the effects of parenterally ingested DMT; the time of onset is ~35–40 min after ingestion, and the effects, which are less intense than parenterally administered synthetic DMT, last

~4 hr. The subjective effects of ayahuasca include phosphene imagery seen with the eyes closed, dream-like reveries, and a feeling of alertness and stimulation. Peripheral autonomic changes in blood pressure, heart rate, etc., are also less pronounced in ayahuasca than parenteral DMT. In some individuals, transient nausea and episodes of vomiting occur, while others are rarely affected in this respect. When ayahuasca is taken in a group setting, vomiting is considered a normal part of the experience and allowances are made to accommodate this behavior (Callaway et al., 1999).

The amounts of β-carbolines present in a typical dose of ayahuasca are well above the threshold for activity as MAOI. It is likely that the main contribution of the β-carbolines to the acute effects of ayahuasca results from their facilitation of the oral activity of DMT, through their action as MAOI at peripheral sites. It is worthy of note that β-carbolines are highly selective inhibitors of MAO-A, the form of the enzyme for which serotonin, and presumably other tryptamines including DMT, are the preferred substrates (Yasuhara et al., 1972; Yasuhara, 1974). This selectivity of β-carbolines for MAO-A over MAO-B, combined with their relatively low affinity for liver MAO, may explain why no reports have appeared of hypertensive crises or peripheral autonomic stimulation associated with the ingestion of ayahuasca in combination with foods containing tyramine (Callaway et al., 1999). On the other hand, Suzuki et al. (1981) have reported that DMT is primarily oxidized by MAO-B; it is possible, therefore, that high concentrations of β-carbolines, partially inhibit MAO-B as well as MAO-A; but the greater affinity of tyramine for MAO-B enables it to compete for binding to the enzyme and displace any residual β-carbolines.

DMT and its derivatives and β-carboline derivatives are widespread in the plant kingdom (Smith, 1977; Allen & Holmstedt, 1980) and both classes of alkaloids have been detected as endogenous metabolites in mammals, including man (Barker et al., 1980; Airaksinen & Kari, 1981; Bloom et al., 1982). Methyl transferases, which catalyze the synthesis of DMT, 5-methoxy-DMT, and bufotenine, have been characterized in human lung, brain, blood, cerebrospinal fluid, liver, and heart, and also in rabbit lung, toad, mouse, steer, guinea pig, and baboon brains, as well as in other tissues in these species (McKenna & Towers, 1984). Endogenous psychotogens have been suggested as possible etiological factors in schizophrenia and other mental disorders, but the evidence remains equivocal (Fischman, 1983). Although the occurrence, synthesis, and degradative metabolism of DMT in mammalian systems has been the focus of scientific investigations (Barker et al., 1980, 1981), the candidacy of DMT as a possible endogenous psychotogen essentially ended when experiments showed comparable levels in both schizophrenics and normal subjects (Uebelhack et al., 1983). At present, the possible neuroregulatory functions of this "psychotomimetic" compound are incompletely understood, but Callaway (1988) has presented an interesting hypothesis regarding the possible role of endogenous DMT and β-carbolines in regulating sleep cycles and rapid eye movement states.

β-Carbolines are tricyclic indole alkaloids that are closely related to tryptamines, both biosynthetically and pharmacologically. They are readily synthesized via the condensation of indoleamines with aldehydes or α-keto acids and their biosynthesis probably also proceeds via similar reactions (Callaway et al., 1994; Fig. 2). β-Carbolines have also been identified in mammalian tissue including human plasma and platelets and rat whole brain, forebrain, arcuate nucleus, and adrenal glands (Airaksinen & Kari, 1981). 6-Methoxy-tetra-

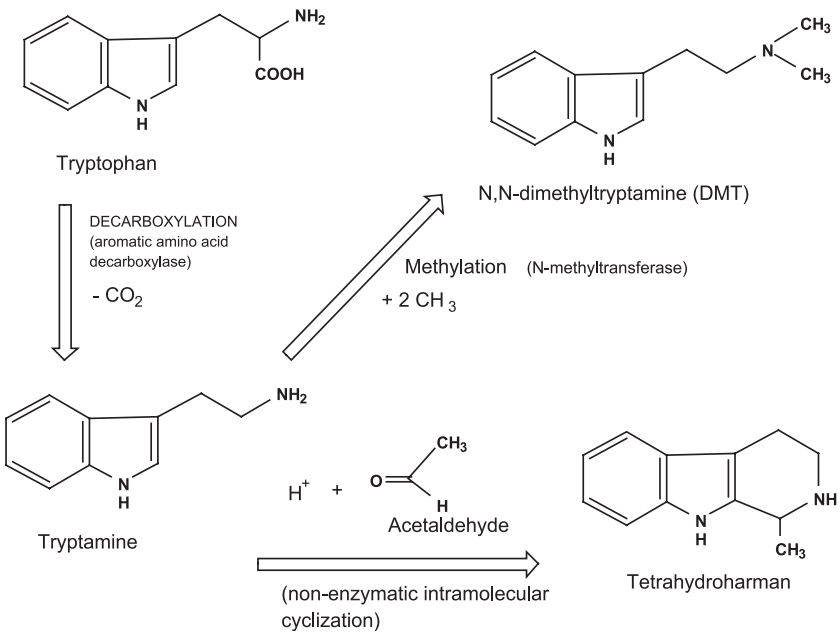


Fig. 2. Biosynthesis of DMT and a tetrahydro-β-carboline from tryptophan.

hydro-β-carboline has been recently identified as a major constituent of human pineal gland (Langer et al., 1984). This compound inhibits the high-affinity binding of [^{3}H]-imipramine to 5-HT receptors in human platelets (Langer et al., 1984), and also significantly inhibits 5-HT binding to 5-HT$_1$ type receptors in rat brain; the compound has a low affinity to 5-HT$_2$ receptors, however (Taylor et al., 1984). 2-Methyltetrahydro-β-carboline and harman have been detected in human urine following ethanol loading, (Rommelspacher et al., 1980) and it was once speculated that endogenous β-carbolines and other amine-aldehyde condensation products might be related to the etiology of alcoholism (Rahwan, 1975). At least one β-carboline has been identified as a by-product of the oxidative metabolism of DMT in rat brain homogenates (Barker et al., 1980).

β-Carbolines exert a variety of neurophysiological and biological effects (McKenna & Towers, 1984). β-Carboline derivatives are selective, reversible, competitive inhibitors of MAO-A (Buckholtz & Boggan, 1976, 1977). Other neurophysiological actions of β-carbolines include competitive inhibition of the uptake of 5-HT, dopamine, epinephrine, and norepinephrine into synaptosomes (Buckholtz & Boggan, 1976; Pahkla et al., 1997), inhibition of Na^+-dependent membrane ATPases (Canessa et al., 1973), interference with biosynthesis of biogenic amines (Ho, 1979), and vasopressin-like effects on sodium and water transport in isolated toad skin (de Sousa & Grosso, 1978). β-Carboline-3-carboxylate and various esterified derivatives have been implicated as possible endogenous ligands at benzodiazepine receptors (Lippke et al., 1983). β-Carboline ligands of these receptors can induce epileptiform seizures in rats and in chickens homozygous for the epileptic gene (Johnson et al., 1984; Morin, 1984); this proconvulsant action can be blocked by other receptor ligands, including diazepam and β-carboline-carboxylate propyl ester (Johnson et al., 1984; Morin, 1984).

β-Carbolines also exhibit other biological activities in addition to their effects on neurophysiological systems. For instance, Hopp et al. (1976) found that harmine exhibited significant anti-trypanosomal activity against *Trypanosoma lewisii*. This finding may explain the use of ayahuasca in mestizo ethnomedicine as a prophylactic against malaria and internal parasites (Rodriguez et al., 1982). Certain β-carbolines are known to exert mutagenic or co-mutagenic effects and the mechanism responsible may be related to their interactions with nucleic acids (Hayashi et al., 1977; Umezawa et al., 1978). The UV light-activated photocytotoxic and photogenotoxic activity of some β-carbolines has also been reported (McKenna & Towers, 1981; Towers & Abramosky, 1983).

8. Recent biomedical investigations of ayahuasca

Although achieving some notoriety in North American literature through the popular press and the writings of Burroughs and Ginsberg (1963), the psychological and physiological phenomena induced by ayahuasca have received little rigorous study. Various travelers to the Amazon have reported their own first hand experiences with ayahuasca (Weil, 1980; Davis, 1996), while both formal and informal ethnographic narratives have excited the public imagination (Lamb, 1971; Luna & Amaringo, 1991). Interest in the exotic origins and effects of ayahuasca have attracted a steady stream of North American tourists, often enticed by articles and advertisements in popular and New Age magazines (Krajick, 1992; Ott, 1993). Concern over possible adverse health effects resulting from the use of ayahuasca by such naive travelers has recently been expressed by a noted authority on mestizo ayahuasca use (Dobkin de Rios, 1994). These concerns are in marked contrast to testimonials of improved psychological and moral functioning by the adherents of the syncretic hoasca churches in Brazil.

The individuals who are attracted to the UDV seem to belong to a somewhat more educated socioeconomic class than those who join the Santo Daime (Grob et al., 1996). Of the ~7000 members of the UDV in Brazil, perhaps 5–10% are medical professionals, among them physicians, psychiatrists, psychologists, chiropractors, and homeopathic physicians. Most of these individuals are fully aware of the psychologically beneficial aspects of the practice and evince a great interest in the scientific study of hoasca, including its botany, chemistry, and pharmacology. The medically educated members can discuss all of these aspects with sophistication equal to that of any US-trained physician or other medical professional. At the same time, they do have a genuine spiritual reverence for the hoasca tea and the experiences it evokes. The UDV places a high value on the search for scientific truth and sees no conflict between science and religion; most members of the UDV express a strong interest in learning as much as possible about how the tea acts on the body and brain. As a result of this unique circumstance, the UDV presents an ideal context in which to conduct a biomedical investigation of the acute and long-term effects of hoasca/ayahuasca.

8.1. The "Hoasca Project"

Due to a fortunate combination of circumstances, my colleagues and I were invited to conduct such a biomedical investigation of long-term hoasca drinkers by the Medical Studies section of the UDV (Centro de Estudos Medicos). This study, which was conducted by an international consortium of scientists from Brazil, the United States, and Finland, was financed through private donations to various nonprofit sponsoring groups, notably Botanical Dimensions, which provided major funding, the Heffter Research Institute, and the Multidisciplinary Association for Psychedelic Studies. Botanical Dimensions is a nonprofit organization dedicated to the study and preservation of ethnomedically significant plants, and the Multidisciplinary Association for Psychedelic Studies (http://www.MAPS.org) and the Heffter

Research Institute (http://www.Heffter.org) are nonprofit organizations dedicated to the investigation of the medical and therapeutic uses of psychedelic agents. The field phase of the study was conducted during the summer of 1993 at one of the oldest UDV temples, the Nucleo Caupari located in the Amazonian city of Manaus, Brazil. Subsequent laboratory investigations took place at the respective academic institutions of some of the principle investigators, including the Department of Psychiatry, Harbor UCLA Medical Center, the Department of Neurology, University of Miami School of Medicine, the Department of Psychiatry, University of Rio de Janeiro, Department of Internal Medicine, University of Amazonas Medical School, Manaus, and the Department of Pharmaceutical Chemistry, University of Kuopio, Finland. This study has since become anecdotally known as "the Hoasca Project" in subsequent presentations of the results in various public venues.

Because this study was the first of its kind, there was virtually no preexisting data on the objective measurement of the physical and psychological effects of ayahuasca in human subjects. As a result, this study was in some respects a pilot study; its primary objectives were modest, representing an effort to collect a basic body of data, without attempting to relate the findings to either possible detrimental effects of ayahuasca, or to possible therapeutic effects. The study had 4 major objectives:

- Assessment of acute psychological and physiological effects of hoasca in human subjects;
- Assessment of serotonergic functions in long-term users of hoasca tea;
- Quantitative determination of active constituents of hoasca teas in plasma;
- Quantitative determination of active constituents of hoasca teas.

Most of these objectives were achieved, and the results have been published in various peer-reviewed scientific journals (Callaway et al., 1994, 1996, Grob et al., 1996; Callaway et al., 1999). Some key findings are summarized briefly below.

8.2. Assessment of acute and long-term psychological effects of hoasca teas

The subjects in all of the studies consisted of a group of 15 healthy, male volunteers, all of whom had belonged to the UDV for a minimum of 10 years, and who ingested hoasca once every 2 weeks, on average, in the context of the UDV ritual. None of the subjects actively used tobacco, alcohol, or any drugs other than hoasca. For some comparative aspects of the study, a control group of 15 age-matched males was also used; these individuals were recruited from among the friends and siblings of the volunteer subjects, and like them, were local residents of Manaus having similar diets and socioeconomic status. None of the control subjects were members of the UDV, and none had ever ingested hoasca tea.

The psychological assessments, administered to both groups, consisted of structured psychiatric diagnostic interviews, personality testing, and neuropsychological evaluations. Measures administered to the UDV hoasca drinkers, but not to the hoasca-naive group, included semistructured and open-ended life story interviews. A phenomenological assessment of the altered state elicited by hoasca was quantified using the Hallucinogen Rating Scale developed by Dr. Rick Strassman in his work with DMT and psilocybin in human subjects (Strassman et al., 1994).

The UDV volunteers showed significant differences from the hoasca-naive subjects in the Tridimensional Personality Questionnaire and the WHO-UCLA Auditory Verbal Learning Test. The Tridimensional Personality Questionnaire assesses 3 general areas of behavior, viz., novelty-seeking, harm avoidance, and reward dependence. With respect to novelty-seeking behaviors, UDV members were found to have greater stoic rigidity versus exploratory excitability, greater regimentation versus disorderliness, and a trend toward greater reflection versus impulsivity; but there was no difference between the groups on the spectrum between reserve and extravagance. On the harm reduction scale, UDV subjects had significantly greater confidence versus fear of uncertainty and trends toward greater gregariousness versus shyness and greater optimism versus anticipatory worry. No significant differences were found between the 2 groups in criteria related to reward dependence.

The 15 UDV volunteers and the control subjects were also given the WHO-UCLA Auditory Learning Verbal Memory Test. Experimental subjects performed significantly better than controls on word recall tests. There was also a trend, although not statistically significant, for the UDV subjects to perform better than controls on number of words recalled, delayed recall, and words recalled after interference.

The Hallucinogen Rating Scale, developed by Strassman et al. (1994) for the phenomenological assessment of subjects given intravenous doses of DMT, was administered to the UDV volunteers only (since control subjects did not receive the drug). All of the clinical clusters on the HRS were in the mild end of the spectrum compared with intravenous DMT. The clusters for affect, intensity, cognition, and volition were comparable to an intravenous DMT dose of 0.1–0.2 mg/kg, and the cluster for perception was comparable to 0.1 mg/kg iv DMT; the cluster for somatesthesia was less than the lowest dose of DMT measured by the scale, 0.05 mg/kg.

The most striking findings of the psychological assessment came from the structured diagnostic interviews and the semistructured open-ended life story interviews. The Composite International Diagnostic Interview was used for the structured diagnostic interview. None of the UDV subjects had a current psychiatric diagnosis, whereas 2 of the control subjects had an active diagnosis of alcohol misuse and

hypochondriasis. Only 1 subject among the controls had a past psychiatric disorder that was no longer present; an alcohol misuse disorder that had remitted 2 years previously. However, prior to membership in the UDV, 11 of the UDV subjects had diagnoses of alcohol misuse disorders, 2 had past major depressive disorders, 4 had past histories of drug misuse (cocaine and amphetamines), 11 were addicted to tobacco, and 3 had past phobic anxiety disorders. Five of the subjects with a history of alcoholism also had histories of violent behavior associated with binge drinking. All of these pathological diagnoses had remitted following entry into the UDV. All of the UDV subjects interviewed reported the subjective impression that their use of hoasca tea within the context of the UDV had led to improved mental and physical health and significant improvements in interpersonal, work, and family interactions.

8.3. Assessment of the acute physiological effects of hoasca tea

The major focus of the biochemical and physiological measurements carried out for the study was on the acute effects subsequent to consuming hoasca tea. One of the objectives was simply to measure the effects of hoasca on standard physiological functions, such as heart rate, blood pressure, and pupillary diameter, subsequent to ingestion (Callaway et al., 1999). We found that all of these responses were well within normal parameters. Hoasca, not surprisingly, caused an increase in pupillary diameter from baseline (predose) levels of 3.7 to ~4.7 mm at 40 min, which continued to 240 min after ingestion, at which point measurements were discontinued. Respiration rate fluctuated throughout the 240 min, from a low of 18.5 at baseline to a high of 23 at 100 min. Temperature rose from a baseline low of 37 °C at baseline to a high of 37.3 °C at 240 min (although the ambient temperature also increased comparably during the course of the experiments, which were conducted from 10:00 to 16:00 h). Heart rate increased from 71.9 bpm at baseline to a maximum of 79.3 bpm by 20 min, decreased to 64.5 bpm by 120 min, then gradually returned toward basal levels by 240 min. There was a concomitant increase in blood pressure; both systolic and diastolic pressure increased to maxima at 40 min (137.3 and 92.0 mm Hg, respectively) over baseline values (126.3 and 82.7 mm Hg, respectively) and returned to basal values by 180 min. We also measured neuroendocrine response for plasma prolactin, cortisol, and growth hormone; all showed rapid and dramatic increases over basal values from 60 (cortisol) to 90 (growth hormone) to 120 min (prolactin) after ingestion. The observed response is typical of serotonergic agonists, and is comparable to the values reported by Strassman and Qualls (1994) in response to injected DMT. In our study, the neuroendocrine response to oral DMT was delayed by a factor of 4 or 5 compared with the almost immediate (<15 min) response to injected DMT.

8.4. Characterization of the pharmacokinetics of hoasca alkaloids in human subjects

The fourth objective of the study was to measure pharmacokinetic parameters of the hoasca alkaloids in plasma following ingestion of hoasca tea and to correlate this to the amounts of alkaloids ingested (Callaway et al., 1996, 1999). The UDV collaborators held a special "preparo" to prepare the sample of hoasca that was used for all subjects in the study. The mestres confirmed the activity in the usual manner, via ingestion, and pronounced it active and suitable for use in the study. Subsequent analysis by high-performance liquid chromatography found the tea to contain (in mg/mL): harmine, 1.7; harmaline, 0.2; THH, 1.07; and DMT 0.24. Each subject received an aliquot of tea equivalent to 2 mL/kg body weight, which was consumed in a single draught. Based on the average body weight (74.2 ± 11.3 kg), the average dose of tea was 148.4 ± 22.6 mL, containing an average of 35.5 mg DMT, 158.8 mg THH, 29.7 mg harmaline, and 252.3 mg harmine. These doses are above the threshold level of activity for DMT as a psychedelic, and for harmine and THH as MAO inhibitors; harmaline is essentially a trace constituent of hoasca tea (Callaway et al., 1996, 1997).

Only 12 of the 15 volunteers had sufficient plasma levels of DMT to permit pharmacokinetic measurements, possibly due to early emesis during the course of the session. Of these, the maximum plasma concentration (C_{max}; 15.8 ng/mL) occurred at 107 min after ingestion, while the half-life ($T_{1/2}$) was 259 min. THH was measured in 14 of the 15 subjects; the C_{max} was 91 ng/mL, which was reached at 174 min. This compound displayed a prolonged half-life of 532 min, in contrast to harmine, which had a half-life of 115.6 min. The C_{max} for harmine and harmaline was 114.8 and 6.3 ng/mL, respectively, and time of maximum concentration (T_{max}) was 102 and 145 min, respectively. The $T_{1/2}$ for harmaline could not be measured (Callaway et al., 1999).

This study was conceived because of the need to collect some basic data on the physiological and pharmacokinetic characteristics of hoasca, since none had existed previously. The conclusions to be drawn from the results, if any, are interesting and potentially significant, particularly in that these findings may offer a physiological rationale for the marked improvements in psychological health that are correlated with long-term hoasca use. Not surprisingly, the highest plasma concentrations of DMT correlated with the most intense subjective effects; however, the psychological measurement (Hallucinogen Rating Scale) indicated that comparable plasma levels of injected DMT in Strassman and Qualls' (1994) study resulted in a more intense subjective experience than the effects reported from the hoasca tea. One possible explanation is that THH, by acting as a 5-HT reuptake inhibitor, may have resulted in a greater availability of 5-HT at the synapse, and this may have competed with DMT for occupancy at serotonergic synapses. Alternatively, the rapid increase in brain levels of DMT following intra-

venous administration may result in a more intense subjective effect compared with that experienced when DMT is absorbed more slowly following oral administration.

Another point worthy of remark is that the activity of THH in hoasca is apparently more a function of its inhibition of 5-HT uptake than to its MAOI action. THH is a poor MAOI compared with harmine ($EC_{50} = 1.4 \times 10^{-5}$ M for THH vs. 8×10^{-8} M for harmine), and while the plasma levels for harmine are well above the EC_{50} values, those for THH are well below the EC_{50} value for this compound as an MAOI.

8.5. Assessment of serotonergic functions in long-term users of hoasca

Another objective of the study was to investigate whether long-term use of hoasca resulted in any identifiable "biochemical marker" that was correlated with hoasca consumption, particularly with respect to serotonergic functions, since the hoasca alkaloids primarily affect functions mediated by this neurotransmitter. Ideally, such a study could be carried out on postmortem brains; since this was not possible, we settled on looking at serotonin transporter sites in blood platelets, using [^{3}H]-citalopram to label the transporters in binding assays (Table 1). The up- or down-regulation of peripheral platelet recognition sites has been proposed to be indicative of similar biochemical events occurring in the brain, although there is some controversy about the correlation between platelet transporter changes and changes in central nervous system transporters in patients receiving antidepressant medications (Stahl, 1977; Pletscher & Laubscher, 1980; Rotman, 1983);. However, platelet-binding assays were deemed suitable for the purposes of our study, as our objective was not to resolve this controversy but simply to determine if some kind of long-term biochemical marker could be identified. Neither did we postulate any conclusions about the possible "adverse" or "beneficial" implications of such a marker, if detected. We conducted the assays on platelets collected from the same group of 15 volunteers after they had abstained from consuming the tea for a period of 1 week. We also collected platelet specimens from the age-matched controls who were not hoasca drinkers. We were surprised to find a significant up-regulation in the density of the citalopram binding sites in the hoasca drinkers compared with control subjects. Although the hoasca drinkers had a higher density of transporters (B_{max} = 993 fmol/mg protein in hoasca drinkers vs. 724 fmol/mg protein in matched controls; cf. Table 1), there was no change in their affinity for the labeled citalopram binding site. The significance of this finding, if any, is unclear. There is no other pharmacological agent that is known to cause a similar up-regulation, although chronic administration of 5-HT uptake inhibitors has been reported to *decrease* both B_{max} (the density of binding sites) (Hrinda, 1987) and 5-HT transporter mRNA in rat brain (Lesch et al., 1993). Increases in B_{max} for the uptake site in human platelets have been correlated with old age (Marazziti et al., 1989) and also to the dark phase of the circadian cycle in rabbits (Rocca et al., 1989). It has been speculated (Marazziti et al., 1989) that up-regulation of 5-HT uptake sites in the aged may be related to the natural course of neuronal decline. Although our sample size was limited, we found no correlation with age, and the mean age of the sample was 38 years. Also, none of our subjects showed evidence of any neurological or psychiatric deficit. In fact, in view of their exceptionally healthy psychological profiles, one of the investigators speculated that perhaps the serotonergic up-regulation is associated, not simply with age, but with "wisdom"—a characteristic often found in the aged, and in many hoasca drinkers.

Another interesting self-experiment related to this finding was carried out by one of the investigators, Jace Callaway, following his return to Finland after the field phase of the study was completed. Dr. Callaway has access to single photon emission computerized tomography scanning facilities in the Department of Pharmacology at the University of Kuopio. Suspecting that the causative agent of the unexpected up-regulation might be THH, Dr. Callaway took single photon emission computerized tomography scans of his own brain 5-HT uptake transporters prior to beginning a 6-week course of daily dosing with THH, repeating the scan after the treatment period. He found that the density of central 5-HT receptors in the prefrontal cortex had increased; when he discontinued THH, their density gradually returned to previous levels over the course of several weeks. While this experiment only had 1 subject, if it is indicative of a general effect of THH that can be replicated and confirmed, the implications are potentially significant. A severe *deficit* of 5-HT uptake sites in the frontal cortex has been found to be correlated with aggressive disorders in violent alcoholics (Tiihonen et al., 1997; Hallikainen et al., 1999); if THH is

Table 1
Age and kinetic parameters of platelet 5-HT uptake activity as measured by [^{3}H]-citalopram for tea drinkers and controls (from Callaway et al., 1994)

Tea drinkers			Controls		
Age (years)	B_{max} (fmol/mg protein)	K_d (nM)	Age	B_{max} (fmol/mg protein)	K_d (nM)
28	1179	2.91	21	1022	2.29
30	914	3.01	24	458	1.63
35	971	2.97	24	593	2.39
36	1153	3.05	29	653	2.15
38	831	2.73	29	856	2.96
38	1470	4.88	36	888	2.89
39	688	1.48	38	718	2.46
40	790	2.17	39	674	3.38
40	847	3.11	43	766	2.17
40	1096	3.42	45	614	2.37
43	855	1.53			
46	771	2.70			
48	1346	3.05			
Mean = 38.5	993*	2.84	32.8	724*	2.47
SEM = 1.6	69	0.25	2.8	55	0.16

Unpaired student's *t*-test.
*P = 0.006.

able specifically to reverse this deficit, it may have applications in the treatment of this syndrome. These findings are especially interesting when viewed in the context of the psychological data collected in the hoasca study (Grob et al., 1996). The majority of the subjects had had a previous history of alcoholism, and many had displayed violent behavior in the years prior to joining the UDV; virtually all attributed their recovery and change in behavior to their use of hoasca tea in the UDV rituals. While it can be argued that their reformation was due to the supportive social and psychological environment found within the UDV, the finding of this long-term change in precisely the serotonin system that is deficient in violent alcoholism argues that biochemical factors may also play a role.

9. Current legal and regulatory status of ayahuasca

In recent decades, ayahuasca has been integrated into the religious practices of several syncretic religions in Brazil, where it is consumed as a sacrament in large group rituals, sometimes involving up to several hundred people. There are 3 primary Brazilian religions that employ ayahuasca as a sacrament: the UDV, the Santo Daime, and the Barquinia. The Santo Daime and UDV churches include about 10,000 members each throughout Brazil. The Barquinia is a smaller group, consisting of only a few hundred members, primarily in the Rio Branco area of the Amazon. The religious practices of these groups, and their sacramental use of ayahuasca, are sanctioned and legally permitted by CONFEN, the Brazilian regulatory agency that fulfills a role similar to the FDA and DEA in this country.

Ayahuasca and its source plants are not internationally prohibited under the 1971 Convention on Psychotropic Substances although one of them contains a Schedule I controlled substance, DMT. The source plant *B. caapi* contains β-carboline alkaloids, which are not listed as controlled substances either internationally or in the United States, so the controversy centers around the occurrence of DMT in the admixture plant, *P. viridis*, or other DMT-containing admixtures that may occasionally be substituted for *P. viridis*. Pure DMT is a Schedule I controlled substance under US law and is listed as a controlled substance under the International Convention on Psychotropic Substances. However, neither *P. viridis* nor any of the numerous other plant species that are known to contain DMT (cf. Smith, 1977; Ott, 1993) are specifically controlled or regulated as controlled substances. Many of these plants are freely and legally available from Internet web sites and elsewhere (Halpern & Pope, 2001). A recent opinion letter was issued by Herbert Schaepe, the Secretary of the Board for the United Nations International Narcotics Control Board (INCB) that has been helpful in clarifying at least the international legal status of plants containing DMT and other hallucinogens (Schaepe, 2001). In response to an inquiry by Mr. Lousberg, Chief, Inspectorate for Health Care of the Ministry of Public Health in the Netherlands regarding whether plant materials and their decoctions are covered by the Convention on Psychotropic Substances. The inquiry was sent by Mr. Lousberg. The opinion letter (dated 17 January 2001) states:

> No plants (natural materials) containing DMT are at present controlled under the 1971 Convention on Psychotropic Substances. Consequently, preparations (e.g., decoctions) made of these plants, including *ayahuasca*, are not under international control, and, therefore, not subject to any of the articles of the 1971 convention.

A copy of this letter was submitted in support of a suit filed by the North American chapter of the UDV seeking a preliminary injunction against attempts by the Department of Justice to suppress the group's religious use of hoasca (see below).

The UDV and Santo Daime churches that originated in Brazil now have legally incorporated American chapters that use *ayahuasca* in religious ceremonies. In November 2000, the US chapter of the UDV, based in Santa Fe, petitioned the Department of Justice in the US District Court for the District of New Mexico for the return of some 30 gal of its sacramental hoasca. This material had been seized by the DEA from the home of the UDV leader in May 1999. The UDV appealed for a special exemption to allow for the religious use of ayahuasca under the provisions of the Religious Freedom Restoration Act (see http://www.nvo.com/cd/nss-folder/pubfiles/PltnfsComplaint.pdf) (O Centro Espirita Beneficiente UDV et al., 2000). A favorable ruling by that court in 2002 allowed for the religious use of hoasca tea by the UDV Church (US District Court for the District of New Mexico: No CV 00-1647 JP/LRP (see "Federal Court Rules in Favor of Ayahuasca-Using Church," http://www.cognitiveliberty.org/dll/udv_pj_granted.htm; Center for Cognitive Liberty and Ethics, 2002a, 2002b). The government filed an emergency stay, and the favorable ruling was temporarily contravened (see http://www.nvo.com/cd/nss-folder/pubfiles/emerstay.htm) (Westlaw Citation # 31862699, 2002). In September 2003, a 3-judge review panel in the 10th US Circuit Court of Appeals in Denver again returned a ruling in favor of the UDV. The ultimate resolution of this case remains pending and may eventually require review by the US Supreme Court. A complete chronology of this case and links to relevant public documents filed in the process can be found on the web site of the Center for Cognitive Liberty and Ethics (http://www.cognitiveliberty.org/dll/udv_index.htm).

Although the ultimate outcome of the UDV's efforts to secure legal sanction for their religious use of ayahuasca may entail years of litigation, it is clear that increasing numbers of people in the United States are using ayahuasca on a regular basis in rituals and in religious ceremonies. The growing use of ayahuasca for religious (and recreational) purposes in the United State has public health implications. Additionally, the growth of "ayahuasca tourism" in the Amazon is attracting increasing numbers of American and

foreign travelers (Dobkin de Rios, 1994). The uncertain legal and regulatory status under US law of ayahuasca, or more specifically, its DMT-containing component, *P. viridis*, also may pose challenges for the eventual pursuit of clinical investigations of ayahuasca under an Investigational New Drug (IND) application.

10. The rationale for clinical studies of ayahuasca

There are currently 2 primary rationales supporting the need for controlled clinical investigations of ayahuasca:

1. Ayahuasca is becoming increasingly popular in this country for both religious and recreational purposes. The currently pending legal challenges to the religious use of ayahuasca, described above, have so far resulted in rulings favorable to the UDV, the religious organization that has petitioned the government for the legal right to employ ayahuasca as a sacrament under the Religious Freedom Restoration Act. This raises the possibility that religious use of ayahuasca may eventually become legally sanctioned in the United States. Yet, increasing use of ayahuasca, whether ritually or recreationally, has public health implications. Despite a long history and tradition of indigenous use that indicates that the preparation can be used safely, very little actual clinical data have been accumulated, which provides a scientific basis for the notion that ayahuasca is safe for human use. Such studies are needed to inform the currently pending legal challenges to its use for religious purposes. In addition, such studies would provide health professionals with a body of information as to its safety, possible side effects, potential drug interactions, potential for adverse reactions, and possible toxicity. Such data may be essential for health providers in the event they are required to treat individuals who have ingested ayahuasca.
2. A second rationale is that ayahuasca may have therapeutic applications, and these require investigation within the context of well-designed clinical studies. Ideally, such studies should be conducted under an FDA-approved IND protocol, together with whatever regulatory permissions are required from the DEA and an institutional IRB affiliated with the institution where the study is to be conducted.

11. Ayahuasca clinical research to date

11.1. Key findings from the "Hoasca Project"

Grob et al. (1996) studied the short- and long-term toxicity profile of hoasca use in the Brazilian UDV. They reported that there was no evidence of acute toxicity during the sessions or of long-term toxicity or other adverse health effects. Hoasca, in the context of the UDV, is consumed regularly by men and women ranging in ages from 13 to 90 and appears to be safe. Many of the older members of the UDV, who are now well into their 80s, have used hoasca regularly since their teenage years and are remarkable for their mental acuity, lack of serious disease history, and physical vigor (Callaway et al., 1999). Psychological screening tests and evaluations have found no evidence of long-term mental or cognitive impairment in long-term hoasca drinkers (UDV members). In fact, most members performed slightly better than control subjects on measures of cognitive function, verbal facility and recall, mathematical ability, motivation, and emotional well-being and personality adjustment (Grob et al., 1996). The study protocol included the psychological assessment of 15 long-term UDV members utilizing hoasca as a legal, psychoactive sacrament, as well as 15 matched controls with no prior history of hoasca ingestion. Measures administered to both groups included structured psychiatric diagnostic interviews, personality testing, and neuropsychological evaluation. Overall assessment revealed high functional status. Implications of these findings and the need for further investigation are discussed (Grob et al., 1996). In addition to psychological parameters, the study included pharmacokinetic measurements of the major active alkaloids (Callaway et al., 1999). Three key findings have emerged from the study, which has come to be known as the "Hoasca Project." These have been described in detail in the previous sections.

12. Potential therapeutic applications of ayahuasca

Two kinds of evidence argue that ayahuasca may have therapeutic applications. A considerable body of anecdotal evidence, coupled with a long history of ethnomedical use, indicates that ayahuasca may be useful for the treatment of abuse disorders, such as alcoholism and substance abuse, as well as for physical maladies such as cancer. In addition, the results of a 1993 biomedical study of long-term members of the UDV in Brazil have provided data that may indicate directions for the future direction of clinical studies of ayahuasca.

12.1. Treatment of alcoholism and substance abuse

In the right circumstances, meaning within appropriate supportive settings and social milieus such as the Brazilian UDV, regular and long-term hoasca use may result in profound, lasting, and positive behavioral and lifestyle changes. The most dramatic example is the finding that, prior to their joining the UDV church, most members that were interviewed had histories of alcoholism, substance abuse, domestic violence, and other maladaptive behaviors and lifestyles. These dysfunctional behaviors resolved themselves on subsequent induction into the UDV and regular use of the hoasca sacrament (Grob et al., 1996).

Dr. Micheal Winkelman coined the term *psychointegrator plants* for describing the psychological benefits of ayahuasca administration (Winkelman, 1995). There is increasing interest in shamanistic forms of healing and ayahuasca's reputed psychological, medical, and spiritual benefits have stirred interest among North American scientists, physicians, and the intellectual lay public. Many North Americans and Europeans travel to the Amazon to participate in ayahuasca ceremonies led by traditional shamans (also called *ayahuasqueros*) (Dobkin de Rios, 1994). A recent report on BBC radio (http://news.bbc.co.uk/2/hi/programmes/crossing_continents/3243277.stm) discusses the successful use of ayahuasca to treat cocaine addition at a Peruvian clinic. The results of the Hoasca study, described above, also argue that ayahuasca treatment, within an appropriate psychotherapeutic context, may also be applicable to the treatment of alcoholism.

12.2. Treatment of serotonergic deficits

Coupled with these positive psychological and behavioral changes, was an unexpected finding. Apparently, regular ayahuasca use results in a long-term modulation of serotonin systems in the brain; specifically, that populations of serotonin transporters exhibit an elevated density in platelets and in the brain, an effect that may be due to one of the β-carbolines in the ayahuasca mixture (see Table 1). The parameter measured in the hoasca study was an elevation in the density of 5-HT transporters in platelets, and did not directly measure brain levels. However, psychopharmacologists have long-used platelets as a peripheral marker of similar biochemical events occurring within the brain (Stahl, 1977; Pletscher & Laubscher, 1980). The serotonin transporter is the membrane bound protein in serotonin containing neurons that manages the reuptake of this neurotransmitter from the synapse, and is the site of action of Prozac and other selective serotonin reuptake inhibitors. Hence, the 5-HT transporter is intimately involved with syndromes such as depression and other mood disorders, suicidal behavior, etc. (Coccaro et al., 1989; Roy et al., 1991; Tiihonen et al., 1997). Callaway et al. (1994) have hypothesized that the elevation of 5-HT transporters seen with long-term ayahuasca use and the positive behavioral changes are linked. Work by other investigators have found severe deficits in the density of these transporters in people with behavioral disorders, especially patients with a history of alcoholism linked with violence, and in those prone to suicidal behavior (Tiihonen et al., 1997; Hallikainen et al., 1999). Based on our finding that 5-HT transporters are significantly elevated in long-term users of ayahuasca, we speculate that ayahuasca may be able to reverse these deficits over time (Callaway et al., 1994).

Serotonergic deficits have been linked to a variety of functional, behavioral, and neurodegenerative disorders, ranging from alcoholism to depression, autism, schizophrenia, attention deficit hyperactivity disorder, and senile dementias. Recent advances in the cloning of neurotransmitter transporter genes and the development of transporter-deficient knockout mice have given neurobiologists powerful new tools for investigating the role of monoamine transporters in the regulation of neurotransmitter functions and their potential links to neurobiological and behavioral disorders (Blakely & Bauman, 2000). Genetic polymorphisms in transporter genes and their expression have been linked to anxiety states (Lesch et al., 1996), autism (Cook et al., 1997), affective disorders (Sher et al., 2000), and alcohol and cocaine abuse (Little et al., 1998). The results from the UDV study indicate that one or more constituents in ayahuasca may be able to modulate gene expression for the serotonin transporters and that the long-term changes observed are correlated with positive behavioral changes. If these preliminary findings are borne out by more rigorous clinical studies neurobiologists may have a new compound that may be applied to the study of serotonin transporter expression and regulation. Eventually, compounds developed form this work may yield new treatments for substance abuse and psychiatric disorders.

12.3. Immune modulation

The third piece of evidence that has emerged is more anecdotal than scientific, but is nonetheless intriguing. This is that ayahuasca may have significant immunomodulatory effects. A number of users of ayahuasca in North America have reported that they have experienced remissions of cancers and other serious illnesses in conjunction with regular use of the tea (Topping, 1998). Additionally, the longevity, physical vigor, and mental acuity evidenced by many *ayahuasqueros* in Peru has long been noted as remarkable. Many of these shamans living in developing nations are well into their 70s, 80s, and 90s and yet appear to live out their years in a state of physical and mental health that would be the envy of many in the so-called developed countries. Certainly, some of this is due to dietary factors and a physically vigorous and demanding lifestyle; but some of it may be the result of exceptional immune functions due to their years of working with ayahuasca. Many of these practitioners are accustomed to consuming it several times a week in the performance of their healing practices and have done so most of their adult lives (Luna, 1984a, 1984b, 1986). In the context of mestizo folk medicine and indigenous shamanic practice, ayahuasca has long enjoyed a reputation as a healing medicine, with properties that transcend its acute, psychological effects. These properties may be attributable to its immune potentiating effects, if they are found to exist. Although the evidence at this point is anecdotal and speculative, this hypothesis can be easily resolved empirically. Many plants are known to possess immune-potentiating properties and both in vivo and in vitro methodologies have been developed to measure the immune-modulating properties of plant extracts (Wilasrusmee et al., 2002a, 2002b; Klein et al., 2000). A recent study characterized the immunopotentiating and antitumor properties of another

well-known indigenous hallucinogen, the peyote cactus (Franco-Molina et al., 2003).

12.4. Clinical studies by Spanish investigators

Subsequent to the completion of the "Hoasca Project" and the publication of its results, a group of investigators at the Universitat Autonoma de Barcelona in Spain have conducted clinical investigations examining various aspects of the pharmacology of ayahuasca in healthy volunteers. Using a freeze-dried, preparation of Brazilian ayahuasca standardized to contain known quantities of DMT and β-carboline alkaloids, these investigators initially conducted safety assessments and dose-response studies in a small sample of healthy volunteers (Riba et al., 2001a, 2001b). After documenting physiological and subjective changes induced by the lyophilized ayahuasca preparation, the investigators concluded that it was well tolerated and presented an acceptable side-effect profile. Subsequent investigations focused on characterization of the pharmacokinetic profiles and effects on cardiovascular parameters (Yritia et al., 2002; Riba et al., 2003). Electrophysiological and psychological effects were investigated using topographic pharmaco-EEG mapping, (Riba et al., 2002a), while effects on the startle reflex and sensory and sensorimotor gating were assessed using suppression of the P50 auditory evoked potential and prepulse inhibition of the startle reflex (Riba et al., 2002b). In contrast to studies with other psychedelic agents, such as psilocybin, these investigators found a decremental effect on sensory gating as measured by the auditory evoked potential and a lack of effect on sensorimotor gating measured by prepulse inhibition. More relevant, perhaps, to the immediate concerns regarding the safety of using ayahuasca in humans are that no serious adverse reactions, evidence of toxicity, or lack of tolerability was experienced by the subjects over the series of studies.

13. The pathway to clinical studies

There exists both a need and an opportunity to investigate these questions in a more rigorous scientific framework that conforms to currently accepted medical paradigms for conducting clinical research. The evidence from the Brazilian UDV study is intriguing, but not compelling; the sample of subjects studied was small, and given the exigencies of conducting this kind of research in a foreign country and a nonclinical venue, inevitably many factors could not be controlled. Additionally, the question of immune potentiation has yet to be investigated in any manner beyond anecdotal reports and casual observations. The safety and efficacy of ayahuasca has not been definitively verified, and safety concerns in the United States grow as use of ayahuasca as a spiritual sacrament grows. The American medical community will insist, rightly so, on more rigorous investigations in the form of controlled clinical trials before any possible therapeutic investigations can proceed.

A number of hurdles must be overcome before ayahuasca can be studied in a clinical context in the United States. First of all, a clarification of its legal status is needed to determine what kinds of permissions, if any, are needed from the DEA and other relevant law-enforcement agencies to pursue clinical studies. Although the opinion issued by the Secretary for the UN Narcotics Control Board (Schaepe, 2001) indicates that neither the preparation nor the source plants are internationally regulated, the status of ayahuasca under US law remains in limbo. The UDV's case for an exemption for the sacramental use of hoasca under the Religious Freedom Restoration Act has received favorable rulings in 2 Federal courts and appeal to the Supreme Court is likely. It is to be hoped that a favorable resolution in this case will simplify the regulatory challenges to biomedical studies of ayahuasca.

13.1. Step I: preclinical study—phytochemistry and pharmacology

Assuming the regulatory dilemmas are eventually resolved, it remains unlikely that the FDA will approve an initial IND application for a clinical study without an extensive body of data derived from preclinical investigations. Although the clinical studies by Riba et al. (2001a, 2001b, 2002a, 2002b, 2003) and Yritia et al. (2002) have produced evidence that ayahuasca can be used safely in a clinical setting, FDA regulators may insist on generating preclinical data within the United States.

Ayahuasca has been poorly studied in animal models, and such a requirement on the part of the FDA could contribute to better quality research in subsequent clinical phases. Many of the questions relating to the understanding of ayahuasca's pharmacology can and should be addressed first in appropriate in vitro and animal models. The results of those preclinical studies will then be useful in terms of framing and focusing subsequent clinical studies.

Ayahuasca, like all botanical medicines, is a complex combination of hundreds, or perhaps even thousands, of biologically active compounds. Although there have been extensive phytochemical investigations of ayahuasca over the years, almost all of them have focused exclusively on the alkaloids, since they are the psychoactive constituents. Not even all of the alkaloids have been investigated thoroughly; for example, the pharmacology of the pyrrolidine alkaloids shihunine and dihydroshihunine in *B. caapi* remains a black box although the compounds were isolated over 30 years ago. Ayahuasca and its source plants are likely to contain flavonoids, tannins, lignans, saponins, volatile oils, and many of the other classes of secondary metabolites that are widespread in plants. All of these have enormous molecular diversity and all have been shown to posses an equally wide spectrum of pharmacological activities. None of those in ayahuasca, however, have been investigated from this perspective. Ad-

ditionally, much of the early phytochemical work and preclinical pharmacology on *Banisteriopsis* extracts was conducted over 30 years ago (Deuloufeu, 1967; O'Connell, 1969; Stull et al., 1971; Ferguson, 1972). There have been important advances in both phytochemical analysis methods and preclinical pharmacology since this work was carried out, and there is a need to revisit these questions using more current methodologies. A thorough phytochemical characterization of ayahuasca and its source plants then becomes the first order of business for any preclinical studies. Extracts of each plant should be made and fractionated using standard phytochemical procedures. The biological activities of the fractions can be further characterized using appropriate in vitro assays. These investigations will provide guidance for further studies in animal models and eventually in clinical trials; it may also detect activities, even toxicity, that is currently unsuspected or uncharacterized. It is important that these investigations be conducted using source plants of known provenance, documented by herbarium voucher collections, so that the data will be applicable to the development of a standardized preparation for eventual use in IND-authorized clinical studies.

Preclinical studies will also enable investigators to examine aspects of ayahuasca's pharmacology that may be relevant to its suspected therapeutic activity. For example, a recent in vitro investigation reported activities in *Banisteriopsis* extracts potentially relevant to the treatment of Parkinson's disease (Schwarz et al., 2003) in which the β-carboline components alone did not sufficiently account for all of the observed activity. Techniques, such as in vitro receptor binding and autoradiography, can be applied to characterize the effects of ayahuasca alkaloids and extracts on modulation of the serotonin transporter and other neurobiological functions. Similarly, questions about the possible immune-potentiating effects of ayahuasca can be initially addressed in animal or in vitro test systems. Relatively standard in vitro and in vivo techniques for measuring immune response have been applied to a variety of medicinal plants (cf. Klein et al., 2000; Wilasrusmee et al., 2002a, 2002b).

13.2. Step II: investigational new drug application

The phytochemical profiling and preclinical pharmacological and toxicological data generated in the preclinical phase will lay the foundation for the IND application.

The FDA has issued a draft of guidelines, still under review, for the development of botanical drugs (FDA/CDER, 2002). The current draft can be viewed at http://www.fda.gov/cder/guidance/1221dft.htm. The study medication to be developed must conform to these guidelines, in whatever form may be current at the time the application is submitted.

Ayahuasca as prepared and traditionally consumed in the Amazon is a decoction prepared from fresh plant materials that is consumed orally as a beverage. The suggested preparation under the IND protocol differs from the traditional preparation in that it will be a freeze-dried, aqueous extract made from fresh plant materials and administered in capsules. Although not "traditional," this preparation can be more easily standardized and is likely to have longer term stability than an aqueous decoction. Moreover, use of a freeze-dried, encapsulated, standardized preparation will make the clinical and preclinical results more readily comparable with the work of Riba et al. (2001a, 2001b). These investigators have set a standard of sorts since they are the only investigators so far to conduct clinical studies with ayahuasca. This extract can be standardized to contain a quantified amount of 3 of the known active alkaloids, viz., DMT, harmine, and THH. The methods will follow those described by Riba et al. (2001b) in the preparation of a freeze-dried extract for clinical study. These investigators reported that 611 g of a freeze-dried, yellowish powder was obtained from a 9.6-L batch of Brazilian ayahuasca, which was used in that study. This represents a solid matter content of 63.6 g/L of soluble solutes from the decoction process. In the present study, we suggest a slightly different methodology be adopted from that followed by Riba et al., in that freeze-dried decoctions of each plant should be prepared separately and quantitatively analyzed. The separate extracts can then be combined in appropriate proportions based on the analytical data, such that the final extract will contain known concentrations of the key alkaloids, harmine, THH, and DMT. This method should minimize the inherent variability created by generating different batches of ayahuasca and then lyophilizing the whole extract. Final volumes and alkaloid concentrations of the combined extracts can be adjusted, if necessary, using inert excipient materials such as lactose.

Advances in phytopharmaceutical technology over the past decade make the preparation of a standardized version of ayahuasca, in which the levels of active constituents are well-characterized, a relatively straightforward proposition. The challenge is to use methods that are similar to traditional indigenous methods of preparation, but carried out under a consistent and replicable protocol. To develop a replicable extraction and standardization procedure, some range-finding work will be needed using small batches of test material to determine the appropriate extraction parameters prior to scaling up to produce the test batch to be used in the study. Specifically, parameters such as the initial and final volumes of solvent (water) to be used, and the effect of boiling time, on the amount of soluble extractives and the alkaloid levels of the final, freeze-dried extract of each plant will need to be determined. Analytical methods, primarily thin-layer chromatography, and high-performance liquid chromatography can be used for the qualitative and quantitative characterization of each freeze-dried extract (McKenna et al., 1984; Callaway et al., 1996).

13.3. Step III: initial clinical studies—safety assessment

Once IND and IRB approvals have been obtained, the initial clinical study should focus on assessing safety in

normal, healthy volunteers. Specific therapeutic outcomes must be deferred to a subsequent, Phase II study in an appropriate patient population. The therapeutic applications to be addressed will in part be determined by the data generated in the animal models and in the initial clinical study in healthy volunteers. In many respects, the objective of this initial study will be to replicate the results of the study of Riba et al. (2001a, 2001b) to establish the safety and tolerability of the standardized ayahuasca preparation. An open-label (i.e., nonplacebo) dose escalation study design will be employed in a minimum of 12 subjects over a 12-week period, for a total of 6 doses per subject at 2-week intervals. In this respect, the proposed dosing protocol differs from that employed by Riba et al. (2001a, 2001b). In that study, subjects received a total of 4 doses of standardized extract ranging from 0 (placebo) to 1.0 mg/kg DMT/kg. Administration was single-blind, in that the doses were known to the investigators for safety reasons, but subjects were told they would receive 1 of 4 doses at random.

In the IND study, we are proposing to administer a total of 6 doses at 2-week intervals over the 12-week course of the study. As with Riba et al.'s study, randomized low, medium, and high doses will be used in a single-blind design. This time course and dosing regimen will be used because it corresponds more closely to the anticipated therapeutic dosing regimen in a subsequent efficacy trial. Table 2 summarizes the proposed clinical guidelines and parameters to be assessed in this initial Phase I safety study.

Table 2
Proposed clinical guidelines and assessment parameters for a Phase I safety study of ayahuasca

Number of subjects	12–15 evaluable subjects (15–25 subjects initially screened)
Subject characteristics	Normal, healthy volunteers, male and female, ages 20–50
Preparation	Subject education, informed consent
Inclusion criteria	Naïve to effects of ayahuasca but prior experience with hallucinogens. No exposure to hallucinogens within previous 6 months; abstinence from alcohol, cannabis, or other illegal drugs for minimum 2 weeks prior to initiation of the study
Exclusion criteria	Evidence of psychological or neurological abnormalities; evidence of abnormal liver, GI, or renal functions or disease; evidence of hypertension or abnormal CV function; evidence of HIV or other infections; pregnancy; evidence of concomitant use of prescription medications
Compliance monitoring	Periodic random urinalysis applied at intervals throughout the study
Dosing regimen	Six total doses administered at 2-week intervals; randomized low, medium, and high doses will be administered in a single-blind model
Controls	Control values will be baseline values for measured parameters, prior to initiation of the study
Measured parameters	Adverse events; CBC; chemistry panel (SMAC-24); EKG; blood pressure; heart rate; temperature; EEG spectral analysis; SF-36 Medical Outcomes Survey; psychological evaluations (see below); platelet 5-HT transporter radioligand binding profiles; plasma levels of DMT, THH, harmine, and harmaline; T/B/NK cell panel; NK cell activity
Psychological evaluations	Hallucinogen rating scale scored at 8 hr postsession; structured psychiatric evaluations

13.4. Step IV: subsequent clinical studies—efficacy of ayahuasca therapy for alcoholism

If the safety and tolerability of ayahuasca is confirmed in the initial study as we anticipate it will be, then the efficacy trial should focus on a clear therapeutic objective. Based on the data generated by the Hoasca study, the treatment of alcohol dependence would appear to be the most logical target. In the UDV subjects in that study (Grob et al., 1996), cessation of alcohol use was linked to ingestion of hoasca tea in a supportive social context and to long-term elevations in the density of platelet 5-HT transporters. Ideally, a clinical efficacy study would seek to replicate these conditions to the extent possible. Candidates with a history of alcohol dependence should be selected for the study. In addition, neuroimaging technologies could be applied to prescreen the subjects for the existence of central serotonin transporter deficits. Subjects found to display the deficits, along with a behavioral history of alcohol dependence, could then be selected for inclusion in the study. The subject's response over the course of the treatment could be assessed by monitoring several parameters, including (1) rates of relapse with respect to drinking; (2) changes in personality, attitudes, and behavior as assessed in psychiatric interviews and by the application of assessment instruments such as the Tridimensional Personality Questionnaire, and (3) long-term modulation (presumably, elevation) of serotonin transporter densities measured with single photon emission computerized tomography or similar appropriate neuroimaging methodology. If supported by the preclinical and initial clinical data, secondary outcome measures might also include the assessment of immune response.

14. Conclusion

The reputation of ayahuasca as a jungle medicine and shamanic psychedelic plant concoction is nearly legendary, but scientific investigations by a variety of investigators over several decades have succeeded in elucidating much of the botany, chemistry, pharmacology, and ethnography that underlies the legend. Clinical investigations of its possible applications in psychiatric practices or in other healing modalities have been few, and formidable legal and technical challenges must be faced and overcome before further studies of its potential as a medicine can be investigated in appropriate clinical settings. It is to be hoped that the completion of recent, albeit preliminary, clinical studies in Brazil and Spain, and the possible approval of its use in a religious context, will open the door to further rigorous scientific investigations of ayahuasca's potential to heal.

References

Airaksinen, M. M., & Kari, I. (1981). β-Carbolines, psychoactive compounds in the mammalian body. *Med Biol 59*, 21–34.

Allen, J. R. F., & Holmstedt, B. (1980). The simple β-carboline alkaloids. *Phytochemistry 19*, 1573–1582.

Anonymous (1855). Journal of a voyage up the Amazon and Rio Negro by Richard Spruce, San Carlos del Rio Negro, June 27, 1853. *Hooker J Bot Kew Garden Misc 6/7*.

Barker, S. A., Monti, J. A., & Christian, S. T. (1980). Metabolism of the hallucinogen *N*,*N*-dimethyltryptamine in rat brain homogenates. *Biochem Pharmacol 29*, 1049–1057.

Barker, S. A., Monti, J. A., & Christian, S. T. (1981). *N*,*N*-Dimethyltryptamine: an endogenous hallucinogen. *Int Rev Neurobiol 22*, 83.

Blakely, R. D., & Bauman, A. L. (2000). Biogenic amine transporters: regulation in flux. *Curr Opin Neurobiol 10*, 328–336.

Bloom, F., Barchus, J., Sandler, M., & Usdin, E. (Eds.). (1982). *β-Carbolines and Tetrahydroisoquinolines*. New York: Alan R. Liss.

Buckholtz, N. S., & Boggan, W. O. (1976). Effects of tetrahydro-β-carbolines on monoamine oxidase and serotonin uptake in mouse brain. *Biochem Pharmacol 25*, 2319–2321.

Buckholtz, N. S., & Boggan, W. O. (1977). Monoamine oxidase inhibition in brain and liver produced by B-carbolines: structure-activity relationships and substrate specificity. *Biochem Pharmacol 26*, 1991–1996.

Burroughs, W. S., & Ginsberg, A. (1963). *The Yage Letters*. San Francisco, CA: City Lights Books.

Callaway, J. C. (1988). A proposed mechanism for the visions of dream sleep. *Med Hypotheses 26*, 119–124.

Callaway, J. C., Airaksinen, M. M., McKenna, D. J., Brito, G. S., & Grob, C. S. (1994). Platelet serotonin uptake sites increased in drinkers of ayahuasca. *Psychopharmacology 116*, 385–387.

Callaway, J. C., Raymon, L. P., Hearn, W. L., McKenna, D. J., Grob, C. S., Brito, G. S., & Mash, D. C. (1996). Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with Ayahuasca. *J Anal Toxicol 20*, 492–497.

Callaway, J. C., McKenna, D. J., Grob, C. S., Brito, G. S., Raymon, L. P., Poland, R. E., Andrade, E. N., Andrade, E. O., & Mash, D. C. (1999). Pharmacokinetics of hoasca alkaloids in healthy humans. *J Ethnopharmacol 65*, 243–256.

Canessa, M., Jaimovich, E., & de la Fuente, M. (1973). Harmaline: a competitive inhibitor of Na^+ ion in the (Na^+/K^+)ATPase system. *J Membr Biol 13*, 263–282.

Center for Cognitive Liberty and Ethics (2002a). *Federal court rules in favor of ayahuasca-using church* [Press release]. Available at: http://www.cognitiveliberty.org/dll/udv_pj_granted.htm.

Center for Cognitive Liberty and Ethics (2002b). *Uniao do Vegetal (UDV) v. United States. Case Chronology as of Dec. 2002.* Available at: http://www.cognitiveliberty.org/dll/udv_index.htm.

Coccaro, E. F., Siever, L. J., Klar, H. M., Maurer, G., Cochrane, K., Cooper, T. B., Mohs, R. C., & Davis, K. L. (1989). Serotonergic studies in patients with affective and personality disorders. Correlates with suicidal and impulsive aggressive behavior. *Arch Gen Psychiatry 46*, 587–599 (Erratum in: Arch Gen Psychiatry).

Cook, E. H. Jr., Courchesne, R., Lord, C., Cox, N. J., Yan, S., Lincoln, A., Haas, R., Courchesne, E., & Leventhal, B. L. (1997). Evidence of linkage between the serotonin transporter and autistic disorder. *Mol Psychiatry 2*, 247–250.

Davis, W. (1996). *One River: Explorations and Discoveries in the Amazon Rainforest*. New York: Simon & Schuster.

de Sousa, R. C., & Grosso, A. (1978). Vasopressin-like effects of a hallucinogenic drug—harmaline—on sodium and water transport. *J Membr Biol 40*, 77–94.

Deuloufeu, V. (1967). Chemical constituents isolated from *Banisteriopsis* and related species. *Psychopharmacol Bull 4*, 17–18.

Dobkin de Rios, M. (1972). Visionary vine: psychedelic healing in the Peruvian Amazon. *Int J Soc Psychiatry 17*, 256–269.

Dobkin de Rios, M. (1973). Curing with *ayahuasca* in an urban slum. In M. Harner (Ed.), *Hallucinogens and Shamanism* (pp. 67–85). London: Oxford Univ. Press.

Dobkin de Rios, M. (1994, January). Drug tourism in the Amazon. *Omni*, 20.

FDA/CDER Center for Drug Evaluation and Research (2002). *Guidance for industry: botanical drug products.* Available at: http://www.fda.gov/cder/guidance/1221dft.htm.

Ferguson, G. G. (1972). Action of alkaloidal extract of *Banisteriopsis quitensis* on rabbit ileum. *J Pharm Sci 61*, 23–234.

Fischman, L. G. (1983). Dreams, hallucinogenic drug states, and schizophrenia: a psychological and biological comparison. *Schizophr Bull 9*, 73–94.

Franco-Molina, M., Gomez-Flores, R., Tamez-Guerra, P., Tamez-Guerra, L., Castillo-Leon, L., & Rodriguez-Padilla, C. (2003). In vitro immunopotentiating properties and tumour cell toxicity induced by *Lophophora williamsii* (Peyote) cactus methanolic extract. *Phytother Res 17*, 1076–1081.

Grob, C. S., McKenna, D. J., Callaway, J. C., Brito, G. S., Neves, E. S., Oberlender, G., Saide, O. L., Labigalini, E., Tacla, C., Miranda, C. T., Strassman, R. J., & Boone, K. B. (1996). Human pharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *J Nerv Ment Dis 184*, 86–94.

Hallikainen, T., Saito, T., Lachman, H. M., Volavka, J., Pohjalainen, T., Ryynanen, O. P., Kauhanen, J., Syvalahti, E., Hietala, J., & Tiihonen, J. (1999). Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. *Mol Psychiatry 4*, 385–388.

Halpern, J. H., & Pope, H. G. Jr. (2001). Hallucinogens on the Internet: a vast new source of underground drug information. *Am J Psychiatry 158*, 481–483.

Hashimoto, Y., & Kawanishi, K. (1975). New organic bases from Amazonian *Banisteriopsis caapi*. *Phytochemistry 14*, 1633–1635.

Hashimoto, Y., & Kawanishi, K. (1976). New alkaloids from *Banisteriopsis caapi*. *Phytochemistry 15*, 1559–1560.

Hayashi, K., Nagao, M., & Sugimura, T. (1977). Interactions of harman and norharman with DNA. *Nucleic Acids Res 4*, 3679–3685.

Heckenberger, M. J., Kuikuro, A., Kuikuro, U. T., Russell, J. C., Schmidt, M., Fausto, C., & Franchetto, B. (2003). Amazonia 1492: pristine forest or cultural parkland?. *Science 301*, 1710–1714.

Ho, B. T. (1979). Pharmacological and biochemical studies with β-carboline analogs. In W. B. Essman, & L. Vazelli (Eds.), *Current Developments in Psychopharmacology*, *vol. 4* (pp. 215–247). New York: Spectrum Press.

Hopp, K. H., Cunningham, L. V., Bromel, M. C., Schermeister, L. J., & Kahlil, S. K. W. (1976). In vitro antitrypanosomal activity of certain alkaloids against *Trypanosoma lewisi*. *Lloydia 39*, 375–377.

Hrinda, P. D. (1987). Regulation of high- and low-[^{3}H]-imipramine sites in rat brain by chronic treatment with antidepressants. *Eur J Pharmacol 138*, 159–168.

Johnson, D. D., Fisher, T. E., Tuchek, J. M., & Crawford, R. D. (1984). Pharmacology of methyl- and propyl-B-carbolines in a hereditary model of epilepsy. *Neuropharmacology 23*, 1015–1017.

Kawanishi, K., Uhara, Y., & Hasimoto, Y. (1982). Shinunine and dihydroshihunine from *Banisteriopsis caapi*. *J Nat Products 45*, 637–638.

Klein, C., Sato, T., Meguid, M. M., & Miyata, G. (2000). From food to nutritional support to specific nutraceuticals: a journey across time in the treatment of disease. *J Gastroenterol Suppl 12*, 1–6.

Krajick, K. (1992, June 15). Vision quest. *Newsweek*, 44–45.

Lamb, F. B. (1971). *Wizard of the Upper Amazon: The Story of Manuel Cordova-Rios*. Boston: Houghton-Miflin.

Langer, S. Z., Lee, C. R., Segnozac, A., Tateishi, T., Esnaud, H., Schoemaker, H., & Winblad, B. (1984). Possible endocrine role of the pineal gland for 6-methoxytetrahydro-*β*-carboline, a putative endogenous neuromodulator of the [^{3}H]imipramine recognition site. *Eur J Pharmacol 102*, 379–380.

Lesch, K. P., Aulakh, C. S., Wolozin, B. L., Tollliver, T. J., Hill, J. L., &

Murphy, D. L. (1993). Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. *Mol Brain Res 17*, 31–35.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., & Murphy, D. L. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science 274*, 1527–1531.

Lippke, K. P., Schunack, W. G., Wenning, W., & Muller, W. E. (1983). *β*-Carbolines as benzodiazepine receptor ligands: 1. Synthesis and benzodiazepine receptor interaction of esters of *β*-carboline-3-carboxylic acid. *J Med Chem 26*, 499–503.

Little, K. Y., McLaughlin, D. P., Zhang, L., Livermore, C. S., Dalack, G. W., McFinton, P. R., DelProposto, Z. S., Hill, E., Cassin, B. J., Watson, S. J., & Cook, E. H. (1998). Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *Am J Psychiatry 155*, 207–213.

Luna, L. E. (1984). The concept of plants as teachers among four mestizo shamans of Iquitos, northeast Peru. *J Ethnopharmacol 11*, 135–156.

Luna, L. E. (1984). The healing practices of a Peruvian shaman. *J Ethnopharmacol 11*, 123–133.

Luna, L. E. (1986). *Vegitalismo: Shamanism Among the Mestizo Population of the Peruvian Amazon*. Stockholm: Almqvist & Wiksell.

Luna, L. E., & Amaringo, P. (1991). *Ayahuasca Visions: The Religious Iconography of a Peruvian Shaman*. Berkeley, CA: North Atlantic Books.

Marazziti, D., Falcone, M., Rotondo, A., & Castrogiovanni, P. (1989). Age related differences in human platelet 5-HT uptake. *Naunyn-Schmiedeberg's Arch Pharmacol 340*, 593–594.

McKenna, D. J. (1999). Ayahuasca: An ethnopharmacologic history. In R. Metzner (Ed.), *Ayahuasca: Hallucinogens, Consciousness, and the Spirit of Nature* (pp. 187–213). New York: Thunder's Mouth Press.

McKenna, D. J., & Towers, G. H. N. (1981). Ultra-violet mediated cytotoxic activity of β-carboline alkaloids. *Phytochemistry 20*(5), 1001–1004.

McKenna, D. J., & Towers, G. H. N. (1984). Biochemistry and pharmacology of tryptamine and β-carboline derivatives: a minireview. *J Psychoact Drugs 16*, 347–358.

McKenna, D. J., & Towers, G. H. N. (1985). On the comparative ethnopharmacology of the malpighiaceous and myristicaceous hallucinogens. *J Psychoact Drugs 17*, 35–39.

McKenna, D., Towers, G. H. N., & Abbott, F. S. (1984). Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of ayahuasca. *J Ethnopharmacol 10*, 195–223.

McKenna, D. J., Luna, L. E., & Towers, G. H. N. (1995). Biodynamic constituents in Ayahuasca admixture plants: an uninvestigated folk pharmacopoeia. In S. von Reis, & R. E. Schultes (Eds.), *Ethnobotany: Evolution of a Discipline* (pp. 349–361). Portland: Dioscorides Press.

Morin, A. M. (1984). β-Carboline kindling of the benzodiazepine receptor. *Brain Res 321*, 151–154.

Naranjo, P. (1979). Hallucinogenic plant use and related indigenous belief systems in the Ecuadorian Amazon. *J Ethnopharmacol 1*, 121–145.

Naranjo, P. (1986). El *ayahuasca* in la arqueología ecuatoriana. *Am Indig 46*, 117–128.

Nichols, D. E., Oberlender, R., & McKenna, D. J. (1991). Stereochemical aspects of hallucinogenesis. In R. R. Watson (Ed.), *Biochemistry and Physiology of Substance Abuse*, *vol. III* (pp. 1–39). Boca Raton, FL: CRC Press, chap. 1.

O Centro Espirita Beneficiente Uñiao do Vegetal, et al., v. Janet Reno, et al. (2000). Case No. CV 00-1647 in the US District Court for the District of New Mexico Plaintiffs' Original Complaint for Declaratory and Injunctive Relief.

O'Connell, F. D. (1969). Isolation of caffeine from *Banisteriopsis inebrians* (Malpighiaceae). *Naturwissenschaften 56*, 139.

Ott, J. (1993). *Pharmacotheon: Entheogenic Drugs, Their Plant Sources and History*. Kennewick, WA: Natural Products.

Pahkla, R., Rago, L., Callaway, J. C., & Airaksinen, M. M. (1997). Binding of pinoline on the 5-hydroxytryptamine transporter: competitive interaction with [^{3}H] citalopram. *Pharmacol Toxicol 80*, 122–126.

Pletscher, A., & Laubscher, A. (1980). Use and limitations of platelets as models for neurons: amine release and shape change reaction. In A. Rotman (Ed.), *Embo Workshop on Platelets: Cellular Response Mechanisms and Their Biological Significance* (pp. 267–276). Wiezmann Institute of Science.

Rahwan, R. G. (1975). Toxic effects of ethanol—possible role of acetaldehyde, tetrahydroisoquinolines, and tetrahydro-*β*-carbolines. *Toxicol Appl Pharmacol 24*, 3–27.

Riba, J., Rodriguez-Fornells, A., Strassman, R. J., & Barbanoj, M. J. (2001a). Psychometric assessment of the Hallucinogen Rating Scale. *Drug Alcohol Depend 62*, 215–223.

Riba, J., Rodriguez-Fornells, A., Urbano, G., Morte, A., Antonijoan, R., Montero, M., Callaway, J. C., & Barbanoj, M. J. (2001b). Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacol (Berl) 54*, 85–95.

Riba, J., Anderer, P., Morte, A., Urbano, G., Jane, F., Saletu, B., & Barbanoj, M. J. (2002a). Topographic pharmaco-EEG mapping of the effects of the South American beverage *ayahuascain* healthy volunteers. *Br J Clin Pharmacol 53*, 613–628.

Riba, J., Rodriguez-Fornells, A., & Barbanoj, M. J. (2002b). Effects of *ayahuascaon* sensory and sensorimotor gating in humans as measured by P50 suppression and prepulse inhibition of the startle reflex, respectively. *Psychopharmacol (Berl) 165*, 18–28.

Riba, J., Valle, M., Urbano, G., Yritia, M., Morte, A., & Barbanoj, M. J. (2003). Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *J Pharmacol Exp Ther 306*, 73–83.

Rivier, L., & Lindgren, J. (1972). *Ayahausca*, the South American hallucinogenic drink: ethnobotanical and chemical investigations. *Econ Bot 29*, 101–129.

Rocca, P., Galzin, A. -M., & Langer, S. A. (1989). Light-dark differences in [^{3}H]-paroxetine binding in rabbit platelet membranes. *Naunyn-Schmiedeberg's Arch Pharmacol 340*, 41–44.

Rodriguez, E., Cavin, J. C., & West, J. E. (1982). The possible role of Amazonian psychoactive plants in the chemotherapy of parasitic worms: a hypothesis. *J Ethnopharmacol 6*, 303–309.

Rommelspacher, H., Strauss, S., & Lindemann, J. (1980). Excretion of tetrahydroharmane and harmane into the urine of man after a load with ethanol. *FEBS Lett 109*, 209–212.

Rotman, A. (1983). Blood platelets in psychopharmacological research. *Prog Neuro-psychopharmacol Biol Psychiatry 7*, 135–151.

Roy, A., Adinoff, B., DeJong, J., & Linnoila, M. (1991). Cerebrospinal fluid variables among alcoholics lack seasonal variation. *Acta Psychiatr Scand 84*, 579–582.

Schaepe, L. (2001). *International control of the preparation "ayahuasca."* United Nations International Control Board. Opinion letter submitted to Mr. Lousberg, Chief, Inspectorate for Health Care, Ministry of Public Health, Den Haag, the Netherlands. Introduced in evidence in O Centro Espirita Beneficiente Uñiao do Vegetal et al. v. Janet Reno et al., Case No. CV 00-1647 JP/RLP (application for preliminary injunction) in the US District Court for the District of New Mexico to the Hon. James A. Parker Chief Judge.

Schultes, R. E. (1957). The identity of the malpighiaceous narcotics of South America. *Bot Mus Leaf Harv Univ 18*, 1–56.

Schultes, R. E. (1972). Ethnotoxocological significance of additives to New World hallucinogens. *Plant Sci Bull 18*, 34–41.

Schultes, R. E., & Hofmann, A. (1980). *The Botany and Chemistry of Hallucinogens* (2nd ed.). Springfield, IL: Charles C. Thomas.

Schwarz, M. J., Houghton, P. J., Rose, S., Jenner, P., & Lees, A. D. (2003). Activities of extract and constituents of *Banisteriopsis caapi* relevant to parkinsonism. *Pharmacol Biochem Behav 75*, 627–633.

Sher, L., Greenberg, B. D., Murphy, D. L., Rosenthal, N. E., Sirota, L. A., & Hamer, D. H. (2000). Pleiotropy of the serotonin transporter gene for seasonality and neuroticism. *Psychiatr Genet 10*, 125–130.

Shulgin, A. T. (1982). Psychotomimetic drugs: structure-activity relationships. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), *Handbook of Psychopharmacology*, *vol. 11* (pp. 243–333). New York: Plenum.

Shulgin, A., Perrine, D. M., & Shulgin, A. (1997). *TIHKAL: The Continuation*. Berkeley, CA: Transform Press.

Smith, T. A. (1977). Tryptamine and related compounds in plants. *Phytochemistry 16*, 17–175.

Stafford, P. (1977). *Psychedelics Encyclopedia*. Berkeley, CA: And/Or Press.

Stahl, S. M. (1977). The human platelet: a diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. *Arch Gen Psychiatry 34*, 509–516.

Strassman, R. J., & Qualls, C. R. (1994). Dose-response study of *N*,*N*-dimethyltryptamine in humans: I. neuroendocrine, autonomic, and cardiovascular effects. *Arch Gen Psychiatry 51*, 85–97.

Strassman, R. J., Qualls, C. R., Uhlenhuth, E. H., & Kellner, R. (1994). Dose-response study of N,N-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry 51*, 98–108.

Stull, R. E., Ferguson, N. M., & Ferguson, G. G. (1971). Selected pharmacological studies of an alkaloidal extract of *Banisteriopsis quitensis*. *J Pharm Sci 60*, 1121–1123.

Suzuki, O., Katsumata, Y., & Oya, M. (1981). Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. *Biochem Pharmacol 30*, 1353–1358.

Szara, S. (1956). Dimethyltryptamine: its metabolism in man; the relation of its psychotic effect to the serotonin metabolism. *Experientia 12*, 411–441.

Taylor, D. L., Silverman, P. B., & Ho, B. T. (1984). Effects of 6-methoxytetrahydro-β-carboline on 5-hydroxytryptamine binding in rat brain. *J Pharm Pharmacol 36*, 125–127.

Tiihonen, J., Kiukka, J. T., Bergstrõm, K. A., Karhu, J., Viinamaki, H., Lehtonen, J., Hallikainen, T., Yang, J., & Hakola, P. (1997). Single-photon emission tomography imaging of monoamine transporters in impulsive violent behavior. *Eur J Nucl Med 24*, 1253–1260.

Topping, D. M. (1998). Ayahuasca and cancer: one man's experience. *Bull Multidiscip Assoc Psychedelic Stud 8*, 22–26 (Available at: http://www.maps.org/news-letters/v08n3/08322top.html).

Towers, G. H. N., & Abramosky, Z. (1983). UV-mediated genotoxicity of furanoquinoline and of certain tryptophan-derived alkaloids. *J Nat Products 46*, 576–581.

Udenfriend, S., Witkop, B., Redfield, B. G., & Weissbach, H. (1958). Studies with reversible inhibitors of monoamine oxidase: harmaline and related compounds. *Biochem Pharmacol 1*, 160–165.

Uebelhack, R., Franke, L., & Seidel, K. (1983). Methylated and unmethylated indolarmine in the cisternal fluid in acute endogenous psychosis. *Biomed Biochem Acta 42*, 1343–1346.

Umezawa, K., Shirai, A., Matsushima, T., & Sugimura, T. (1978). Comutagenic effect of harman and norharman with 2-acetyl-aminoflourine derivatives. *Proc Natl Acad Sci USA 75*, 928–930.

Weil, A. T. (1980). In the land of yage. *The Marriage of the Sun and Moon: A Quest for Unity in Consciousness* (pp. 99–131). Boston: Houghton-Mifflin.

Westlaw Citation # 31862699 (2002). Available at: http://nvo.com/cd/nss-folder/pubfiles/emerstay.htm.

Wilasrusmee, C., Kittur, S., Siddiqui, J., Bruch, D., Wilasrusmee, S., & Kittur, D. S. (2002a). In vitro immunomodulatory effects of ten commonly used herbs on murine lymphocytes. *J Altern Complement Med 8*, 467–475.

Wilasrusmee, C., Siddiqui, J., Bruch, D., Wilasrusmee, S., Kittur, S., & Kittur, D. S. (2002b). In vitro immunomodulatory effects of herbal products. *Am Surg 68*, 860–864.

Winkelman, M. (1995). Psychointegrator plants: their roles in human consciousness, cultures, and health. In M. Winkelman, & W. Andritsky (Eds.), Sacred plants, consciousness, and healing. Cross-cultural and interdisciplinary perspectives. *Yearb Cross-Cult Med Psychother*, 9–53.

Yasuhara, H. (1974). Studies on monoamine oxidase (report XXIV). Effect of harmine on monoamine oxidase. *Jpn J Pharmacol 24*, 523–533.

Yasuhara, H., Sho, S., & Kamijo, K. (1972). Differences in the actions of harmine on the oxidations of serotonin and tyramine by beef brain mitochondrial MAO. *Jpn J Pharmacol 22*, 439–441.

Yritia, M., Riba, J., Ortuno, J., Ramirez, A., Castillo, A., Alfaro, Y., de la Torre, R., & Barbanoj, M. J. (2002). Determination of *N*,*N*-dimethyltryptamine and beta-carboline alkaloids in human plasma following oral administration of ayahuasca. *J Chromatogr B Anal Technol Biomed Life Sci 779*, 271–281.

Bringing Ayahuasca to the Clinical Research Laboratory†

Jordi Riba, Ph.D.* & Manel J. Barbanoj, M.D., Ph.D.**

Abstract—Since the winter of 1999, the authors and their research team have been conducting clinical studies involving the administration of ayahuasca to healthy volunteers. The rationale for conducting this kind of research is twofold. First, the growing interest of many individuals for traditional indigenous practices involving the ingestion of natural psychotropic drugs such as ayahuasca demands the systematic study of their pharmacological profiles in the target species, i.e., human beings. The complex nature of ayahuasca brews combining a large number of pharmacologically active compounds requires that research be carried out to establish the safety and overall pharmacological profile of these products. Second, the authors believe that the study of psychedelics in general calls for renewed attention. Although the molecular and electrophysiological level effects of these drugs are relatively well characterized, current knowledge of the mechanisms by which these compounds modify the higher order cognitive processes in the way they do is still incomplete, to say the least. The present article describes the development of the research effort carried out at the Autonomous University of Barcelona, commenting on several methodological aspects and reviewing the basic clinical findings. It also describes the research currently underway in our laboratory, and briefly comments on two new studies we plan to undertake in order to further our knowledge of the pharmacology of ayahuasca.

Keywords—ayahuasca, clinical pharmacology, DMT, human, psychedelics, review

In 1994, the authors had the unusual opportunity of directly witnessing the arrival of a religious tradition to Spain which involved the use of what was at that time a relatively obscure psychotropic plant concoction called ayahuasca. This tradition was being imported by a very motivated small nucleus of people involved in self-knowledge and spiritual practices who were experimenting with a peculiar mind-modifying tea in the context of a Brazilian religion known as the Santo Daime. Sessions, or "works," as participants called them, were held twice a month and interested outsiders were able to attend. Most participants seriously considered their commitment to the group as a path to self-discovery and personal betterment and had no notion of engaging in "drug taking" or any kind of illicit practices. To the external observer, nothing remarkable occurred during these sessions. After ingesting a small volume of a brownish tea, participants would sit, separated by sex, in rows of chairs where they would remain for most of the session in a kind of slumber that would force them to close their eyes and yawn from time to time. This was only interrupted by periods of chanting and dancing. Sporadically, someone would become upset and start sobbing or

†The current and future studies described in the present article are supported by grant SAF 2002-02746 from the Spanish Ministry of Education and Science. The authors also wish to acknowledge the support of Richard Wolfe who has funded the assessment, currently underway, of ayahuasca's effects on the immune system

*Researcher, Centre d´Investigació de Medicaments, Institut de Recerca, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau; Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Spain.

**Director, Centre d´Investigació de Medicaments, Institut de Recerca, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau; Departament de Farmacologia i Terapèutica, Universitat Autònoma de Barcelona, Spain.

Please address correspondence and reprint requests to Jordi Riba, Centre d´Investigació de Medicaments, Institut de Recerca, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau, St. Antoni Maria Claret 167, Barcelona 08025, Spain; email: jriba@santpau.es

leave the room in order to lie down, or they would be accompanied to the bathroom if they felt nauseated. These rather quiet introspective sessions were watched over by experienced participants. Several hours later, after two or three intakes of the tea, they would end over a snack with everyone vividly discussing what appeared to be remarkable psychotropic effects with powerful visions and overwhelming emotions.

The fact that these ayahuasca sessions did not apparently interfere with the participants' everyday lives and social integration made this pattern of psychotropic drug use a remarkable social phenomenon. Furthermore, the relatively short-lived and a priori controllable nature of the subjective effects suggested that the drug could be amenable to study in a clinical research setting. Virtually no data were available on the effects of ayahuasca in humans, but that same year Strassman's papers on DMT appeared (Strassman & Qualls 1994; Strassman et al. 1994), providing very valuable information on the pharmacology of what had been described as the main psychotropic ingredient in ayahuasca brews. We thus considered that scientific inquiry into the effects on humans would be a stimulating challenge that could provide useful data to both users and the research community.

AYAHUASCA, AN UNUSUAL CHALLENGE TO THE PHARMACOLOGIST

When reviewing the botanical and chemical bibliography on the "ayahuascas," one is immediately confronted with the degree of complexity that these brews can attain. While the *Banisteriopsis caapi* liana appears to be the ubiquitous ingredient, as already described in the nineteenth century by Spruce (1908), the number of additional plants that can be used as additives to the tea is very extensive. Ott (1993) has listed up to 90 different species belonging to 38 families. As is well known, *B. caapi* contributes three major alkaloids to the tea, namely harmine, tetrahydroharmine (THH) and harmaline and, as in the samples we have analyzed, also harmol and harmalol (see below). Among the admixtures identified by Ott (1993) are found powerful psychotropic plants such as the nicotine-containing *Nicotiana tabacum* and *Nicotiana rustica*, *Erythroxylum coca* containing ecgonine alkaloids such as cocaine, *Ilex guayusa* and *Paullinia yoco* (both rich in caffeine), the solanaceous *Brugmansia sauveolens* and *Brugmansia insignis* which contain tropane alkaloids like atropine and scopolamine, and many others. However, the most frequently employed additives throughout the Amazon Basin are the rubiaceous *Psychotria viridis* and the malpighiaceous *Diplopterys cabrerana*, which lend the tea the visionary compound *N,N*-dimethyltryptamine or DMT.

In view of the above, when planning to study ayahuasca, it was important to establish which ayahuasca was be evaluated. Happily, the Santo Daime in Brazil agreed to support this research project by generously donating the ayahuasca samples from which the lyophilizate that was later administered in the clinical trials was obtained. Thus it was possible to focus on brews that were of the same geographical origin, were all elaborated following the same methodology and most importantly, always contained the same plant ingredients, i.e., exclusively *B. caapi* and *P. viridis*.

So far, two batches of ayahuasca from the Santo Daime have been analyzed and freeze-dried in this study. One was "Daime" and the other was "*Daime doblado*" or "double Daime" (which differ in strength, the latter having higher alkaloid concentrations). The present report focuses on the composition of the latter. The "double Daime" batch was the source of the ayahuasca used throughout the pilot and final double-blind studies described below. It had a volume of 9.6 liters and yielded 611 grams of powder after the freeze-drying process. One gram of freeze-dried material contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg THH, which corresponded to the following alkaloid concentrations in the original tea: DMT 0.53 mg/ml, harmine 0.90 mg/ml, harmaline 0.06 mg/ml and THH 0.72 mg/ml. In posterior analyses it was found that the lyophilizate also contained small amounts of harmol (0.28 mg/g) and harmalol (0.08 mg/g), corresponding to concentrations in the tea of 0.02 mg/ml and 0.005 mg/ml, respectively.

Even the study of brews prepared exclusively from *B. caapi* and *P. viridis* would thus involve the interplay of DMT with at least three major and two minor b-carbolines for which MAO inhibition and serotonin reuptake inhibition had been described (Buckholtz & Boggan 1977a, b). As will be discussed below, in the studies undertaken to date the authors have addressed the pharmacology of complete brews in the form of lyophilizates. In future studies, when we have adequately characterized the pharmacological profile of the ayahuasca lyophilizates, these standard preparations could be used as ideal "positive controls" against which the effects of the individual alkaloids or combinations thereof could be compared.

FINDING AN OPTIMAL PHARMACEUTICAL FORM

Before we could start the clinical phase of the project, we spent two years in the planning stage, writing the study protocol, requesting the necessary permits and contacting potential volunteers. A considerable part of this period was also devoted to deciding whether to use "natural" ayahuasca or rather combinations of purified active compounds. Once the former option had been chosen, we had to obtain a pharmaceutical form of ayahuasca that could be administered in accordance with certain methodological requirements. The decision to study natural ayahuasca was based on the fact that this is the product to which users

were normally exposed. However, working with a natural extract like ayahuasca poses a series of problems such as progressive alkaloid degradation with time or the difficulty of establishing accurate dosages. Ideally, variations in alkaloid contents in the final pharmaceutical form should be kept to a minimum and dosing units should be easily stored and administered, and always contain the same amount of product. We were also confronted with the masking problem. Clinical research methodology requires that expectancy on the part of the study participant and on the part of the researcher be the same across experimental sessions. This is related to the fact that expectancy (i.e., knowledge about what one is going to receive or to administer) is believed to influence the effects reported by a volunteer and the experimenter's attention or motivation during the experimental session, respectively. Thus, when we finally decided to go for the completely natural product it became obvious that in its usual liquid form it would easily degrade and with its characteristic taste and smell masking would be virtually impossible. So it was decided that ayahuasca would be administered as a standardized encapsulated lyophilizate. Thus, although we plan to study combinations of pure DMT plus pure β-carbolines in the future in order to assess specific drug-drug interactions, in the research conducted to date we have evaluated the human pharmacology of ayahuasca as a whole. While some findings might not have changed much from one approach to the other, we believe that other aspects of the clinical picture certainly would have. A natural extract such as ayahuasca contains a myriad of substances. Some are pharmacologically active, but inert plant material is likely to influence at least the bioavailability and gastrointestinal tolerability of the tea. The extensive literature on personal experiences with the tea describes vomiting as a very common event. In some rituals and also in certain nonreligious contexts, this behavior is encouraged as it is considered a form of purification or an acceptable form of somatization, an essential part of the ayahuasca session. In contrast, self-reports on the effects of DMT plus harmine combinations describe nausea or vomiting as rare (Ott 1999). Thus, although not ideal for the precise study of drug-drug interactions, the ingestion of the natural tea may at least lead to a different experience in terms of somatic effects, nausea and vomiting, compared with the simple combination of pure compounds in a capsule. The use of the lyophilizate, where only the original water is lost, seemed to be a reasonable compromise allowing for a classical clinical trial design and at the same time being closer to the original tea than pure alkaloid combinations.

Lyophilization, or freeze-drying, is a method of removing water from a substance or material at low temperatures. In this case, ayahuasca is first frozen in an air-tight chamber, after which the frozen material is kept under high vacuum for around 24 hours. Under these conditions, the water in the samples changes directly from ice to vapor, leaving behind a porous solid. The fact that the whole process takes place at a low temperature prevents active compounds from being destroyed by heat, and the remaining material can be analyzed to accurately establish the alkaloid concentrations and encapsulated. Once made, the capsules can be tested for uniformity of weight and content. The use of capsules also allows perfect masking and ayahuasca effects can be compared with those of placebos or positive controls (i.e., capsules containing lactose, for example, or an active compound, respectively).

Despite these advantages, working with the ayahuasca lyophilizate poses a difficulty: as the product is extremely dry, it acts as a sponge to humidity. It attracts any moisture in the atmosphere and becomes a sticky paste which can not be redissolved or freeze-dried over again. Indeed this occurred in front of our very eyes on removing the first samples of ayahuasca from the freeze-drying chamber. The lyophilizate must consequently be stored under vacuum or, alternatively, in a dry atmosphere. Consequently, in subsequent freeze-drying rounds the bulk freeze-dried ayahuasca was first stored under vacuum and the encapsulation process was later conducted under dry nitrogen.

ESTABLISHING THE OBJECTIVES OF THE CLINICAL RESEARCH PROJECT

As mentioned above, this project aimed to obtain basic information on the human pharmacology of a rather standard form of ayahuasca. Specifically, it would study teas obtained exclusively from *Banisteriopsis caapi* and *Psychotria viridis*. We decided to focus on acute effects following a single dose administration and that the experimental part should follow the methodology of a typical controlled clinical trial, i.e., incorporate a placebo and the use of single-blind or double-blind randomized designs. We also decided at this stage not to address any possible adverse psychopathological effects derived from repeated consumption, or study any hypothetical therapeutic potential of ayahuasca.

The design of the clinical trial was underway by late 1996, at a time when virtually all data available on ayahuasca dealt only with its botany and chemistry. The only information on its effects in humans were self-administration reports and no data were available regarding its acute administration to healthy humans in controlled studies. Although as yet unpublished at that time, James Callaway, a researcher at the University of Kuopio, generously shared with us data he and his colleagues had obtained in the Hoasca Project, and this, together with our own field observations, helped to give us an approximate idea of the time course of effects following the administration of a single dose. Furthermore, blood drawing points were based in part on the pharmacokinetic parameters that Callaway and colleagues had calculated and later published (Callaway et al. 1999).

We drew up the following specific objectives:

1. Assess the general tolerability of ayahuasca, i.e., the volunteers' vital signs after the drug administration, and any event, either physical or psychological, regarded as unpleasant by the participant. Evaluate the impact of drug administration on standard hematological and biochemical analytical parameters.
3. Describe the pharmacokinetics of DMT and the β-carbolines present in ayahuasca after the oral administration of various doses of the preparation.
4. Measure *in vivo* the inhibition of MAO provoked by acute ayahuasca administration.
5. Measure the nature and time course of subjective effects elicited by acute ayahuasca administration.
6. Assess the effects of ayahuasca on the central nervous system (CNS) by means of quantitative pharmaco-electroencephalography (q-EEG).
7. Conduct an exploratory analysis of the brain cortical regions responsible for the observed q-EEG effects by means of low resolution electromagnetic tomography (LORETA).
8. Measure the effects of ayahuasca on sensory and sensorimotor gating (theoretical constructs dealing with the capacity of the brain to filter out irrelevant sensory information).

To accomplish these objectives it was necessary to examine the goodness of the main tool used to assess subjective effects, and to validate the analytical technique used to determine the plasma concentrations of the ayahuasca alkaloids. Thus, we also addressed the following:

1. The translation into Spanish of the Hallucinogen Rating Scale (HRS; Strassman et al. 1994), a then recently developed self-report instrument designed to evaluate the subjective effects elicited by psychedelics. The sensitivity of the instrument to psychedelics other than intravenous DMT had still to be tested and certain psychometric properties (i.e., its reliability and construct validity) had not been assessed either for the original American version or any of its translations.
2. The development and validation of an analytical methodology to adequately quantify ayahuasca alkaloids in plasma in order to characterize their pharmacokinetics in humans following oral administration of the tea. As the method developed by Callaway and colleagues (1996) was published, this was used as a starting point for our own methodology.

The steps taken to fulfill the above objectives were as follows. First, the Spanish version of the HRS questionnaire was administered both to ayahuasca users and to users of psychedelics in order to explore the psychometric properties of this instrument. The next stage was the initiation of the clinical studies with a pilot study conducted to explore the general tolerability (i.e., safety) of the lyophilizate, and its capacity to elicit psychotropic effects. The information obtained was used to establish the doses to be administered in the larger final study. This involved a greater number of volunteers and study variables, and the implementing of an optimal design. The analytical method was developed once the clinical phase of the project was completed. Further details can be found in a report by Yritia and colleagues (2002).

Questionnaire Assessment Studies

This involved administering the Spanish version of the HRS to a large sample of volunteers in two different studies, without manipulating their pattern of drug use. In Study 1, 75 users of ayahuasca participated. They were asked to answer the HRS approximately four hours after intake, in what is known as an immediate retrospective assessment of effects. The reliability of the different HRS scales was assessed. In Study 2, 56 volunteers with experience in the use of different kinds of psychotropic drugs were asked to answer the HRS and an already-validated questionnaire, the Addiction Research Center Inventory (ARCI; Martin et al. 1971), recalling the effects of their last previous psychedelic drug intake. This approach is known as a delayed retrospective assessment of effects. Again, reliability was assessed and correlations between HRS and ARCI scales were also calculated.

The result was that four of the six HRS scales were found to have an acceptable level of internal consistency. Two HRS scales also showed significant, though limited, correlations with the ARCI LSD scale, indicating the construct validity of the questionnaire. Finally, the HRS appeared to be sensitive to psychedelic drug effects other than those elicited by intravenous DMT, for which it was originally designed. Perhaps the most important limitation of the HRS is the high degree of intercorrelations found between scales, indicating that what they intend to measure is not totally independent but shows a marked overlap (for more information on the HRS assessment study, see Riba et al. 2001a). However, this problem is also present for other questionnaires measuring psychedelic effects, such as the APZ (see Dittrich 1998).

THE CLINICAL STUDIES, METHODOLOGY AND RESULTS

All participants enrolled in the pilot and final double-blind studies were healthy young volunteers with prior experience in the use of psychedelics. Volunteers were recruited by word of mouth. The extent and nature of their past psychedelic drug use was assessed in an initial interview in which the purposes of the study and the routine of the experimental sessions were explained to them. Insufficient or prior traumatic experience with psychedelics precluded participation. Eligible candidates subsequently underwent an interview with a psychiatrist to

rule out present or past psychiatric disorders, and alcohol and drug dependencies. A psychiatric disorder in first degree relatives was also considered a cause for exclusion. After the psychiatric assessment, volunteers underwent a general medical examination involving anamnesis, physical exploration, electrocardiogram and standard laboratory tests. This selection phase was also useful for the volunteer to become familiar with the research team and the environment where the sessions would be conducted. As an additional precaution and to reduce the anxiety associated with the interventions, it was decided that ayahuasca would not be administered on the first day of participation of any volunteer. In the course of the pilot single-blind study this was accomplished by administering the placebo on the first day of participation, and in the final double-blind study by administering an additional nonrandomized placebo on the first day of participation in a single-blind manner (see below).

All sessions were conducted in a quiet, dimly-lit room. The experimenter stayed with the volunteer, or remained in an adjacent room when no measurements were being conducted if the volunteer preferred. Room temperature and light were also adjusted to the participant's comfort. The volunteers were informed that nausea might occur and that they should try to avoid vomiting as this would modify the experiment results, i.e., alkaloid plasma level measurements. Nevertheless, preparations were made in case this was unavoidable.

Every effort was made to make the experimental sessions as comfortable and pleasant as possible for the volunteers within the inevitable limitations of a clinical research laboratory. An aspect that was important in achieving this goal was that the volunteers share the researchers' view on the value of the information to be gained through this kind of studies. A high degree of trust and empathy has to develop between the participant and the person conducting the study so that the volunteer feels safe. During the initial familiarization phase, potential volunteers who wished to enroll in the clinical trials despite overt distrust towards science in general or towards the clinical research approach to psychedelics were recommended not to participate. All volunteers received detailed information on the nature of ayahuasca and the general effects of psychedelics, including potential adverse effects. A signed informed consent form was required from all volunteers, participation was totally free and anyone could withdraw from the study at any time if they so wished. The clincial trials were approved by the hospital's ethics committee and the Spanish Ministry of Health.

The Pilot Clinical Trial

The first participants enrolled in the clinical phase of this project were six male volunteers, all of them with experience in psychedelic drug use and more specifically with ayahuasca. The aims of this pilot study were to assess the general tolerability, i.e., the safety of ayahuasca administration and to obtain initial information on the time course of effects and intensity thereof, in order to decide the final doses to be used in a larger subsequent study. For safety reasons, the doses were administered in increasing order, i.e., first the placebo, followed by the low dose, the medium dose and the high dose. On this occasion, the more demanding double-blind design was not used, but rather a single-blind approach, in which the experimenter but not the volunteer knew which dose was being administered on each day. The doses used were 0.5, 0.75 and 1.0 mg DMT/kg body weight. To measure subjective effects, the HRS and ARCI questionnaires were used together with visual analogue scales (VAS). Systolic (SBP) and diastolic (DBP) blood pressure and heart rate (HR) were recorded as physiological measures. Furthermore, blood samples were drawn after each experimental session to conduct complete hematological and biochemical determinations.

From this study it became evident that ayahuasca followed a dose response pattern with subjective effect intensity and alkaloid plasma levels increasing with dose. Cardiovascular effects appeared moderate in intensity and did not reach statistical significance for any variable. The laboratory analyses conducted after each session did not find any alterations in hematological indices or in biochemical indicators of liver function or other standard analytical parameters. Given the specific prior experience of this group of volunteers with ayahuasca, the doses to be used in the final study were decided based on their reports. Participants indicated that the 1.0 mg DMT/kg dose was exceedingly high. It was thus determined that the two doses to be used in the final larger study would lie between 0.5 and 0.75 mg/kg (low dose) and between 0.75 and 1.0 mg/kg (high dose). The detailed results of the pilot study have been published by Riba and colleagues (2001b).

THE FINAL DOUBLE-BLIND CLINICAL TRIAL

In this larger study, 18 volunteers (three female) were enrolled. They all had prior experience with psychedelic drug use, although not necessarily with ayahuasca. This time the study followed a double-blind design and doses of 0.6 and 0.85 mg DMT/kg body weight were administered following a randomized balanced order. As study variables, subjective and cardiovascular effects and pharmacokinetics were again measured, together with electroencephalography, evoked potential recordings, and levels of excreted neurotransmitter metabolites. The latter were quantified to evaluate the MAO inhibition pattern of ayahuasca. Hematological and biochemical parameters were determined this time at the beginning and at the end of the study and not after every single session. The main findings of this second larger study are described below.

Tolerability

Cardiovascular effects. Throughout the experimental sessions, cardiovascular measures were obtained with the volunteers seated. In both the pilot and the final study, increases in SBP, DBP and HR mean values were observed at specific time points following ayahuasca administration as compared with mean placebo values. However, these increases did not appear to be a robust effect of ayahuasca as they were not large in magnitude or systematic. Statistically significant results were only obtained for DBP in the final double-blind study, where the sample was larger. This variable showed a moderate 9 mm Hg increase at 75 minutes after the 0.85 mg DMT/kg body weight dose. For SBP the largest, though not significant, differences with placebo were observed at 75 minutes and corresponded to 6 mm Hg increases after the high dose. Heart rate was even less affected. The largest differences with placebo were seen at 60 minutes and corresponded to four beats/minute increases after the high dose. In the pilot study, maximum differences with placebo for cardiovascular measures were observed after the high 1 mg DMT/kg dose and corresponded to 14 mm Hg for SBP, 10 mm Hg for DBP and 9 bpm for HR.

In the pilot and final study combined, two volunteers showed SBP values above 140 mm Hg at some time point and four showed DBP values above 90 mm Hg, the diagnostic criteria of hypertension. One volunteer showed HR values above 100 bpm, the diagnostic criterion of tachycardia. The maximum values recorded at any time point were 146 mm Hg for SBP, 96 mm Hg for DBP and 101 bpm for HR.

In view of these moderate cardiovascular effects, ayahuasca seems relatively safe from a cardiovascular point of view, but it should also be kept in mind that the results obtained refer only to single dose administrations in young healthy volunteers and recorded in the absence of any physical exercise. The cardiovascular picture could differ following repeated dose administration, a common practice in ayahuasca rituals, or were ayahuasca ingested by older individuals or those with cardiovascular conditions, or while performing physical exercise such as dancing.

Other aspects of the physical tolerability of ayahuasca. In the course of the experimental sessions, volunteers commonly reported a series of somatic-dysphoric effects associated with drug intake. The most common were altered bodily sensations, nausea, sensations of heat or cold, pins and needles, and gastrointestinal disturbances. Despite the nausea experienced by many volunteers, actual vomiting was only observed in four of 53 occasions in which ayahuasca was administered (pilot and final studies combined). Regarding laboratory determinations, laboratory blood analyses were conducted after each experimental session in the course of the pilot study without any alteration being found in standard hematological and biochemical parameters.

Unpleasant psychological effects. Most volunteers regarded the ayahuasca experience as pleasant, although they felt some degree of anxiety at some moment or another during the session, most often as effects increased towards a maximum. In no case did subjective effects last longer than the usual four to six hours, nor did any participant become concerned that they might not wear off. The most distressing event was experienced by one volunteer during the pilot study after having received the medium 0.75 mg DMT/dose. The volunteer experienced an intensely dysphoric reaction with transient disorientation and anxiety. Verbal support was sufficient to get him through this state, which lasted around 20 minutes. His displeasure with the experience led him to voluntarily withdraw from the study.

Alkaloid Plasma Levels Following Ayahuasca Administration

As part of the secondary objectives of the research project, analytical methods were validated to determine the plasma concentration of the different alkaloids (for the technical details see Yritia et al. 2002). Following this methodology, three major ayahuasca alkaloids (DMT, harmaline and THH) were measured in plasma following ayahuasca administration. Unexpectedly, however, levels of harmine were negligible and only observed in a small subset of volunteers. Levels of harmol and harmalol, the *O*-demethylated analogues of harmine and harmaline, were detected in all volunteers. For all measured compounds, the maximum plasma concentration (C_{max}) and area under the curve (AUC) values for the alkaloids increased with dose. C_{max} values for DMT were 12.14 ng/ml and 17.44 ng/ml after the low and high ayahuasca doses, respectively. The C_{max} was reached 1.5 hours after both doses and coincided with the peak of subjective effects. Indeed, the time course of DMT plasma concentrations closely paralleled that of subjective effects. The AUC normalized by dose index increased with dose for DMT. Conversely, the apparent volumes of distribution (V_z/F) and clearance (Cl/F) values calculated for DMT decreased with dose. These decreases were statistically significant for V_z/F and showed a tendency for Cl/F (Riba et al. 2003). Such results are suggestive of a nonlinear increment of DMT levels following the administration of larger doses of ayahuasca and could be due to the action of the higher amounts of β-carbolines administered. The authors plan to address the role of the β-carbolines in the possible nonlinearity of DMT levels between ayahuasca doses in a repeated dose administration study.

Based on the intravenous (i.v.) DMT plasma level data generously provided by Rick J. Strassman, we estimated the percentage of the total DMT amount in ayahuasca that finally reached systemic circulation (bioavailability). This calculation yielded a tentative value of around 10% to 15%

(Riba 2003). The approach used to calculate bioavailability was not ideal since i.v and oral data were obtained in different groups of volunteers and calculations had to be performed with grand-mean values rather than on an individual basis. However, if the real value is close to the value we obtained, it would be surprisingly low and would pose questions regarding the metabolism of DMT in vivo. It would mean that a very small fraction of the DMT in ayahuasca actually reaches systemic circulation. MAO activity has either only partially been inhibited by the β-carbolines, or DMT is redirected to other metabolic routes. Indeed, MAO-independent metabolites have been detected in in vitro experiments (Fish et al. 1955). However, the metabolic fate of DMT when administered alone to humans has not been investigated systematically. Szára (1956) found the MAO-dependent 3-indoleacetic acid in the urine of his volunteers after receiving DMT, but this amounted to a very low percentage of the administered dose. An interesting experiment would be to first identify the metabolic products of oral DMT when administered alone and then assess how the metabolic profile is modified by the concomitant administration of increasing doses of the different β-carbolines.

Another intriguing aspect of ayahuasca pharmacology is the absence of measurable levels of harmine in plasma in the present sample. This would suggest the alkaloid has an efficacious metabolism. A rapid turnover of harmine to harmol has been observed in vitro (Yu et al. 2003). However, that this is the cause of the lack of harmine levels cannot be unequivocally established, as low, but nevertheless measurable, levels of harmol and harmalol were found in the ayahuasca sample used in this study (see above). Interestingly, Callaway and colleagues (1999) found high harmine plasma levels in their sample. Ethnical differences in the metabolism of harmine could perhaps be an explanation. Phenotyping or genotyping volunteers for the CYP2D6 and CYP1A1 involved in harmine *O*-demethylation could shed some light on the differences observed between the present sample and that of Callaway and colleagues (1999).

Assessing the MAO-Inhibiting Effects of Ayahuasca in Vivo

Although MAO inhibition is generally acknowledged as the mechanism facilitating the absorption of DMT in the gastrointestinal tract, there is no direct proof that this enzymatic inhibition actually takes place in humans after the ingestion of ayahuasca. In order to measure MAO inhibition in our volunteers in vivo, we quantified the levels of excreted urine monoamine metabolites. This approach is based on the fact that the neurotransmitters noradrenaline, epinephrine and dopamine are themselves physiologically degraded by MAO and another enzyme, cathecol-*O*-methyltransferase (COMT), to produce deaminated and methylated metabolites, respectively. Serotonin, on the other hand, is exclusively metabolized by MAO to produce a deaminated compound. In vivo and in vitro studies have shown that when MAO is pharmacologically inhibited, the levels of MAO-dependent deaminated metabolites decrease while those of COMT-dependent methylated compounds increase. In humans, MAO inhibitors decrease, after acute administration, the urinary excretion of vanilmandelic acid (VMA), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), the deaminated metabolites of noradrenaline/epinephrine, dopamine and serotonin, respectively; while increasing that of metanephrine and normetanephrine, the methylated metabolites of epinephrine and noradrenaline, respectively (Koulu et al. 1989; Pletscher 1966). Monoamine metabolites have both a CNS and a non-CNS origin and their assessment in urine does not give information regarding the organ where MAO was inhibited. Nevertheless, this approach can identify dose-response relationships after drug administration and allows for the study of the time course of MAO-inhibition.

However, the results we obtained after acute administration of ayahuasca could not establish an unequivocal pattern of variation in these levels based on these measures. Ayahuasca increased the excretion of all metabolites measured in 24 h urine. Nevertheless, the only statistically significant increase observed was that of normetanephrine, the noradrenaline COMT-dependent metabolite. Although the increase in normetanephrine is in line with an inhibition of MAO, the increases of deaminated compounds (VMA, HVA, 5-HIAA) are not. With the increase in normetanephrine as the only significant change, it is impossible to conclude there was a systemic inhibition of MAO. The increases in normetanephrine could, for instance, also be due to increased noradrenaline release while its degradation rate remained constant.

The lack of a clear MAO inhibitory profile may have been related to the amounts of β-carboline administered with the ayahuasca doses. These were established in terms of DMT administered per kg body weight. At the 0.85 mg DMT/kg dose which was used in the final study as the high dose, volunteers received 1.45 mg/kg harmine. This value is slightly below the 1.5 mg/kg found by Ott (1999) to be the threshold dose necessary to render DMT orally active in self-experiments. Although 1.45 mg/kg may have been sufficient to allow enough DMT to reach the systemic circulation unmetabolized and to elicit psychotropic effects, it may not have been sufficient to modify the profile of endogenous monoamine metabolites in urine. Thus, the harmine-DMT interaction may have taken place predominantly in the gastrointestinal tract. At least in our experiment, harmine cannot possibly have exerted any effects on the CNS, as it did not even reach the systemic circulation. The in vivo MAO inhibition effect could be reassessed with this methodology in future studies, using

ayahuasca batches with higher amounts of β-carboline. A repeated dose study would perhaps yield a greater degree of MAO inhibition that could be reflected as the expected variations in neurotransmitter metabolite excretion.

Subjective Effects

The approach used in this study to what the participants experienced subjectively in the course of the experimental sessions was several-fold. Interviews were conducted and recorded after any session when volunteers felt they would like to report on something they had experienced. These interviews were basically nonstructured and the interviewer tried to intervene only minimally, mainly to ask the volunteers if they would like to enlarge on a topic that had been raised. Although this approach should a priori provide a richer account of the experience, it relies heavily on the volunteer's ability to verbalize the nuances of the experience. Therefore, while in some cases rich descriptive reports were obtained, in others volunteers only commented on their having been more or less "high." To date, none of this material has been analyzed.

A more structured approach was inevitably needed to record subjective effects. While obviously reductionistic, this approach provides a quantitative measure of various aspects of the experience that may help in the search for dose-response relations. Thus, questionnaires like the ARCI and the HRS were administered. With the ARCI, the experimental drug can be classified as pertaining to a given class based on the subjective effects it elicits. Essentially, it provides information on whether a drug is more sedative-like or stimulant-like or whether it elicits dysphoric effects or euphoriant effects. On the other hand, the HRS was specifically developed to measure different facets of the psychedelic experience and was drafted from reports of subjects taking parenteral DMT, both self-administered and in a clinical research setting. In the present studies, these two questionnaires were always administered after at least four hours had elapsed following the administration of ayahuasca. To measure the time course of effects, i.e. the intensity of a certain effect at a given time point, visual analogue scales were administered.

The overall picture obtained from the structured approach showed that effects exhibited a similar time course between individuals. On average, psychological effects were first noted after 30 to 60 minutes, they peaked between 60 to 120 minutes and were resolved in four to six hours. These effects included feelings of increased activation (ARCI-A, VAS-stimulated), euphoria and well-being (ARCI-MBG, VAS-high, VAS-liking, VAS-good effects) and somatic modifications (ARCI-LSD), in addition to perceptual modifications (HRS-Perception, VAS-visions), changes in thought content (HRS-Cognition) and increased emotional lability (HRS-Affect).

The constellation of subjective effects elicited by ayahuasca could thus be effectively quantified by means of the battery of self-administered instruments used for this purpose. The HRS was sensitive to ayahuasca effects, as it has previously been shown to be sensitive to other psychedelics such as i.v. DMT and oral psilocybin (Gouzoulis-Mayfrank et al. 1999; Strassman et al. 1994). VAS data indicated that ayahuasca shows a distinct duration of effects in comparison with other prototypical psychedelic drugs (i.e., longer than those of i.v. DMT) but shorter than those of oral mescaline or LSD. Finally, ARCI results revealed that ayahuasca effects are not merely perceptual but share common features with psychostimulants and elicit marked somatic-dysphoric symptoms. The coexistence of stimulation with modifications in the sensorium clearly portrayed ayahuasca as a psychedelic, with its subjective effects following a classical dose-dependent pattern. Once this information has been established for ayahuasca, blockade studies with selective antagonists can be proposed to better characterize the neurochemical mechanisms involved in its effects. Additionally, it may now be possible to address the long neglected study of individual β-carbolines in humans and examine which of these and at what doses they can elicit subjective effects analogous to those of ayahuasca, if at all. Besides, the use of quantitative measures of subjective effects in studies involving the administration of pure β-carbolines with pure DMT may help tease apart the contribution of each of the β-carbolines in the facilitation of DMT absorption per os.

Effects on the EEG Power Spectrum

Electroencephalography consists of recording spontaneous brain electrical activity by means of electrodes placed on the scalp surface. Once the recordings are obtained and after adequate preprocessing of the signals, the energy or power present in a series of frequency bands can be quantified. This approach to the study of drug effects on the CNS, termed quantitative pharmacoelectroencephalography, is modest in its objectives despite its long tradition. The power spectrum variables derived from the spontaneous EEG are not directly associated with specific cognitive processes and thus the information gained by these means is somewhat unspecific. This technique essentially detects whether a drug has an effect on the CNS, assesses the time course of this effect, and determines whether there is a dose-response profile when doses are escalated. In some cases it may provide a specific neurophysiologic correlate of the drug's central actions, identifying from among the multiple variables that can be computed those that are most selectively and reliably modified by the drug in question. Furthermore, by comparing these changes with the pattern induced by other better known compounds, inferences can be made regarding the neurochemical mechanism of action of the drug under investigation This technique not only has the advantage of providing a quantitative output, but is objective, relatively inexpensive and it allows for an almost unlimited number

of measurements within a session. However, in order to obtain meaningful results it requires very strictly standardized procedures given the large interindividual variability in EEG measures. Recording conditions and the degree of vigilance during the recording session should be, among other aspects, carefully monitored. Furthermore, placebos should be implemented to control for circadian rhythms, measurements should be repeated along time to determine whether there is a time course of EEG effects compatible with the pharmacokinetics of the drug and finally, various doses of the same drug should be administered to make sure the observed effect varies with dose. Field conditions are not therefore the ideal setting for conducting this kind of research. Additionally, adequate preprocessing of the recordings in order to eliminate artifacts is a critical step that cannot be resolved by the mere visual inspection of the traces, and submitting the data to statistical analysis is mandatory (see Anderer et al. 1992, 1987; Ferber et al. 1999). In recent years, several studies attempting to evaluate the effects of ayahuasca on the CNS under field conditions have overlooked the above requirements. The reproducibility of results is therefore dubious and even more so considering that the only results found in some of these studies were in the frequencies above 30 Hz, where EEG energy is minimal and artifact susceptibility highest, thus imposing even more demanding recording conditions and preprocessing techniques.

Concerning our own research, we are still far from having established a specific neurophysiologic marker of ayahuasca effects on the EEG and we consider our results preliminary. Nevertheless, we have effectively been able to demonstrate that ayahuasca does indeed induce statistically significant changes on the EEG and have measured the time course of these effects. Non-parametric analysis combining all variables and leads together showed that the first changes relative to placebo appeared between 15 and 30 minutes. These are followed by a steep rise at 45 minutes, reaching the maximum effects between 45 and 90 minutes. EEG measures gradually decline thereafter to reach baseline values in around four to six hours. These ayahuasca-induced EEG modifications largely coincide with the time course of subjective effects and DMT plasma levels.

With regard to the individual EEG variables targeted, a general decrease was observed in absolute power in the entire 0.3-30 Hz frequency range studied. This decrease was more pronounced in the low frequency range, leading to a displacement of the center-of-gravity towards the higher frequencies. Absolute power decreases were most prominent in theta, delta and beta-1 bands at 90 to 120 minutes, while the alpha-2 band showed a highly significant decrease in all leads at 60 minutes. Relative power was most prominently increased in the beta-3 and beta-4 bands. Additionally, the alpha/delta-theta ratio, an index of activation, was found to be increased after ayahuasca. All these effects were more intense at the higher dose than at lower ayahuasca dose (Riba et al. 2002a).

The modification pattern of the individual variables is far from simple and further EEG studies will be needed to establish which variable better reflects the effects of ayahuasca on the CNS. However, some comparisons can be established with other psychoactive drugs. Early pharmaco-EEG research also found decreases in delta-theta and alpha activity after acute LSD administration (Itil & Fink 1966). Ayahuasca's effects would be similar to those described for LSD. The fact that ayahuasca decreases delta and theta power, which have traditionally been thought to reflect inhibitory activity and relaxed states, respectively, suggests an excitatory or arousing effect for the brew. This assumption is further supported by the fact that major tranquilizers with D_2 or mixed D_2/5-HT_2 antagonist activity, such as chlorpromazine and risperidone, are characterized by their delta and theta-promoting activity (Lee et al. 1999; Saletu et al. 1993).

Intracerebral Location of the Observed Scalp EEG Changes

Based on the scalp electrical potential distribution (topography) obtained by means of classical EEG measures it is possible to tentatively identify the cortical brain areas responsible for the observed changes. One technique that allows for the calculation of this "inverse solution" is called low resolution electromagnetic tomography or LORETA. This algorithm computes a unique three-dimensional intracerebral power density distribution for the different EEG frequency bands, making no a-priori assumptions about the number of sources involved, in contrast to dipole modeling techniques. Based on the assumption that neighboring neuronal sources are likely to be similarly active, the only constraints imposed by this technique is that of maximal smoothness of the solution and its anatomical restriction to cortical gray matter and hippocampus. Thus, the algorithm provides by definition a sparse map of activated (or deactivated) areas. Hence the "low resolution" (Pascual-Marqui, Michel & Lehmann 1994).

In the present study, intracerebral power density showed the maximum differences with placebo at 60 and 90 minutes. Ayahuasca decreased power density in the alpha-2, delta, theta and beta-1 bands. At 60 minutes, a widespread power reduction in the alpha-2 band was observed in extensive areas around the temporo-parieto-occipital junction in both hemispheres. At 90 minutes, the slow delta rhythm was decreased also in posterior brain regions around the temporo-parieto-occipital junction. Theta was found to be decreased in the medial frontal and medial temporal cortices. Finally, beta-1 decreases were found mainly in the parietal lobe (Riba et al. 2004).

Based on these results, it would seem that bioelectrical changes are found on cortical association areas in temporo-parietal and frontomedial areas, together with

paralimbic areas such as the cingulate and the temporomedial cortices, which play relevant roles in the neurobiology of attention, emotion, and memory. It is worth mentioning that in contrast with the widespread power density decreases observed here for ayahuasca in posterior regions with LORETA, PET and SPECT studies of psychedelic drug administration have shown blood flow and metabolic increases in the frontal cortex (see for instance Vollenweider et al. 1997). However, a recent study has shown that a direct anatomical relation between drug-induced bioelectric and blood perfusion/metabolic changes in the brain may not exist (Gamma et al. 2004; Nuñez & Silberstein 2000).

Effects on Sensory and Sensorimotor Gating

Current human research with psychedelics and entactogens has explored the possibility that drugs displaying agonist activity at the 5-$HT_{2A/2C}$ sites temporally disrupt inhibitory neural mechanisms thought to intervene in the normal filtering of information. Suppression of the P50 auditory evoked potential and prepulse inhibition of startle (PPI) are considered operational measures of sensory and sensorimotor gating, respectively. According to this model, serotonergic psychedelics would interact with brain structures involved in the gating mechanisms, temporarily decreasing their functionality and giving rise to the characteristic perceptual and cognitive effects elicited by these agents. In the final double-blind study we evaluated the effects of ayahuasca on these measures.

At the doses administered, however, ayahuasca induced a different pattern of effects on PPI and P50. Ayahuasca produced dose-dependent reductions of P50 suppression measured both as amplitude difference between the response to the conditioning and testing stimuli and as percent inhibition of the testing response. On the contrary, no statistically significant effects were found on the magnitude of the startle response, its habituation rate or on the percentage prepulse inhibition at any of the prepulse-to-pulse intervals studied (Riba et al. 2002b).

STUDIES CURRENTLY UNDERWAY

Since the research project described above was completed, we have undertaken new experiments to study issues such as changes in regional cerebral blood flow during the peak effects of ayahuasca, the modulation of the neuroendocrine and immune system parameters after acute ayahuasca administration, and the effects of daytime ayahuasca use on sleep architecture. The clinical part of these experiments is now complete and we are currently analyzing the data generated.

We studied regional cerebral blood flow by means of single photon computerized tomography or SPECT. A radiotracer was administered at the peak of subjective effects following the administration of a 1 mg DMT/kg body weight ayahuasca dose. The uneven uptake of the radiotracer in the different brain regions is dependent on blood flow, which is in turn believed to reflect neural activity, with increased uptake reflecting increased activation in a given brain region. In fact, this technique, together with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) are considered the "gold standards" in neuroimaging. By this means we hope to expand our knowledge of which brain areas are involved in the genesis of the effects of ayahuasca.

Another interesting aspect of ayahuasca phamacology we are currently addressing is the effects of the tea on the neuroendocrine and immune systems. We are currently analyzing the variations in cortisol, growth hormone and prolactin release, and lymphocyte subpopulations in blood samples taken after the acute administration of ayahuasca. Although these analyses on the acute effects of the tea on neuroendocrine variables and the immune system will not provide information regarding the changes experienced by users after years of ayahuasca ingestion, important initial information will be gained on the capabilities of ayahuasca to modulate these systems.

Finally, we are also exploring the effects of daytime consumption of ayahuasca on the architecture of sleep on the following night. It is well known that the serotonergic system, which is targeted by ayahuasca alkaloids, plays a relevant role in the regulation of sleep. Our approach to assessing the impact of ayahuasca on sleep regulation is based both on the analysis of variables derived from the classical sleep stage classification (macroscopic changes in a 30 s scale) and on the study of the variations of energy content in the different frequency bands throughout the night (microscopic changes in a continuous scale).

FUTURE STUDIES

Two studies are planned for the near future. We are currently about to begin a repeated dose study in which two identical doses of ayahuasca will be administered following a certain time interval, a pattern of use which is very common in ayahuasca sessions. A primary focus of this study will be on cardiovascular measures and on alkaloid pharmacokinetics in order to determine a possible nonlinearity in the DMT plasma levels associated with the administration of the second dose. After controlling for this possibility, we will explore whether subjective and EEG effects after the second dose are disproportionally increased (indicating sensitization), disproportionally decreased (indicating tolerance), or simply linearly superimposed on the residual effects of the first dose.

In 2006, we plan to conduct a study involving the administration of ayahuasca after pretreatment with a serotonin-2 receptor antagonist and evaluate how this

modifies subjective effects and EEG measures. This study should help clarify whether mechanisms other than the activation of this receptor are responsible for the entire or at least part of the pharmacological picture of ayahuasca.

Finally, in the more distant future, we plan to undertake the study of the individual ayahuasca alkaloids and their interactions. Here, as already mentioned, the ayahuasca lyophilizate could be used as a potential positive control. This approach could help tease apart the respective contributions of the different alkaloids to the final psychotropic effects of this fascinating Amazonian preparation.

REFERENCES

Anderer, P.; Semlitsch, H.V.; Saletu, B. & Barbanoj, M.J. 1992. Artifact processing in topographic mapping of electroencephalografic activity in neuropsychopharmacology. *Psychiatry Research Neuroimaging* 45:79-93.

Anderer, P.; Saletu, B.; Kinsperger, K. & Semlitsch, H. 1987. Topographic brain mapping of EEG in neuropsychopharmacology - Part I. Methodological aspects. *Methods and Findings in Experimental and Clinical Pharmacology* 9:371-84.

Buckholtz, N.S. & Boggan, W.O. 1977a. Monoamine oxidase inhibition in brain and liver produced by β-carbolines: Structure-activity relationships and substrate specificity. *Biochemical Pharmacology* 26: 1991-96.

Buckholtz, N.S. & Boggan, W.O. 1977b. Inhibition by β-carbolines of monoamine uptake into a synaptosomal preparation: structure-activity relationships. *Life Science* 20: 2093-99.

Callaway, J.C.; McKenna, D.J.; Grob, C.S.; Brito, G.S.; Raymon, L.P.; Poland, R.E.; Andrade, E.N.; Andrade, E.O. & Mash, D.C. 1999. Pharmacokinetics of *hoasca* alkaloids in healthy humans. *Journal of Ethnopharmacology* 65: 243-56.

Callaway, J.C.; Raymon, L.P.; Hearn, W.L.; McKenna, D.J.; Grob, C.S. & Brito, G.S. 1996. Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *Journal of Analytical Toxicology* 20: 492-97.

Dittrich, A. 1998. The standardized psychometric assessment of altered states of consciousness (ASCs) in humans. *Pharmacopsychiatry* 31 (Suppl 2): 80-4.

Ferber, G.; Abt, K.; Fichte, K. & Luthringer, R. 1999. IPEG guideline on statistical design and analysis for pharmacodynamic trials. International Pharmaco-EEG group. *Neuropsychobiology* 39: 92-100.

Fish, M.S.; Johnson, N.M.; Lawrence, E.P. & Horning, E.C. 1955. Oxidative N-dealkylation. *Biochemical and Biophysical Acta* 18: 564-65.

Gamma, A.; Lehmann, D.; Frei, E.; Iwata, K.; Pascual-Marqui, R.D. & Vollenweider, F.X. 2004. Comparison of simultaneously recorded [$H_2{}^{15}O$]-PET and LORETA during cognitive and pharmacological activation. *Human Brain Mapping* 22: 83-96.

Gouzoulis-Mayfrank, E.; Thelen, B.; Habermeyer, E.; Kunert, H.J.; Kovar, K.A.; Lindenblatt, H.; Hermle, L.; Spitzer, M. & Sass, H. 1999. Psychopathological, neuroendocrine and autonomic effects of 3,4-methylenedioxyethylamphetamine (MDE), psilocybin and *d*-methamphetamine in healthy volunteers. *Psychopharmacology* 142: 41-50.

Itil, T. & Fink, M. 1966. Klinische Untersuchungen und quantitative EEG-Daten bei experimentellen Psychosen. Arzneimittelforschung 16: 237-39.

Koulu, M.; Scheinin, M.; Kaarttinen, A.; Kallio, J.; Pyykkö, K.; Vuorinen, J. & Zimmer, R.H. 1989. Inhibition of monoamine oxidase by moclobemide: effects on monoamine metabolism and secretion of anterior pituitary hormones and cortisol in healthy volunteers. *British Journal of Clinical Pharmacology* 27 :243-55.

Lee, D.Y.; Lee, K.U.; Kwon, J.S.; Jang, I.J.; Cho, M.J.; Shin, S.G. & Woo, J.I. 1999. Pharmacokinetic-pharmacodynamic modeling of risperidone effects on electroencephalography in healthy volunteers. *Psychopharmacology* 144: 272-78.

Martin, W.R.; Sloan, J.W.; Sapira, J.D. & Jasinski, D.R. 1971. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. *Clinical Pharmacology and Therapeutics* 12:245-258.

Nuñez, P.L. & Silberstein, R.B. 2000. On the relationship of synaptic activity to macroscopic measurements: does co-registration of EEG with fMRI make sense? *Brain Topography* 13: 79-96

Ott, J. 1999. Pharmahuasca: Human pharmacology of oral DMT plus harmine. *Journal of Psychoactive Drugs* 31: 171-77.

Ott, J. 1993. *Pharmacotheon: Entheogenic Drugs, Their Plant Sources and History*. Kennewick, Washington: Natural Products Co.

Pascual-Marqui, R.D.; Michel, C.M. & Lehmann, D. 1994. Low resolution electromagnetic tomography: A new method for localizing electrical activity in the brain. *International Journal of Psychophysiology* 18: 49-65.

Pletscher, A. 1966. Monoamine oxidase inhibitors. *Pharmacological Reviews* 18: 121-29.

Riba, J. 2003. Human pharmacology of ayahuasca. Doctoral dissertation, Barcelona, Universitat Autònoma de Barcelona.

Riba, J.; Anderer, P.; Jané, F.; Saletu, B. & Barbano, M.J. 2004. Effects of the South American psychoactive beverage *Ayahuasca* on regional brain electrical activity in humans: A functional neuroimaging study using low resolution electromagnetic tomography (LORETA) *Neuropsychobiology* 50: 89-101.

Riba, J.; Valle, M.; Urbano, G.; Yritia, M.; Morte, A. & Barbanoj, M.J. 2003. Human pharmacology of *Ayahuasca*: subjective and cardiovascular effects, monoamine metabolite excretion and pharmacokinetics. *Journal of Pharmacology and Experimental Therapeutics* 306: 73-83.

Riba, J.; Anderer, P.; Morte, A.; Urbano, G.; Jané, F.; Saletu, B. & Barbanoj, M.J. 2002a. Topographic pharmaco-EEG mapping of the effects of the South American psychoactive beverage *Ayahuasca* in healthy volunteers. *British Journal of Clinical Pharmacology* 53:613-628.

Riba, J.; Rodríguez-Fornells, A. & Barbanoj, M.J. 2002b. Effects of *Ayahuasca* on sensory and sensorimotor gating in humans, as measured by P50 suppression and prepulse inhibition of the startle reflex, respectively. *Psychopharmacology* 165: 18-28.

Riba, J.; Rodríguez-Fornells, A,; Strassman, R.J. & Barbanoj, M.J. 2001a. Psychometric assessment of the Hallucinogen Rating Scale. *Drug and Alcohol Dependence* 62: 215-23.

Riba, J.; Rodríguez-Fornells, A.; Urbano, G.; Morte, A.; Antonijoan, R.; Montero, M.; Callaway, J.C. & Barbanoj, M.J. 2001b. Subjective effects and tolerability of the South American psychoactive beverage *ayahuasca* in healthy volunteers. *Psychopharmacology* 154: 85-95.

Saletu, B.; Barbanoj, M.J.; Anderer, P.; Sieghart, W. & Grünberger, J. 1993. Clinical-pharmacological study with two isomers (*d*-, *l*-) of fenfluramine and its comparison with chlorpromazine and *d*-amphetamine: Blood levels, EEG mapping and safety evaluation. *Methods and Findings in Experimetal and Clinical Pharmacology* 15: 291-312.

Spruce, R. 1908. *Notes of a Botanist on the Amazon and Andes, Vol 2*. London: Macmillan.

Strassman, R.J. & Qualls, C.R. 1994. Dose-response study of *N,N*-dimethyltryptamine in humans, I. Neuroendocrine, autonomic and cardiovascular effects. *Archives of General Psychiatry* 51: 85-97.

Strassman, R.J.; Qualls, C.R.; Uhlenhuth, E.H. & Kellner, R. 1994. Dose-response study of *N,N*-dimethyltryptamine in humans, II. Subjective effects and preliminary results of a new rating scale. *Archives of General Psychiatry* 51: 98-108.

Szára, S. 1956. Dimethyltryptamine: Its metabolism in man; the relation of its psychotic effect to the serotonin metabolism. *Experientia* 12: 441-42.

Vollenweider, F.X.; Leenders, K.L.; Scharfetter, C.; Maguire, P.; Stadelmann, O. & Angst, J. 1997. Positron emission tomography and fluorodeoxyglucose studies of metabolic hyperfrontality and psychopathology in the psilocybin model of psychosis. *Neuropsychopharmacology* 16: 357-72.

Yritia, M.; Riba, J.; Ortuño, J.; Ramirez, A.; Castillo, A.; Alfaro, Y.; de la Torre, R. & Barbanoj, M.J. 2002. Determination of *N,N*-dimethyltryptamine and b-carboline alkaloids in human plasma following oral administration of *Ayahuasca*. *Journal of Chromatography: Biomedical Applications* 779:271-281.

Yu, A.; Idle, J.R.; Krausz, K.W.; Küpfer, A. & Gonzalez, F.J. 2003. Contribution of individual cytochrome P450 isozymes to the *O*-demethylation of the psychotropic β-carboline alkaloids harmaline and harmine. *Journal of Pharmacology and Experimental Therapeutic* 305: 315-22.

doi:10.1111/j.1360-0443.2006.01652.x

Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids

Robert S. Gable

Claremont Graduate University, Claremont, CA, USA

ABSTRACT

Aim To extend previous reviews by assessing the acute systemic toxicity and psychological hazards of a dimethyltryptamine and β-carboline brew (ayahuasca/hoasca) used in religious ceremonies. **Method** A systematic literature search, supplemented by interviews with ceremony participants. **Results** No laboratory animal models were located that tested the acute toxicity or the abuse potential of ayahuasca. Separate animal studies of the median lethal dose of dimethyltryptamine (DMT) and of several harmala alkaloids indicated that a lethal dose of these substances in humans is probably greater than 20 times the typical ceremonial dose. Adverse health effects may occur from casual use of ayahuasca, particularly when serotonergic substances are used in conjunction. DMT is capable of inducing aversive psychological reactions or transient psychotic episodes that resolve spontaneously in a few hours. There was no evidence that ayahuasca has substantial or persistent abuse potential. Long-term psychological benefits have been documented when ayahuasca is used in a well-established social context. **Conclusion** A decoction of DMT and harmala alkaloids used in religious ceremonies has a safety margin comparable to codeine, mescaline or methadone. The dependence potential of oral DMT and the risk of sustained psychological disturbance are minimal.

Keywords Abuse potential, ayahuasca, dimethyltryptamine, DMT, toxicity.

Correspondence to: Robert S. Gable, 2738 Fulton Street, Berkeley, CA 94705, USA. E-mail: robert.gable@cgu.edu
Submitted 19 February 2006; initial review completed 12 June 2006; final version accepted 11 July 2006

INTRODUCTION

Among substances suspected of having abuse potential, N,N-dimethyltryptamine (DMT) has received relatively little attention. However, in 2006, a case was decided by the US Supreme Court that involved the question as to whether a DMT and β-carboline plant decoction (ayahuasca/hoasca) is safe for ceremonial use by members of a small spiritualist Christian church [1]. The church prevailed in a unanimous decision in part because the government, which opposed the use of DMT, could not meet its burden of showing that hoasca posed a serious health risk to church members. This paper is a review of the known acute systemic risks of oral DMT and concomitantly ingested harmala alkaloids.

Risk refers to the quantified probability of a future harm. The concept of risk requires a causal relationship between a hazard (such as a drug overdose) and a known unfavorable outcome (such as illness or death). In order to establish this causal relationship, a stimulus must produce an effect more often than would normally occur in the absence of that stimulus. Thus, the probabilistic estimate of risk is often an intrinsic aspect of the research process by which a causal relation is demonstrated. In the case of oral DMT, probabilistic estimates can be made for very few of the drug's effects at the present time. Some effects, both favorable and unfavorable, that have been attributed to DMT may amount to little more than plausible associations. Given the limited amount of published scientific data regarding oral DMT, this paper takes a broad view of potential hazards with the expectation that future research will better establish the risks that might actually exist.

Ayahuasca (EYE-ah-WAS-ka) or hoasca (WAS-ka) is a mixture consisting essentially of two compounds. One is an amine—the simplest and most common being the tryptamine DMT. The second compound is a monoamine oxidase inhibitor (MAOI) in the form of a β-carboline such as harmine, harmaline or tetrahydroharmine. DMT alone has predicable and significant psychological

activity when smoked, injected or insufflated; but when used orally it only becomes, or remains, psychoactive in combination with an MAOI [2–4].

Ayahuasca/hoasca is prepared customarily by combining the leaves from the *Psychotria viridis* bush along with bark scraped from the stem of the *Banisteriopsis caapi* vine [4,5]. The ingredients are boiled for several hours and then decanted. The resulting thick, brown, oily liquid (referred to colloquially in some locales as 'vine of the souls' or 'vine of the dead') has been used throughout the Amazon Basin as a medicinal and ceremonial beverage since antiquity [6].

Various sacramental brews containing DMT and β-carbolines, used primarily in Amazonia, are generally known as ayahuasca. The term 'hoasca', a Portuguese transliteration of ayahuasca, is limited historically and properly to a subclass of ayahuasca beverages associated with a specific sacramental use ([5], chapter 16, 'Hoasca versus Ayahuasca'). The most commonly recognized sacramental use of ayahuasca occurs among members of two churches in Brazil: the O Centro Espirita Beneficiente Uniao Do Vegetal (UDV), founded in 1961 with approximately 8000 current members, and the Santo Daime, founded around 1940 with approximately 2500 current members. Hoasca preparation occurs as part of a religious ritual known as *preparo* in the UDV. The Santo Daime church also uses a decoction of *P. viridis* and *B. cappi* called *santo daime* or simply *daime*, and its preparation ritual is known as *feitio* [7]. In the present paper, 'hoasca' will refer exclusively to UDV's sacramental decoction of plant DMT and harmala alkaloids.

The UDV cultivates the plants that are used in their hoasca 'tea' on land that the church owns and maintains for that purpose. The preparo ceremony can involve up to 200 people working together at a UDV temple over a period of several days. Photographs of the process appear in Metzner ([8], pp. 20, 21, 205). In 1992, the Brazilian Federal Narcotics Council granted legal status for the use of hoasca in religious contexts.

METHOD

The present paper extends previous reviews [9,10] that compared the acute lethal toxicity and the abuse potential of various psychoactive substances. A simple inductive and iterative procedure was used to locate English-language serial publications, from 1969 to 2005, accessible through six databases: Biosis Previews, Digital Dissertations, Google, PsychAbstracts, Pubmed and Toxline. The Science Citation Index was occasionally used to follow-up unusually salient reports. Six primary search terms—'ayahuasca', 'hoasca', 'dimethyltryptamine', 'harmine', 'harmaline' and 'tetrahydroharmine'—were keyed into the search engines and scanned for the topics 'overdose', 'lethal dose', 'lethality', 'toxicity', 'death', 'therapeutic index', 'abuse potential' and 'dependency'. The most relevant books, reference works and special journal issues (e.g. [5,8,11–12]) were also consulted, thus expanding the information sources beyond those located only by the search descriptors. In 2003, this author met with 16 people 2 hours before and periodically for 12 hours after an ayahuasca ceremony led by a Peruvian shaman. In 2005, the author had access to a detailed legal document [13] consisting of more than 1000 pages that served as the evidentiary basis for health information about hoasca for use in the US Supreme Court case mentioned previously [1].

An item retrieved during the literature search was considered potentially relevant if it met at least two of four criteria: (1) DMT or one of the monoamine oxidase inhibitors was quantified with respect to an effective or a toxic dose; (2) the health status of the individual was indicated; (3) the possible use of concomitant substances was mentioned; and (4) the data source appeared to be technical or scholarly in nature.

RESULTS

Fewer than 100 scholarly articles that focused specifically on ayahuasca/hoasca were located in English-language serial publications. The majority of these articles involved inapplicable topics such as neurological experiments, ethnographic descriptions or potential medical uses. The descriptors 'dimethyltryptamine', 'harmine', 'harmaline' and 'tetrahydroharmine' resulted in approximately 200 citations retrieved from the databases, excluding Google and the Science Citation Index. However, after cross-indexing the keyword 'toxicity' with these four descriptors, the number of non-redundant citations that met the screening criteria was fewer than 25. When all relevant citations from serial publications were supplemented with excerpts from scholarly books, monographs and published reports, the total number of documents printed and filed was approximately 140.

Description of ayahuasca/hoasca brew

The DMT alkaloid has been reported as ranging from 0.1% to 0.66% dry weight in *P. viridis* leaves [14]. Surprisingly, the DMT in leaf samples from a single *P. viridis* plant has been shown to vary from approximately 3 mg/g to 9.5 mg/g dry weight in the course of one day. The concentrations of the β-carboline alkaloids in *B. caapi* have been reported as ranging from 0.05% to 1.95% dry weight [15,16].

Substantial variation in concentrations and proportions of the constituents of ayahuasca brews can be expected as a consequence of the varying chemical

Table 1 Quantity (in milligrams) of alkaloids in ayahuasca/hoasca brews.[1]

Dose size [2]	*DMT*	*Harmine*	*Harmaline*	*THH*	*Reference*
140 ml	35.6	238	28	150	[2,14,18]
237 ml	33	17	26	Not stated	[75]
60 ml	36	280	25	96	[15]
Gelatin capsule	30	120	None	None	[4]
100 ml	9.1	47.5	Trace	4.2	[17]
100 ml[3]	8.8	9.2	Not stated	26.5	[17]
Gelatin capsule	42	71	4.8	57	[30]
200 ml	25	30	trace	10	[16]

[1]Adapted, in part, from Riba [76]. [2]Estimated average given to a 70 kg adult in the study referenced. [3]Two samples of ayahuasca reported in the same study.

composition of the source plants as well as different methods of preparation. Average DMT doses in assayed ayahuasca brews have ranged from 8.8 mg [17] to 42 mg [18]. The content of other alkaloids also varied widely (see Table 1). This fivefold variance of DMT quantities in ayahuasca brews might be compared to the common fivefold variance in caffeinated beverages which range typically from 0.2 mg/ml of caffeine in green tea to 1.0 mg/ml of caffeine in filtered coffee [19].

The ritual structure in which hoasca is ingested provides some control of dosage and subsequent psychological effects. Because the natural sources used in preparation of the tea does not allow UDV members to standardize their hoasca brew with respect to DMT or β-carbolines, the person conducting the ceremony drinks the brew before administering it to UDV members as a means of testing for potency. Different amounts of the brew are initially offered to individual participants and, depending upon reactions, a participant may be offered a second cup at his or her request.

A clear distinction must be made between preparations consisting exclusively of DMT that are injected, smoked or insufflated versus DMT preparations that are oral mixtures of DMT and MAOI. Injected, smoked or insufflated DMT is noted for its very rapid activity. Peak cognitive effects last only 3–10 minutes, and normal consciousness returns within 30 minutes. In contrast, initial somatic effects (e.g. nausea, tingling, increased body temperature) after ingesting oral DMT appear in approximately 20 minutes, followed by the onset of cognitive effects that peak between 60 and 120 minutes [20]. The cognitive effects diminish gradually to a normal state in approximately 4 hours. At normally used doses, the psychological effects of oral DMT are less intense than those of injected, smoked or insufflated DMT.

Acute lethality

A traditional and standard criterion for assessing the relative toxicity of various substances has been the lethal dose of a substance. The median lethal dose (LD_{50}) is the statistically derived quantity of a substance given in a single dose that causes death in 50% of the experimental animals. One early study by Trevan [21] used more than 900 mice to assess the toxicity of cocaine and of insulin. More humane standards, such as the minimal lethal dose or the maximal non-lethal dose, are being gradually implemented by toxicologists, although many regulatory agencies still rely on the traditional LD_{50}. Some test procedures have been advocated that can reduce the number of killed animals to as few as six [22].

The criterion of single-dose acute lethality is an extremely limited estimate of human toxicity because it does not take into account variables such as interspecies differences, repeated dosing, environmental conditions, prior health status and psychological factors. None the less, the influence that such variables might have on an established dose–response relationship should not obscure the fact that the LD_{50} is a clearly defined, replicable and important benchmark of toxicity.

The LD_{50} of DMT in mice is reported as 47 mg/kg intraperitoneally and 32 mg/kg intravenously [23]. No other LD_{50} data on DMT are believed to be available in the English language at this time. Five compounds with structural resemblance to DMT—serotonin, psilocin, psilocybin, bufotenin and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT)—all have an intravenous LD_{50} among rodents that are similar to, or substantially less toxic than, DMT. In two rare studies reporting oral lethal doses of these related compounds, the LD_{50} of serotonin in mice was 60 mg/kg [23]; the LD_{50} of 5-MeO-DMT in mice was 278 mg/kg [24].

The principal β-carboline alkaloids added to DMT in ayahuasca mixtures are harmine, harmaline and tetrahydroharmine. In addition to the *B. caapi* vine, these alkaloids can be found in the *Peganum harmala* (Syrian rue) shrub, a desert plant native to Central Asia. An aqueous extract of *P. harmala* seeds administered orally to rats resulted in a LD_{50} of 2 g/kg [25]. Because the β-carboline admixtures in ayahuasca appear to be less

Table 2 Heart rate and blood pressure increases after administration of DMT or other psychoactive substances.

Substance	*Dose (mg/kg)*[1]	*Peak interval*	*Heart rate (bpm)*	*Systolic (mmHg)*	*Diastolic (mmHg)*	*Reference*[2]
DMT						
Intravenous	0.4	2 minutes	26	35	30	[27][2]
Oral	0.48	20 minutes	7.4	9.0	9.3	[3]
Oral	0.5	45 minutes	6.4	8.8	10.4	[20]
Oral	0.75	45 minutes	8.0	13.4	9.8	[20]
Oral	1.0	45 minutes	9.2	13.8	8.6	[20]
Alcohol oral	1157	15 minutes	9	−2	1	[77]
Caffeine oral[3]	3.3	15 minutes	4.0	5.0	5.0	[29]
Cocaine insufflated[4]	1.37	55 minutes	17	14	14	[78]
Marijuana smoked	(98.6 ng/ml)[5]	15 minutes	11.6	(See footnote 6)		[79]
MDMA oral	1.5	60 min	28	25	7	[80]

[1]Assumes an individual who has not developed tolerance to the indicated substance. [2]The reference listed is the primary, but not the exclusive, source for the data summarized in the table. Some original data were averaged. [3]Caffeine powder mixed with grapefruit juice; caffeine dose was equivalent to two or three cups of instant coffee. [4]Administered as 120 mg of white powder (cocaine and lactose) in four 'lines', snorted two lines per nostril. [5]Plasma THC from 10 puffs, at 1-minute intervals, from a marijuana cigarette containing 1.75% THC. [6]Typically, a slight increase in blood pressure when in a supine position, a decrease when in an upright position.

toxic than DMT, our attention should focus on the DMT component.

A simple extrapolation of DMT lethality data from mice to humans is obviously untenable. We are therefore forced to make some intrepid, but not original, assumptions. One traditional rule for scaling unknown differences between humans and non-human animal species is simply to assume that humans are 10 times more sensitive, based on body weight, than rodents [26]. To be even more cautious in light of the absence of LD_{50} data regarding oral DMT, let us assume that humans are 20 times more sensitive than rodents. This would result in a human LD_{50} of 1.6 mg/kg for DMT administered intravenously, or a total intravenous dose of 112 mg for a typical 70 kg person. Because we are interested in the potential toxicity of oral DMT in ayahuasca, it is necessary to convert our estimated intravenous LD_{50} dose of 1.6 mg/kg DMT to an oral equivalent. Usually, an intravenously administered dose of a substance is assumed to have bioavailability of 100%. The bioavailability of an oral dose is significantly less. An oral DMT dose of 1.0 mg/kg has been reported to increase blood pressure and heart rate comparable to a 0.1–0.2 mg/kg intravenous dose [20]. With respect to psychological reactions, an intravenous DMT bolus of 0.4 mg/kg has been documented carefully as 'highly psychedelic' ([12], p. 138), while an oral dose of DMT 'in excess of 2.0 mg/kg' in ayahuasca is known to produce maximal effects ([4], p. 175). Let us assume an intravenous-to-oral conversion factor of 1 : 5, based on the assumption that 0.4 mg intravenously is equivalent to approximately 2.0 mg of oral DMT. If 1.6 mg/kg is a median lethal dose of intravenous DMT, then a median lethal dose of oral DMT would be 8 mg/kg, or a total dose of 560 mg for a 70 kg individual. Note, however, that 560 mg is the estimated median dose, and therefore one-half of potential DMT fatalities would occur at an oral dose less than 560 mg.

A safety ratio or safety margin for ayahuasca can be estimated by comparing the calculated LD_{50} to the customary effective dose. The average ceremonial dose of DMT, as listed in Table 1, is about 27 mg; therefore, the safety margin for ayahuasca is approximately 20 (560/27 = 20.7).

Cardiac stress

Intravenous DMT is known to increase heart rate rapidly, as well as systolic and diastolic blood pressure. Strassman & Qualls [27] reported that a 0.4 mg/kg dose of DMT at the postinjection interval of 2 minutes raised the heart rate approximately 26 beats per minute (bpm), and raised systolic pressure by 35 mmHg and diastolic pressure by 30 mmHg. DMT plus β-carbolines in ayahuasca ingested in doses ranging from 0.48 mg/kg to 1.0 mg/kg were shown to induce peak increases in heart rate and blood pressure at about one-third that of intravenous DMT after 90 and 120 minutes [3,20]. Table 2 summarizes these data along with comparisons of some commonly used psychoactive substances. The reference cited for each of the substances in Table 2 is the primary, but not exclusive, source of the information presented.

On the basis of acute heart rate and blood pressure data, the hemodynamic effects of a typical dose of ayahuasca appear to be less potentially hazardous than many commonly used psychoactive substances. This tentative conclusion assumes that any substance listed in

Table 2 is administered once by the route and in the quantity indicated without any concomitant pharmacologically active substances. Occasional stroke, myocardial infarction and other adverse cardiovascular events can be expected to be associated with, even if not directly caused by, the use of ayahuasca or other drugs or foods that induce acute hemodynamic changes. Individual differences in metabolism and health status often result in a wide range of reactions to psychoactive substances. For example, the heart rate of some of the participants in the Strassman & Qualls intravenous DMT study [27] peaked at 150 bpm, while the heart rate of other participants was raised no higher than 95 bpm. Furthermore, the hemodynamic reaction to ayahuasca may depend on the relative concentrations of DMT to the β-carbolines. In decoctions tested by Pomilio, Vitale & Ciprian-Ollivier [17], an exceptionally low concentration of DMT compared to β-carbolines significantly decreased heart rate. An experiment with dogs found that harmala alkaloids decreased heart rate but were inconsistent in their effect on blood pressure [28].

Because heart rate increases with ayahuasca are so minimal, the differences may be the result of unrelated physical activity of the participants. Such changes might be put into perspective by a study conducted by Hartley, Lovallo & Whitselt [29]. A beverage containing a 230 mg dose of caffeine (equivalent to two or three 150 ml cups of instant coffee) resulted in only negligible increases in heart rate (4 bpm) and blood pressure (5 mmHg). Later in the same study, the researchers asked the 77 study participants to deliver a 3-minute speech 'to a video camera in front of 2 experimenters wearing white coats' ([29], p. 1023). Under this anxiety-producing condition, the average increase in heart rate was 30 bpm; the systolic pressure increased an average of 28 mmHg and the diastolic pressure increased an average of 21 mmHg. These increases are more than twice those occurring with oral DMT.

It is generally held that the β-carbolines in ayahuasca are reversible and highly selective inhibitors of MAO [30]. The ayahuasca-induced blockade of MAO presumably allows a larger quantity of serotonin to accumulate in nerve terminals. Excessive accumulation can produce a range of adverse physiological symptoms, a 'serotonin syndrome', that includes tremor, diarrhea, autonomic instability, hyperthermia, sweating, muscle spasms and possible death [31].

A few irreversible or non-selective MAO inhibitors (e.g. phenelzine, tranylcypromine) are associated strongly with instances of severe serotonin syndrome, but a large number of opiates, analgesics, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and anti-migraine agents have also been implicated [31]. Individuals who have recently used ginseng, St John's wort, dextromethorphan or 3,4-methylenedioxymethamphetamine (MDMA: ecstasy) should be cautious about using ayahuasca. Physical discomfort or chronic pain (e.g. backache) may be exacerbated by ayahuasca, so potential users should be warned of this side effect.

Table 3 lists six reported instances in which there were severe toxic reactions to a psychoactive tryptamine and/or a β-carboline. The death of a 25-year-old male caused by amine intoxication [32] involved 5-MeO-DMT in addition to DMT and β-carboline. No laboratory animal studies were located during the present review that compared the toxicity of orally active 5-MeO-DMT with orally active DMT. However, 5-MeO-DMT is reported to be more potent than DMT when smoked [5] or taken orally with harmaline [4]. Experienced researchers have cautioned that these substances should not be casually interchanged in ayahuasca-like preparations. The male decedent ingested an unknown quantity of 'herbal tonics' that presumably contained MAOIs in addition to the DMT and 5MeO-DMT because he was found to have a blood concentration of tetrahydroharmine three times higher than that found among 14 volunteers in a UDV hoasca study [2]. The autopsy was performed the day after the body was discovered, so post-mortem drug redistribution makes it difficult to determine what the peak cardiac or peripheral blood concentration of tetrahydroharmine or 5-MeO-DMT might have actually been.

The briefly described fatality of a 71-year-old diabetic female, listed in Table 3, reported that only tobacco leaves and *B. caapi* were the constituents of the so-called 'ayahuasca' brew [33]. The abstract did not document the concentration of any β-carbolines, but did report measurements (at unstated time intervals) of 1900 ng/ml and 710 ng/ml of blood nicotine. These concentrations exceed by at least 20 times the average post-mortem nicotine concentration (34 ng/ml) reported by Ekwald & Clemedson [34]. 'The cause of death [of the 71-year-old] was attributed to acute nicotine intoxication' ([33], p. 287). Administration of the unorthodox nicotine/β-carboline brew by enema to this older person, who was a non-smoker, precludes the relevance of this case to typical ayahuasca/hoasca use.

The quantity of *P. harma* seeds consumed as a single intoxicant by the two individuals cited in Table 3 [36,37] should be distinguished from the lower quantity of *B. caapi* or *P. harmala* that is used customarily in an ayahuasca brew. These individuals ingested 15–50 times more β-carboline alkaloids than the 3 g needed to maintain the oral activity of DMT in an ayahuasca preparation [5]. Herraiz [38] has noted that coffee may act as a MAOI, and the caffeine used by one of the decedents probably intensified the effect of the harmala seeds.

Table 3 Acute toxic reactions to ayahuasca-related compounds.

Age/sex	*Primary*	*Estimated*	*Effects*	*Toxicological*	*Factors*	*Reference*
25 M	5-MeO-DMT	Unknown quantity of 'herbal tonics'	Death caused by 'amine intoxication'	5-Meo-DMT 1.88 mg/l, THH 0.38 mg/l, harmine 0.17 mg/l	Camping in park, autopsy examination unremarkable	[32]
71 F	Ayahuasca brew	Not reported	Death caused by 'nicotine intoxication'	Nicotine 1900 mg/l and 710 mg/l	Non-smoker, no emesis, brew administered by enema	[33]
36 M	Ayahuasca brew	100 ml of brew	Gross motor tremors, confusion, symptoms resolved without treatment after 4 hours	No blood chemistry reported	Daily use of 20 mg of oral fluoxetine	[35]
35 M	*Peganum harmala*	150 g of *P. harmala* seeds	Heart rate 100 bpm, bp 80/40 mmHg, convulsion, stable in few hours after infusion NaCl/glucose solution	β-carbolines not reported	Gastric ulcer, mild anemia but other parameters normal	[36]
27 F	*P. harmala*	50 g of *P. harmala* seeds	Hallucinations, GI disturbance, bradycardia, resolved in a few hours	β-carbolines, caffeine level not reported	Seeds ingested with coffee[1]	[37]
20–50[2]	Harmine, atropine scopolamine	A brew of harmine 27 mg, atropine atropine 4 mg, scopolamine 78 mg	Tachycardia, coma, amnesia, hallucination, all patients recovered with supportive treatment	Harmine 179 mg/l, atropine 27 mg/l scopolamine 515 mg/l	Ingested alkaloids had 'synergistic actions'	[39]

[1]Coffee brews contain β-carboline alkaloids. [2]'More than 30 people aged 20–50 years'. No information provided regarding sex or health status of patients.

The last item listed in Table 3 involved more than 30 individuals between the ages of 20 and 50 years who 'were participating in a meditation session named "releasing autohypnosis of forest medicine men"' ([39], p. 50). The published report does not detail the number of participants who were hospitalized, their gender or their health status following the ingestion of 100–200 ml of herbal tea, although all the patients recovered satisfactorily. Fatalities from atropine or scopolamine are rare, and from harmine are extremely rare. The reported dose of atropine (4 mg) and of scopolamine (78 mg) received by the meditation session participants is eight to 13 times less than the estimated minimal lethal dose of these substances [40,41]. The severe reactions of at least a few of the individuals who required mechanical ventilation was attributed by the report authors to the combined synergistic actions of harmine, atropine and scopolamine. This incident provides additional evidence of the potential danger of combining pharmacologically active substances in non-traditional ways.

To this author's knowledge, there have been no deaths caused by hoasca or any other traditional DMT/β-carboline ayahuasca brews. The probability of a toxic overdose of ayahuasca is seemingly minimized by serotonin's stimulation of the vagus nerve which, in turn, induces emesis near the level of an effective ayahuasca dose. The risk of overdose appears to be related primarily to the concurrent or prior use of an additional serotonergic substance. People who have an abnormal metabolism or a compromised health status are obviously at a greater risk than the normal population, and might prudently avoid the use of ayahuasca preparations.

Psychological dysfunction

The general sequence of psychological and cognitive responses to ayahuasca is dose-dependent and predictable; however, the reactions of any particular individual at any one session are not. With a medium dose of DMT, objects in the environment appear to vibrate and increase in brightness. Rapidly moving patterns and scenes emerge that are visible with eyes either open or closed. This experience is referred to as being a 'visionary state' ([42], p. 109). It should be noted that these altered perceptions and cognitions do not usually cause users to become unaware of their surroundings or lose the ability to speak coherently. Many users walk to the restroom as a result of diarrhea.

Riba and colleagues [20] described the reactions of six volunteers after they each received doses of 0.05, 0.75 and 01.0 mg/kg of ayahuasca. Results obtained on Addiction Research Center Inventory (ARCI) scales showed that as the doses increased, emotions of happiness, sadness, awe and amazement also increased. At medium and high doses, the volunteers agreed that the experience seemed similar to a dream and that the sense of self and the passing of time were deeply affected. For example, the awareness between individuals can become so interlinked that typical self-centered judgements about the contents of another person's statement are simply absent. With respect to time perception, some people report that during an ayahuasca session they are swept into a conscious state where the usual orderly progression of time becomes obviously non-existent, and they experience 'eternity' ([43], p. 45). This sensation/perception was often associated with the emotion of well-being, but it can also be accompanied by feelings of terror. Virtually all data sources indicate that the DMT or ayahuasca experience has a substantial degree of unpredictability with respect to both aversive and positive aspects, depending on variables such as dosage, participant's intention and setting. Reactions during ayahuasca ceremonies have ranged from profound calmness [44] to anguished cries for forgiveness [45].

In interviews with 150 informants, Shannon [46] reported that, even among individuals without academic education or philosophical training, the ayahuasca experience prompted users to reflect seriously on the phenomena of life, nature and human consciousness. A pilot study by Grob and colleagues [47] comparing 15 members of the UDV with a control group of 15 non-members revealed that the UDV members showed more rigidity, regimentation, reflection and word-recall on various tests than non-members. According to Grob ([47], p. 90), 'all of the 15 UDV subjects claimed that their experience with ritual use of hoasca as a psychoactive ritual sacrament had had a profound [positive] impact on the course of their lives'. Most of the UDV members had a history of moderate to severe alcohol use prior to joining the UDV. This variable was not reported for the control group, so the generalization of this study's result to the general population is uncertain.

Certain perceptual characteristics of the ayahuasca experience overlap those of schizophrenia, and a few researchers have reported that urinary or blood levels of DMT are above normal in schizophrenic individuals [48,49]. This has led to the hypothesis that the non-destruction of dimethylated indolealkyalamines such as DMT could play a role in progressive deterioration of cognitive processes [50]. Other studies have produced conflicting results and alternative conclusions [51,52]. Jacob & Presti ([53], p. 935) proposed that increased DMT, acting at the G-protein-coupled trace amine receptor, might actually serve in schizophrenics as 'a homeostatic response to calm or suppress psychotic activity, rather than exacerbate it'. At present, the proposition that endogenous DMT in schizophrenia is related biochemically to ayahuasca-induced states of consciousness remains a speculative hypothesis.

The hallucinogenic effect of ayahuasca and other tryptamine derivatives can precipitate severe adverse psychological reactions, and this is especially true when administered outside established ceremonial practices [54]. For example, two cases of unsupervised use of 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT, 'foxy') have been reported in which an unknown amount of 5-MeO-DIPT caused sensory hallucinations requiring several hours of hospitalization [55,56]. Transient psychotic episodes are also known to occur with high doses of psilocybin [57] and LSD [58]. LSD, psilocybin and 5-MeO-DIPT are, however, orally active and more potent than DMT; thus the comparability of these three substances with DMT is somewhat problematic.

Over a period of 5 years, the medical studies section of the UDV documented between 13 and 24 cases in which ayahuasca might have been a contributing factor in a psychotic incident ([13], p. 701). The incidents documented by the UDV occurred from an estimated total of 25 000 servings of the hoasca tea. Although the prevalence rate of psychosis or schizophrenia among adults in the United States varies according to the way in which diagnostic criteria are applied, the generally accepted estimate is approximately 1.3% [59]. A reported UDV rate of psychotic episodes under 1% suggests that the use of hoasca is not a triggering event for sustained psychosis. Many or most of the UDV psychotic episodes were transient in nature and resolved spontaneously ([13], p. 623).

Dependence and abuse potential

The extent to which ayahuasca might lead to physiological dependence or compulsive drug-seeking is an

important public health concern. The physiological dependence potential of DMT and ayahuasca remains to be documented convincingly. Hallucinogenic substances structurally similar to DMT are rarely used in a compulsive manner that would meet the dependence criteria of the *Diagnostic and Statistical Manual* version IV (DSM-IV) [60]. No studies were located during the present review that reported that the termination of DMT resulted in an abstinence syndrome.

With respect to drug tolerance, however, a few non-human animal studies have been conducted that show varying degrees of tolerance to behavioral and physiological responses over a period of 3 or 4 weeks [61–63]. Little or no tolerance to emotional or autonomic effects was reported in one study, where human volunteers were administered DMT four times at 30-minute intervals [64], and in another study where DMT was administered twice daily for 5 days [52].

Despite the presumed absence of physiological dependency and tolerance, ayahuasca might, nonetheless, function as a positive reinforcer leading to significant abuse potential. We would want to know, for example, what proportion of individuals who try DMT or ayahuasca once or twice fall into a pattern of chronic use that they then find difficult to quit. The UDV has reported that 15–20% of first-time participants in hoasca ceremonies become UDV members ([13], p. 700). This subsequent participation in the UDV church is within the reported range of first-time visitors who become members of Christian churches in the United States [65].

Future consumption patterns of ayahuasca are difficult of determine because, in part, the frequency of present use in the general population is so low, but its general psychopharmacological profile suggests that it lacks the abuse potential of amphetamines, cocaine, opiates or other widely abused substances. Indeed, accounts of any strong and sustained reinforcing effects of tryptamine compounds are rare in experimental literature. More typically, non-human animal studies conclude that administration of tryptamine derivatives such as mescaline, psilocybin or DMT result in erratic patterns of self-administration indicating that 'these compounds have weak reinforcing effects, or alternatively, mixed reinforcing and aversive effects' ([66], p. 156).

DISCUSSION

Any attempt to characterize the possible acute adverse health effects of ayahuasca is hampered by the very limited number of relevant scientific studies of DMT and β-carboline decoctions. This situation invites an easy commingling of facts, speculations, inferences, biases, conjectures and hidden agendas. Arguments made in the course of litigation are especially prone to linguistic obfuscation of empirical weaknesses. None the less, legal and policy decisions must be made in the light of what almost always seems to be insufficient evidence. Data available at this time indicate that the acute systemic toxicity of ayahuasca is, by comparison, substantially less than alcohol. The average acute lethal dose of ethyl alcohol is well-documented at approximately 330 g [67], 10 times the normal recreational dose. The acute lethal dose of ayahuasca was calculated in this review as 20 times the effective dose. This safety margin is similar to codeine, mescaline and methadone [9]. Previous estimates of the LD_{50} of DMT have been as high as 40 or 50 times the customary dose [9,68]; the difference is primarily a result of varying the safety factors that are used to extrapolate data from non-human laboratory animal studies to humans.

No acute health hazards, excluding potential serotonergic reactions, have been documented as a routine, serious threat from ayahuasca when ingested within the range of customary dosages. Possible chronic health effects of ayahuasca were not considered in the present paper.

Most public attention is focused on hazards that we want to avoid, such as accidents, heart attacks or bankruptcy. However, there are hazards that people accept, perhaps grudgingly, because they perceive a potential benefit—for instance, crossing a street against the traffic light, speeding to a hospital with a sick child, agreeing to an adjustable mortgage. Andritzky [69] has described the traditional use of ayahuasca in Amazonia during healing rituals. A more contemporary North American illustration was provided by a university administrator who reported that he previously considered Schedule I drugs to be a taboo option until he was diagnosed with metastatic liver cancer [70]. When potential benefits are considered, the option with the least risk is not necessarily the best choice.

Indeed, some risks are not merely accepted, but actively sought. Examples include hang-gliding, road racing and alcohol drinking contests. Recently, the internet has become a favored source of information for individuals seeking drug-related experiences. Boyer, Shannon & Hibberd [71] have profiled 12 adolescents in a drug treatment program who reported being influenced detrimentally by drug information, including a description of Syrian rue, that they found on the internet. However, the internet also contains descriptions of intoxicated misadventures that might serve as a cautionary tale (e.g. a self-reported ayahuasca overdose caused by the user's self-described 'swaggering arrogance' ([72], p. 1). Virtually all poisoning reports with tryptamine/MAOI mixtures involve individuals who prepared their own brew and/or who ingested an additional psychoactive substance [35,39,72,73].

The relative lack of abuse potential of ayahuasca in social settings seems very plausible. The unpredictable occurrence of frightening images and thoughts, plus predictable nausea and diarrhea, makes it a very unlikely candidate for a 'club drug'.

Finally, it might be noted that the discipline of toxicology has its own queasiness—especially about spiritual concepts such as 'transcendence', 'ineffability' and 'grace' that often appear in descriptions of ayahuasca sessions by physically and psychologically healthy individuals [74]. Many reported experiences are similar to descriptions of *samadhi* in advaitan Hinduism, *satori* in Zen Buddhism or *beatific vision* in Christianity. Such alleged beneficial experiences lie outside the pathology-oriented realm of toxicology, but not necessarily or completely beyond the scientific requirement of falsifiability. Variations in consciousness are, at least in theory, worthy of serious scientific study because of their central place in human endeavors.

Acknowledgements

This review was funded, in part, by the Life Science Research Group, Inc., an independent non-profit research trust.

References

1. *Gonzales* v. *O Centro Espirita Beneficiente Uniao Do Vegetal* 546 US 2006. US Supreme Court, no. 04–1084, decided 21 February 2006.
2. Callaway J. C., McKenna D. J., Grob C. S., Brito G. S., Raymon L. P., Poland R. E. Pharmacokinetics of h*oasca* alkaloids in healthy humans. *J Ethnopharmacol* 1999; **65**: 243–56.
3. McKenna D. J., Callaway J. C., Grob C. S. The scientific investigation of ayahuasca: a review of past and current research. *Heffter Rev Psychedel Res* 1998; **1**: 65–76.
4. Ott J. Pharmahuasca: human pharmacology of oral DMT plus harmine. *J Psychoact Drugs* 1999; **31**: 171–7.
5. Shulgin A., Shulgin A. *TIHKAL. The Continuation*. Berkeley: CA. Transform Press; 1997.
6. Schultes R. E., Hofmann A. *Plants of the Gods: Origins of Hallucinogenic Use*. New York: Alfred van der Marck Editions; 1992.
7. Santo Daime. Religion of the forest: What is our religion? (n.d.) Available at: http://www.santodaime.org/doctrine/whatis.htm (accessed 23 September 2005).
8. Metzner R., editor. *Ayahuasca: Human Consciousness and the Spirits of Nature*. New York: Thunder's Mountain Press; 1999.
9. Gable R. S. Comparison of acute lethal toxicity of commonly abused psychoactive substances. *Addiction* 2004; **99**: 686–96.
10. Gable R. S. Acute toxic effects of club drugs. *J Psychoact Drugs* 2004; **36**: 303–13.
11. Seymour R., editor. Ayahuasca: cross-cultural perspectives. *J Psychoact Drugs* 2005;Special Issue: 37.
12. Strassman R. *DMT: The Spirit Molecule*. Rochester, VT: Park Street Press; 2001.
13. Joint Appendix, *O Centro Espirita Beneficiente Uniao Do Vegetal et al.* v. *John Ashcroft*, no. CV02-2323, on Writ of Certiorari to the US Court of Appeals for the 10th Circuit, New Mexico, 2002 .
14. Callaway J. C., Brito G. S., Neves E. S. Phytochemical analyses of *Banisteriopsis caapi and Psychotria viridis*. *J Psychoact Drugs* 2005; **37**: 145–50.
15. McKenna D. J., Towers G. H., Abbott F. S. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and *B*-carboline constituents of ayahuasca. *J Ethnopharmacol* 1984; **10**: 195–223.
16. Rivier L., Lindgren J. *Ayahuasca*, the South American hallucinogenic drink: ethnobotanical and chemical investigations. *Econ Bot* 1972; **29**: 101–29.
17. Pomilio A. B., Vitale A. A., Ciprian-Ollivier J. Cult-hoasca: a model for schizophrenia. *Mol Med Chem* 2003; **1**: 1–7.
18. Callaway J. C. Various alkaloid profiles in decoctions of *Banisteriopsis caapi*. *J Psychoact Drugs* 2005; **37**: 151–5.
19. Primack W. A. Caffeine consciousness. *Arch Pediatr Adolesc Med* 2004; **158**: 1092.
20. Riba J., Rodriguez-Fornells A., Urbano G., Morte A., Antonijoan R., Montero M. *et al.* Subjective effects and tolerability of the South American psychoactive beverage *ayahuasca* in healthy volunteers. *Psychopharmacology (Berl)* 2001; **154**: 85–95.
21. Trevan J. The error of determination of toxicity. *Proc Roy Soc Sect B* 1927; **101**: 438–514.
22. Gad S. C., Chengelis C. P. *Acute Toxicology Testing*, 2nd edn. San Diego, CA: Academic Press; 1998.
23. ChemIDplus Advanced. N,N-dimethyltryptamine or serotonin. Bethesda, MD: National Library of Medicine, Specialized Information Services; 2005. Available at: http://chem.sis.nim.nih.gov/chemidplus/jsp/common/Toxicity.jsp (accessed 25 September 2005).
24. Gillin J. C., Tinklenberg J., Stoff D. M., Stillman R., Shortlidge J. S., Wyatt R. J. 5-methoxy- N,N-dimethyltryptamine: behavioral and toxicological effects in animals. *Biol Psychiatry* 1976; **11**: 355–8.
25. Lamchouri F., Settaf A., Cherrah Y., El Hamidi M., Tigui N., Lyoussi B. *et al.* Experimental toxicity of *Peganum harmala* seeds. *Ann Pharm Fr* 2002; **60**: 123–9.
26. Neubert D. Risk assessment and preventative hazard minimization. In: Marquardt H., Schafer S. G., McClellan R., Welsch F., editors. *Toxicology*. San Diego, CA: Academic Press; 1999, p. 1153–89.
27. Strassman R., Qualls C. R. Dose–response study of N,N-Dimethyltryptamine in humans. I. Neuroendocrine, autonomic, and cardiovascular effects. *Arch Gen Psychiatry* 1994; **51**: 85–97.
28. Aarons D. H., Rossi G. V., Orzechowski R. F. Cardiovascular actions of three harmala alkaloids: harmine, harmaline, and harmalol. *J Pharm Sci* 1977; **66**: 1244–8.
29. Hartley T. R., Lovallo W. R., Whitsett T. L. Cardiovascular effects of caffeine in men and women. *Am J Cardiol* 2004; **93**: 1022–6.
30. Riba J., Barbanoj M. J. Bringing ayahuasca to the clinical research laboratory. *J Psychoact Drugs* 2005; **37**: 219–29.
31. Boyer E. W., Shannon M. The serotonin syndrome. *N Engl J Med* 2005; **352**: 1112–20.
32. Sklerov J., Levin B., Moore K. A., King T., Fowler F. A fatal intorxication following the ingestion of 5 methoxy-*N,N*-dimethyltryptamine in an ayahuasca preparation. *J Anal Toxicol* 2005; **29**: 838–41.

33. Warren R. J. Fatal intoxication resulting from the ingestion of 'ayahuasca'. *J Anal Toxicol* 2004; **28**: 287 [abstract].
34. Ekwall B., Clemedson C. *Time-Related Lethal Blood Concentrations Form Acute Human Poisoning of Chemicals.* Part 2. *The Monographs*, no. 18. *Nicotine.* Stockholm: Scandinavian Society for Cell Toxicology; 1997.
35. Callaway J. C., Grob C. S. Ayahuasca preparations and serotonin reuptake inhibitors: a potential combination for severe adverse interactions. *J Psychoact Drugs* 1998; **30**: 367–9.
36. Mahmoudian M., Jalipour H., Salehian P. Toxicity of *Peganum harmala*: review and a case report. *Iran J Pharmacol Ther* 2002; **1**: 1–4.
37. Salah N. B., Amamou M., Jerbi Z., Salah F. B. Un cas de surdosage en *Peganum harmala* [A case of overdosage with *Peganum harmala*]. *J Toxicol Clin Exp* 1986; **6**: 319–22.
38. Herraiz T. Human monoamine oxidase is inhibited by tobacco smoke. *Biochem Biophys Res Commun* 2005; **326**: 378–86.
39. Balikova M. Collective poisoning with hallucinogenous [sic] herbal tea. *Forensic Sci Int* 2002; **128**: 50–2.
40. Lewis R. J. *Sax's Dangerous Properties of Industrial Materials*, 8th edn. New York: Van Norstrad Reinhold Co.; 1994 [CD-ROM].
41. Smith E. A., Meloan C. E., Pickell J. A., Oehme F. W. Scopolamine poisoning from homemade 'moon flower' wine. *J Anal Toxicol* 1991; **15**: 216–19.
42. Schinzinger A. Mysterious tea. In: Roberts T. B., editor. *Psychoactive Sacramentals: Essays on Entheogens and Religion*. San Francisco, CA: Council on Spiritual Practice; 2001, p. 103–12.
43. Shanon B. Altered temporality. *J Conscious Stud* 2001; **8**: 35–58.
44. Barbosa P. C., Giglio J. S., Dalgalarrondo P. Altered states of consciousness and short-term psychological after-effects induced by the first time ritual use of ayahuasca in an urban context in Brazil. *J Psychoact Drugs* 2005; **37**: 193–201.
45. Lovetree I. M. Liquid plum'r for the soul. In: Metzner R., editor. *Ayahuasca: Human Consciousness and the Spirits of Nature*. New York: Thunder's Mouth Press; 1993, p. 116–23.
46. Shanon B. The divine within. *J Conscious Stud* 2001; **8**: 91–6.
47. Grob C. S., McKenna D. J., Callaway J. C., Brito G. S., Neves E. S., Oberlaender G. *et al.* Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *J Nerv Ment Dis* 1996; **184**: 86–94.
48. Checkley S. A., Murray R. M., Oon M. C., Rodnight R., Birley J. L. A longitudinal study of urinary excretion of N,N-dimethyltryptamine in psychotic patients. *Br J Psychiatry* 1980; **137**: 236–9.
49. Lipinski J. F., Mandel L. R., Ahn H. S., Vanden Heuvel W. J., Walker R. W. Blood dimethyltryptamine concentrations in psychotic disorders. *Biol Psychiatry* 1974; **9**: 89–91.
50. Ciprian-Ollivier J., Cetkovich-Bakmas M. G. Altered consciousness states and endogenous psychoses: a common molecular pathway? *Schizophr Res* 1997; **28**: 257–63.
51. Forsstrom T., Tuominen J., Kakkainen J. Determination of potentially hallucinogenic N,N-dimethylated indoleamines in human urine by HPLC/ESI-MS-MS. *Scand J Clin Lab Invest* 2001; **61**: 547–56.
52. Gillin J. S., Kaplan J., Stillman R., Wyatt R. J. The psychedelic model of schizophrenia: the case of N,N-dimethyltryptamine. *Am J Psychiatry* 1976; **133**: 203–8.
53. Jacob M. S., Presti D. E. Endogenous psychoactive tryptamines reconsidered: an anxiolytic role for dimethyltryptamine. *Med Hypoth* 2005; **64**: 930–7.
54. de Rios M. D. Interview with Guillermo Arrevala, a Shipibo urban shaman, by Roger Rumrrill. *J Psychoact Drugs* 2005; **37**: 203–7.
55. Meatherall R., Sharma P. Foxy, a designer tryptamine hallucinogen. *J Anal Toxicol* 2003; **27**: 313–7.
56. Wilson J. M., McGeorge F., Smolinske S., Meatherall R. A foxy intoxication. *Forensic Sci Int* 2005; **148**: 31–6.
57. Hyde C., Glancy G., Omerod P., Hall D., Taylor G. S. Abuse of indigenous psilocybin mushrooms: a new fashion and some psychiatric complications. *Br J Psychiatry* 1978; **132**: 602–4.
58. Klock J. C., Boerner U., Becker C. E. Coma, hyperthermia, and bleeding associated with massive LSD overdose: a report of eight cases. *Clin Toxicol* 1975; **8**: 191–203.
59. Department of Health and Human Services. *Mental Health*. A report of the Surgeon General. Washington, DC: Author; 1999.
60. Morgenstern J., Langenbucher J., Labouvie E. W. The generalizability of the dependence syndrome across substances: an examination of some properties of the proposed DSM-IV dependence criteria. *Addiction* 1994; **89**: 1105–13.
61. Cole J. M., Pieper W. A. The effects of N,N-dimethyltryptamine on operant behavior in squirrel monkeys. *Psychopharmacology* 1973; **29**: 107–13.
62. Gillin J. C., Cannon E., Magyar R., Schwartz M., Wyatt R. J. Failure of N,N-dimethyltryptamine to evoke tolerance in cats. *Biol Psychiatry* 1973; **7**: 213–20.
63. Kovacic B., Domino E. F. Tolerance and limited cross-tolerance to the effects of N,N-dimethyltryptamine (DMT) and lysergic acid diethylamide-25 (LSD) on food-rewarded bar pressing in the rat. *J Pharmacol Exp Ther* 1976; **197**: 495–502.
64. Strassman R. J., Qualls C. R., Berg L. M. Differential tolerance to biological and subjective effects of four closely spaced doses of N,N-dimethyltryptamine in humans. *Biol Psychiatry* 1994; **39**: 784–95.
65. Miller H. *How to Build a Magnet Church*. Nashville, TN: Abingdon Press; 1987.
66. Fantegrossi W. E., Woods J. H., Winger G. Transient reinforcing effects of phenylisopropylamine and indolealkylamine hallucinogens in rhesus monkeys. *Behav Pharmacol* 2004; **15**: 149–57.
67. Jones A. W., Holmgren P. Comparison of blood-ethanol concentrations in deaths attributed to acute alcohol poisoning and chronic alcoholism. *J Forensic Sci* 2003; **48**: 874–9.
68. Carvalho J. E., Costa M., Dies P. C., Antonio. M. A., Brito A. R. Pharmacological effects of the decoction (hoasca) of *Banisteriopsis caapi* and *Psychotria viridis* in guinea pigs. Referenced in: Callaway J. C., editor. A report from the International Conference of Hoasca Studies, Rio de Janeiro, 2–4 November 1995. Available at: http://www.maps.org/news-letters/v06n3/06336udv.html (accessed 30 January 2006).
69. Andritzky W. Sociopsychotherapeutic functions of ayahuasca healing in Amazonia. *J Psychoact Drugs* 1989; **21**: 77–89.
70. Topping D. M. Ayahuasca and cancer: one man's experience. *Bull Multidisc Assoc Psychedel Stud* 1998; **8**: 22–6.
71. Boyer E. W., Shannon M., Hibberd P. L. The internet and psychoactive substance use among innovative drug users. *Pediatrics* 2005; **115**: 302–6.

72. Ontological meltdown. Lycaeum website (n.d.). Available at: http://leda.lycaeum.org/Trips/Ontological_Meltdown.4972.shtml (accessed 21 October 2005).
73. Brush D. E., Bird S. B., Boyer E. W. Monoamine oxidase inhibitor poisoning resulting from Internet misinformation on illicit substances. *J Toxicol Clin Toxicol* 2004; **42**: 191–5.
74. Roberts T. B. *Psychoactive Sacramentals: Essays on Entheogens and Religion*. San Francisco, CA: Council on Spiritual Practices; 2001.
75. De Marderosian A. H., Pinkley H. V., Dobbins M. F. Native use and occurence [sic] of N,N- dimethyltryptamine in the leaves of *Banisteriopsis rusbyana*. *Am J Pharm* 1968; **140**: 137–47.
76. Riba J. *Human pharmacology of ayahuasca*. Doctoral thesis, Departament de Farmacologia i Terapeutica. Barcelona, Spain: Universitat Autonoma de Barcelona; 2003.
77. Riff D. P., Join A. C., Doyle J. T. Acute hemodynamic effects of ethanol on normal human volunteers. *Am Heart J* 1969; **78**: 592–7.
78. Foltin R. W., Haney M. Intranasal cocaine in humans: acute tolerance, cardiovascular and subjective effects. *Pharmacol Biochem Behav* 2004; **78**: 93–101.
79. Azorlosa J. L., Heishman S. J., Stitzer M., Mahaffey J. M. Marijuana smoking: effects of varying Δ^9–tetrahydrocannabinol content and number of puffs. *J Pharmacol Exp Ther* 1992; **261**: 114–21.
80. Lester S. J., Baggott M., Welm S., Schiller N. B., Jones R.T., Foster E. *et al.* Cardiovascular effects of 3,4-methylenedioxy methamphetamine: a double-blind, placebo-controlled trial. *Ann Intern Med* 2000; **133**: 969–73.

Transworld Research Network
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

The Ethnopharmacology of Ayahuasca, 2011: 55-63 ISBN: 978-81-7895-526-1
Editor: Rafael Guimarães dos Santos

3. An overview of the literature on the pharmacology and neuropsychiatric long term effects of ayahuasca

José Carlos Bouso and Jordi Riba
Human Experimental Neuropsychopharmacology and Centre d'Investigació de Medicaments (CIM-Sant Pau), Institut d'Investigacions Biomèdiques Sant Pau (IIB-Sant Pau), Barcelona, Spain

Abstract. The last two decades have seen a steady increase in the number of publications devoted to ayahuasca. This fascinating psychotropic plant tea has attracted the attention of biomedical and psychological scientists. Researchers have gathered data on the physiological impact of ayahuasca administration to humans and have assessed the consequences of long term regular use. Acute administration studies have provided information on the fate of the alkaloids in the organism, the modification of vital signs, neuroendocrine and immunological parameters and neurophysiological variables. For the first time, neuroimaging techniques have shown us which brain areas "light up" during the most intense phases of the ayahuasca experience. Also, the popularization of ayahuasca has raised concerns that its regular use may cause neuropsychiatric and addiction-related problems for

Correspondence/Reprint request: Dr. Jordi Riba, Human Experimental Neuropsychopharmacology and Centre d'Investigació de Medicaments (CIM-Sant Pau), Institut d'Investigacions Biomèdiques Sant Pau (IIB-Sant Pau), Barcelona, Spain. E-mail: JRiba@santpau.cat

users. An increasing number of studies have tried to address these concerns. In the present chapter we aim to give an overview of the available literature on the human pharmacology of acute ayahuasca intake and on the neuropsychiatric and psychosocial consequences of its long-term use.

1. The human pharmacology of ayahuasca

By temporarily modifying serotonergic neurotransmission, ayahuasca exerts a powerful action on the central nervous system. These neurochemical modifications constitute the basis of the unique experience reported by users. Scientific inquiry into the workings of ayahuasca, and all psychoactive drugs in general, is greatly advanced by the study of these substances in the only species that can accurately report on the diverse facets of the psychedelic-induced experience, i.e., human beings. By administering these compounds in known dosages to carefully selected individuals valuable information has been obtained on their impact on the human body and psyche.

The clinical investigation of ayahuasca was initiated by a field study conducted in the early 1990s by Callaway, Grob, McKenna and colleagues, and aimed to assess the subjective and physiological impact of acute ayahuasca administration in regular users. In the study a 2 ml/kg dose of ayahuasca was given to a group of 15 long-term members of a Brazilian ayahuasca church known as União do Vegetal. Subjective effects were measured with the Hallucinogen Rating Scale (HRS; an instrument originally developed by Strassman and colleagues[1] to assess the effects of intravenous dimethyltryptamine [DMT])[2]. The authors also assessed various physiological parameters and the pharmacokinetic profile of ayahuasca alkaloids[3]. The HRS measurements provided information on six different spheres of the psychedelic-induced experience: *Somaesthesia*, reflecting somatic effects; *Affect*, sensitive to emotional and affective responses; *Volition*, indicating the volunteer's degree of incapacitation; *Cognition*, describing modifications in thought processes or content; *Perception*, measuring visual, auditory, gustatory, and olfactory experiences; and, finally, *Intensity*, which reflects the strength of the overall experience. Scores on the six subscales showed that at the administered dose ayahuasca was able to induce distinct psychedelic effects, with the intensity of the experience falling on "the mild end of the spectrum when contrasted to the highly potent, short-acting intravenous DMT"[2].

This pioneering study found measurable plasma levels of the four main alkaloids (DMT, harmine, harmaline and tetrahydroharmine [THH]), described their pattern of variation with time and how this related with time-dependent modifications which for the most part reached a maximum

between one and two hours and had disappeared at 24 hours after ayahuasca ingestion[3].

More recently, research has been conducted by Riba and coworkers at the Hospital de Sant Pau in Barcelona. Since 1999 this team has performed a series of studies which have tried to better characterize the neuropharmacological profile of ayahuasca. The group started by analyzing the psychometric characteristics of the HRS and obtaining subjective ratings of ayahuasca[4]. A pilot study was then undertaken to assess the tolerability of ayahuasca within a range of dosages, from which safe and pharmacologically effective doses were selected for subsequent studies involving a larger number of volunteers[5]. A method to determine ayahuasca alkaloids in plasma was perfected to now include several metabolites which had not been measured previously in humans[6]. These initial efforts were followed by a series of clinical trials which have provided information on the pharmacokinetics, the subjective and cardiovascular effects of different doses of ayahuasca[7], and various psychophysiological measures[8-11]. For the first time in the centuries-long history of ayahuasca use, the neuroimaging study conducted by this team[12] identified the brain areas specifically involved in the genesis of ayahuasca effects. A review of their studies and findings between 1999 and 2004 can be found in Riba and Barbanoj (2005)[13]. In the last five years the same group has conducted additional studies on the sleep, neuroendocrine and immunological effects of ayahuasca, as well as on the pharmacology of repeated ayahuasca intake. Several of these studies are still pending publication[14].

As would be expected from conventional drugs and somewhat in contrast to popular belief, when administered in a clinical setting and carefully controlling for expectancy (blind designs) ayahuasca was found to act in a dose-dependent manner. This was the case for physiological (cardiovascular), pharmacokinetic and psychological variables (assessed with the HRS; with the ARCI – Addiction Research Centre Inventory, another rating scale to assess subjective effects of drugs; and with VAS – Visual Analogue Scales, a simple method to assess subjective effects of drugs consisting of 100-mm horizontal lines with different labels as “any effect”, “good effects”, “liking”, “visions”, etc., that subjects must mark depending on the intensity of a given effect as experienced while under the effects of the drug). In these studies, DMT plasma concentrations reached their peak coinciding with the maximum intensity of the subjective effects. An unexpected result was the very low levels of harmine found in plasma for the majority of participants. This is suggestive of intense metabolism and also indicative that, at least in some people, the contribution of harmine to the overall central effects of ayahuasca would be small[7]. Ayahuasca induced cardiovascular effects, basically consisting of elevations of

diastolic blood pressure. While these increases were moderate, caution should be exerted by people who have elevated blood pressure or other cardiovascular problems. This is even more relevant considering recent reports in the media concerning the unexplained deaths of people participating in ayahuasca rituals[15-17]. It should be noted that the clinical data which has been published to date is from young healthy volunteers. Safety results might be different in older people or individuals with pre-existing conditions.

At the psychophysiological level ayahuasca induces significant effects, shifting the energy distribution in the electroencephalogram (EEG), i.e., the spontaneous electrical activity of the brain, towards the higher end of the power spectrum. This shift towards the so-called faster frequencies of the EEG can be measured as an increase in the relative power of the EEG beta band[10]. While this effect can be interpreted as reflecting enhanced Central Nervous System (CNS) activity, this activation is unique to psychedelics and different from that induced by traditional psychostimulants. Unpublished data from a study comparing ayahuasca with *d*-amphetamine (*d*-AMPH), a classical psychostimulant enhancing dopaminergic and noradrenergic neurotransmission, show that *d*-AMPH has no effect whatsoever on relative beta power[14]. Whereas both drugs share some sympathomimetic effects, such as increasing pupillary diameter and elevating blood pressure, the distinct effects of ayahuasca on the EEG would relate to its specific serotonergic mechanism. Differences in neurochemical mechanism are also evidenced by ayahuasca, but not *d*-AMPH, significantly increasing prolactin levels (a hormone whose release is enhanced by serotonergic drugs and inhibited by dopaminergic drugs). Despite these differences, both ayahuasca and *d*-AMPH induce a stress-like reaction increasing cortisol levels, the increment induced by ayahuasca being higher. Another interesting finding is that ayahuasca is able to modulate the cell immune system. This effect appears to be non-specific as both ayahuasca and amphetamine induce similar time-dependent modifications on lymphocyte subpopulations: the percentages of CD4 and CD3 cells decrease, while the percentage of NK cells increase. These changes reach a maximum at around 2 hours post-administration and return to baseline levels at 24 hours[14]. No studies have yet assessed the possible impact of these acute physiological modifications on the health of long term ayahuasca users.

Perhaps the most interesting finding from the mentioned clinical trials is the identification of the brain areas where ayahuasca acts. Using the neuroimaging technique SPECT (single photon emission tomography) researchers found that ayahuasca acts almost exclusively on the cerebral cortex without acting on subcortical areas. Ayahuasca increases the activity of the anterior insula bilaterally, with greater intensity in the right

hemisphere. It also hyperactivates the anterior cingulate/frontomedial cortex of the right hemisphere, areas previously known to be implicated in somatic awareness, subjective feeling states, the processing of emotional information and emotional arousal. Additional increases were observed in the left amygdala/parahippocampal gyrus, structures also involved in emotional arousal and the processing of memories[12].

2. Neuropsychiatric long term effects of ayahuasca

Since personality and neuropsychological function are to a great extent regulated by the prefrontal cortex, the study of personality, psychopathological status and neuropsychological functions in long term ayahuasca users is essential to ascertain whether regular ayahuasca use has some impact on mental health.

A few studies have been conducted assessing the consequences of regular ayahuasca use in the long term. The data available is limited and would need replication in larger samples. One preliminary study led by Charles Grob assessed personality and neuropsychological function using the TPQ (Tridimensional Personality Questionnaire) and the WHO-UCLA Auditory Verbal Learning Test. The questionnaires were administered to a sample of 15 regular users with more than 10 years of experience with ayahuasca and to a comparison group of 15 non-users. No personality alterations or neuropsychological deficits were found in the ayahuasca-using subjects, though there were personality differences between groups, which the authors did not interpret as pathological. No information was given as to whether the scores fell within the normal range according to normative data[2]. A typical problem with this kind of studies lies in the interpretation of results. It is difficult to establish whether the scores obtained with the TPQ reflect the impact of ayahuasca use or rather pre-use personality. In the study by Grob and coworkers the authors also used the structured psychiatric interview known as CIDI (Composite International Diagnostic Interview) and found that 11 out of the 15 participants had a history of moderate to severe past alcohol use. Five of them reported episodes of associated violent behavior and a diagnosis of alcohol abuse disorder prior to their involvement with an ayahuasca church. Four subjects also reported previous use of other drugs of abuse, including cocaine and amphetamines, and 8 of the 11 subjects who had a history of alcohol and other drug use and misuse were addicted to nicotine at the time of their first ayahuasca session. According to the authors, all these addiction problems resolved after they began their regular use of ayahuasca. Ayahuasca participants did not meet diagnostic criteria either for

addiction or for any other psychiatric disorder at the moment of the assessment.

A recent study on 32 regular ayahuasca users belonging to the Igreja do Santo Daime in Oregon, USA, did not find psychiatric alterations as measured by a series of rating scales and compared to normative US data. As occurred in the study by Grob and colleagues[2], most of the ayahuasca users had shown some psychiatric disorder or some drug or alcohol abuse disorder in the past, which at the time of the assessment was not present. This was interpreted again as a direct benefit of participating in the Santo Daime ceremonies[18]. Since the subjects in this study were not compared with matched non-users, the findings should be interpreted with caution.

Finally, two papers have been published regarding the long term psychopathological and neuropsychological effects of regular ayahuasca in adolescents. Each study involved 40 adolescents with a two-year history of ayahuasca use, and a comparison group of 40 matched non-users. No statistical differences were found in psychopathology scores[19] or in measures of neuropsychological function[20]. In sum, while no deletereous effects have been demonstrated, due to the small number of studies conducted on regular ayahuasca users the potential impact of sustained ayahuasca use on mental health remains an open question.

3. Additional studies

Several studies have assessed the impact of acute ayahuasca on psychological traits and measures, psychopathology, personality and spirituality, all in a naturalistic context.

In one study of first-time users of ayahuasca in the ritual context of the Brazilian churches of the Santo Daime (19 subjects) and the União do Vegetal (nine subjects), significant reductions of minor psychiatric symptoms and positive changes in behavior were found in the four days following ayahuasca use[21]. Another study found reductions in the scores of panic and hopelessness one hour after ayahuasca ingestion, as compared to baseline[22]. Still in another study, 49 participants without previous experience with ayahuasca attended different ayahuasca ceremonies after which quantitative and qualitative assessments of spiritual experiences were conducted. The rating scales measuring changes in spirituality were not significantly modified after the sessions, though many subjects experienced spiritual themes according to the qualitative data obtained[23]. One study did a six-month follow-up of participants who had consumed ayahuasca for the first time in the context of a Brazilian ayahuasca church (Santo Daime and União do Vegetal). The study found a general improvement in several

psychological measures at the end of the study. Most of the subjects continued to consume ayahuasca after the six-month study period. Additionally, the authors found positive correlations between ayahuasca use and positive psychological attitudes. Ayahuasca use did not appear to cause any adverse effects[24]. Finally, a naturalistic study found that ayahuasca alters binocular rivalry, a perceptual measure of cognitive processing[25].

One recently published study used the Addiction Severity Index (ASI) as a measurement instrument. The ASI is a semi-structured interview designed to assess the impact of drug use in a multi-dimensional fashion. It assesses the participant's Medical Status, Employment/Support, Drug and Alcohol Use, Legal Status, Family/Social Relationships, and Psychiatric Status, and provides general information on the participant's current condition and his/her level of deterioration. The ASI was administered to two different samples of regular ayahuasca users. This study assessed the largest sample studied to date, i.e., a total of 112 regular ayahuasca users. These users belonged to two different ayahuasca churches – the Santo Daime and the Barquinha – and they were assessed in two different settings – jungle and urban-based, respectively. They had a 15-year history of use and they were compared with 115 matched controls. Assessments were repeated one year later as a follow-up. The study concluded that "the ritual use of ayahuasca, as assessed with the ASI in currently active users, does not seem to be associated with the psychosocial problems that other drugs of abuse typically cause"[26]. This research group recently presented a conference paper[27] reporting that they did not find evidence of neuropsychological deficits or personality and psychiatric disorders in their sample.

4. Final remarks

Though no serious adverse events were attributed either to acute or chronic ayahuasca use in the published studies reviewed, a note of caution should be made regarding ayahuasca safety. The clinical trials cited in this chapter were performed in healthy young volunteers who had extensive experience in psychedelic drug use and did not present any sequelae derived from this use. The conclusions cannot be extrapolated to the general population, and especially not to ayahuasca-naive individuals. Ayahuasca has shown to moderately increase several cardiovascular parameters and such increases could have deletereous effects on people with cardiovascular conditions. Furthermore, although only one subject in the clinical trials suffered an episode of disorientation, a case report describes a patient who presented a psychotic breakdown after acute ayahuasca intake. Antipsychotic

medication was needed until its remission, and the same individual suffered a second psychotic crisis after subsequent ayahuasca use[28]. Other cases of psychiatric adverse psychiatric reactions, including psychotic disorders, have been reported following acute ayahuasca ingestion[29]. It is necessary to take into account the anecdotal evidence available on its potential dangers in order to get a complete picture of the possible negative psychiatric consequences.

Regarding the studies of long term effects, it should be noted that the participant samples studied in the reviewed papers may have suffered from a self-selection bias. This would mean that the assessed individuals may have been those who did not experience any negative neuropsychiatric consequences derived from their maintained ayahuasca use. Subject experiencing adverse consequences might have given up ayahuasca use altogether and would consequently not be accessible to researchers.

To conclude, the scientific investigation of ayahuasca has only found a moderate risk associated to acute ayahuasca administration and has even reported psychological improvements after long-term use. Future investigation into the neuropsychiatric safety of regular ayahuasca use should ideally also include people who used ayahuasca regularly in the past but decided to discontinue its use.

References

1. Strassman, R.J., Qualls, C.R., Uhlenhuth, E.H., and Kellner, R. 1994, Arch. Gen. Psychiatry, 51, 98.
2. Grob, C.S., McKenna, D.J., Callaway, J.C., Brito, G.S., Neves, E.S., Oberlaender, G., Saide, O.L., Labigalini, E., Tacla, C., Miranda, C.T., Strassman, R.J., and Boone, K.B. 1996, J. Nerv. Ment. Dis., 184, 86.
3. Callaway, J.C., McKenna, D.J., Grob, C.S., Brito, G.S., Raymon, L.P., Poland, R.E., Andrade, E.N., Andrade, E.O., and Mash, D.C. 1999, J. Ethnopharmacol., 65, 243.
4. Riba, J., Rodríguez-Fornells, A., Strassman, R.J., and Barbanoj, M.J. 2001, Drug Alcohol Depend., 62, 215.
5. Riba, J., Rodríguez-Fornells, A., Urbano, G., Morte, A., Antonijoan, R., Montero, M., Callaway, J.C., and Barbanoj, M.J. 2001, Psychopharmacology (Berl), 154, 85.
6. Yritia, M., Riba, J., Ortuño, J., Ramirez, A., Castillo, A., Alfaro, Y., de la Torre, R., and Barbanoj, M.J. 2002, J. Chromatogr. B., 779, 271.
7. Riba, J., Valle, M., Urbano, G., Yritia, M., Morte, A., and Barbanoj, M.J. 2003, J. Pharmacol. Exp. Ther., 306, 73.
8. Barbanoj, M.J., Riba, J., Clos, S., Giménez, S., Grasa, E., and Romero, S. 2008, Psychopharmacology (Berl), 196, 315.
9. Riba, J., Rodríguez-Fornells, A., and Barbanoj, M.J. 2002, Psychopharmacology (Berl), 165, 18.

10. Riba, J., Anderer, P., Morte, A., Urbano, G., Jané, F., Saletu, B., and Barbanoj, M.J. 2002, Br. J. Clin. Pharmacol., 53, 613.
11. Riba, J., Anderer, P., Jané, F., Saletu, B., and Barbanoj, M.J. 2004, Neuropsychobiology, 50, 89.
12. Riba, J., Romero, S., Grasa, E., Mena, E., Carrió, I., and Barbanoj, M.J. 2006, Psychopharmacology (Berl), 186, 93.
13. Riba, J., and Barbanoj, M.J. 2005, J. Psychoactive Drugs, 37, 219.
14. Santos, R.G., Valle, M., Bouso, J.C., Nomdedéu, J.F., Rodríguez-Espinosa, J., McIlhenny, E.H., Barker, S.A., Barbanoj, M.J., and Riba, J. 2011. [Submitted]
15. Neto, J.S. 2009, Polícia de Goiás investiga morte de universitário após tomar chá do Santo Daime. O Globo, November 18.
16. Gomes, H. 2010, A encruzilhada do Daime. Isto É, February 05.
17. Vera, G.H. 2010, Hombre que quería “arreglar su matrimonio”, murió tomando yagé. El Espacio, November 16.
18. Halpern, J.H., Sherwood, A.R., Passie, T., Blackwell, K.C., and Ruttenber, A.J., 2008. Med. Sci. Monit., 14, SR15.
19. Da Silveira, D.X., Grob, C.S., de Rios, M.D., Lopez, E., Alonso, L.K., Tacla, C., and Doering-Silveira, E., 2005. J. Psychoactive Drugs 37, 129.
20. Doering-Silveira, E., Lopez, E., Grob, C.S., de Rios, M.D., Alonso, L.K., Tacla, C., Shirakawa, I., Bertolucci, P.H., and Da Silveira, D.X., 2005. J. Psychoactive Drugs, 37, 123.
21. Barbosa P.C., Giglio J.S., and Dalgalarrondo, P. 2005, J Psychoactive Drugs, 37, 193.
22. Santos, R.G., Landeira-Fernandez, J., Strassman, R.J., Motta, V., and Cruz, A.P. 2007, J. Ethnopharmacol., 112, 507.
23. Trichter, S., Klimo, J., and Krippner, S. 2009, J. Psychoactive Drugs, 41, 121.
24. Barbosa, P.C., Cazorla, I.M., Giglio, J.S., and Strassman, R. 2009, J. Psychoactive Drugs, 41, 205.
25. Frecska, E., White, K.D., and Luna, L.E. 2004, Psychopharmacology (Berl), 173, 79.
26. Fábregas, J.M., González, D., Fondevila, S., Cutchet, M., Fernández, X., Barbosa, P.C., Alcázar-Córcoles, M.A., Barbanoj, M.J., Riba, J., and Bouso, J.C. 2010, Drug Alcohol Depend., 111, 257.
27. Fábregas, J.M. Long Term Effects on Mental Health of Ayahuasca Ritual Use. Psychedelic Science in the 21st Century. MAPS, San Jose, CA, April 16-18.
28. Santos, R.G., and Strassman, R.J. 2008, Br. J. Psychiatry (Online), 3 December. [*See the final article in the present book*]
29. Lima, F,. Naves, M., Motta, J., Migueli, J., Brito, G., et cols. 2002, Rev. Bras. Psiquiatr., 24, suppl. 2.

International Center for Ethnobotanical
Education, Research & Service
Nieuwe Zandstraat 4
4661AP Halsteren
Netherlands
tel.: +31624841232
email: info@iceers.org
www.iceers.org

Literature Research conducted by
José Carlos Bouso
jcbouso@gmail.com