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ORIGINAL INVESTIGATION

Pharmacology of ayahuasca administered in two repeated doses

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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.

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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

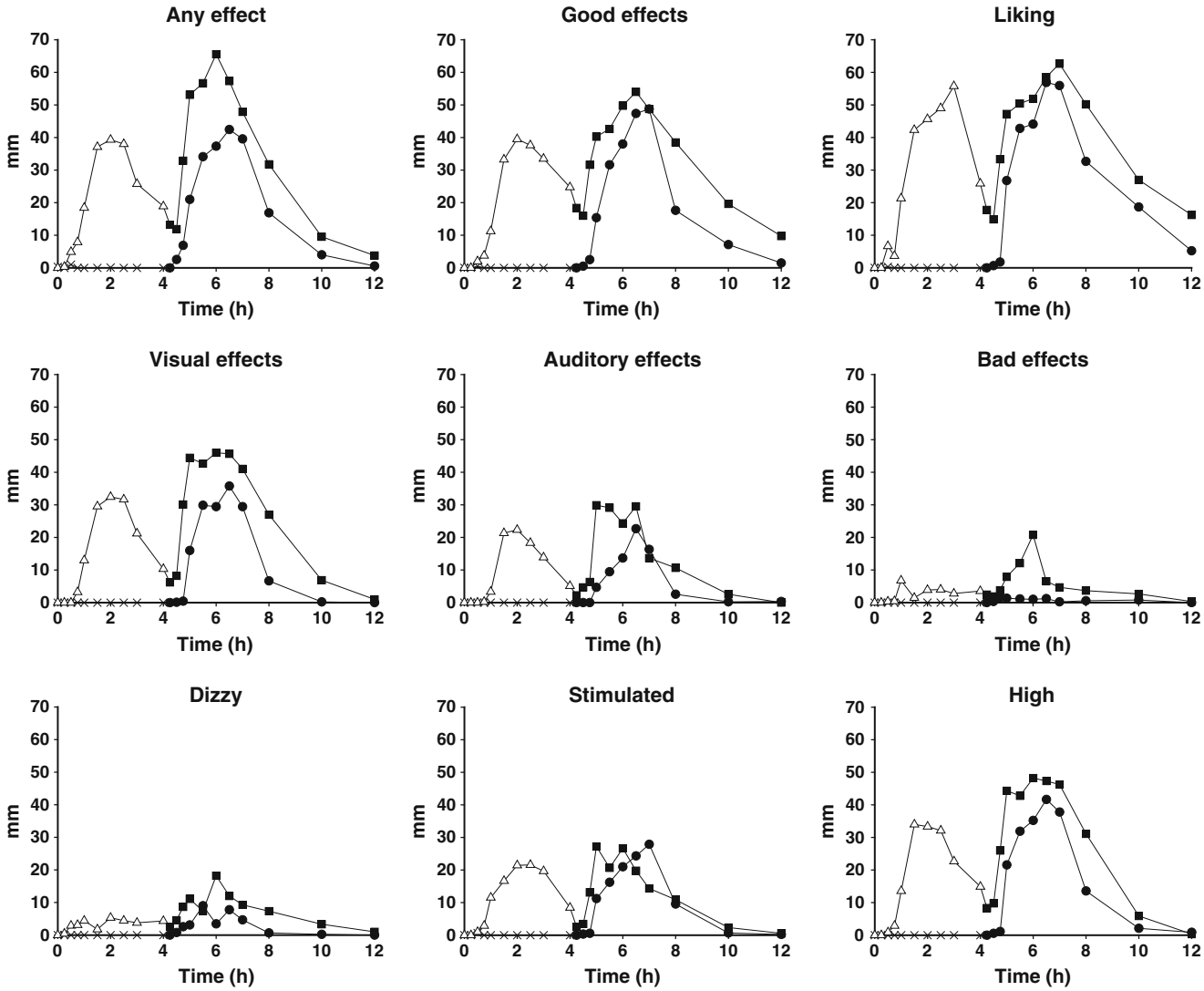

Fig. 1 Time course of scores on the nine VAS items (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*) and *Aya2* (*filled square*). *Aya0* was preceded 4 h by the placebo 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 $p<0.1$

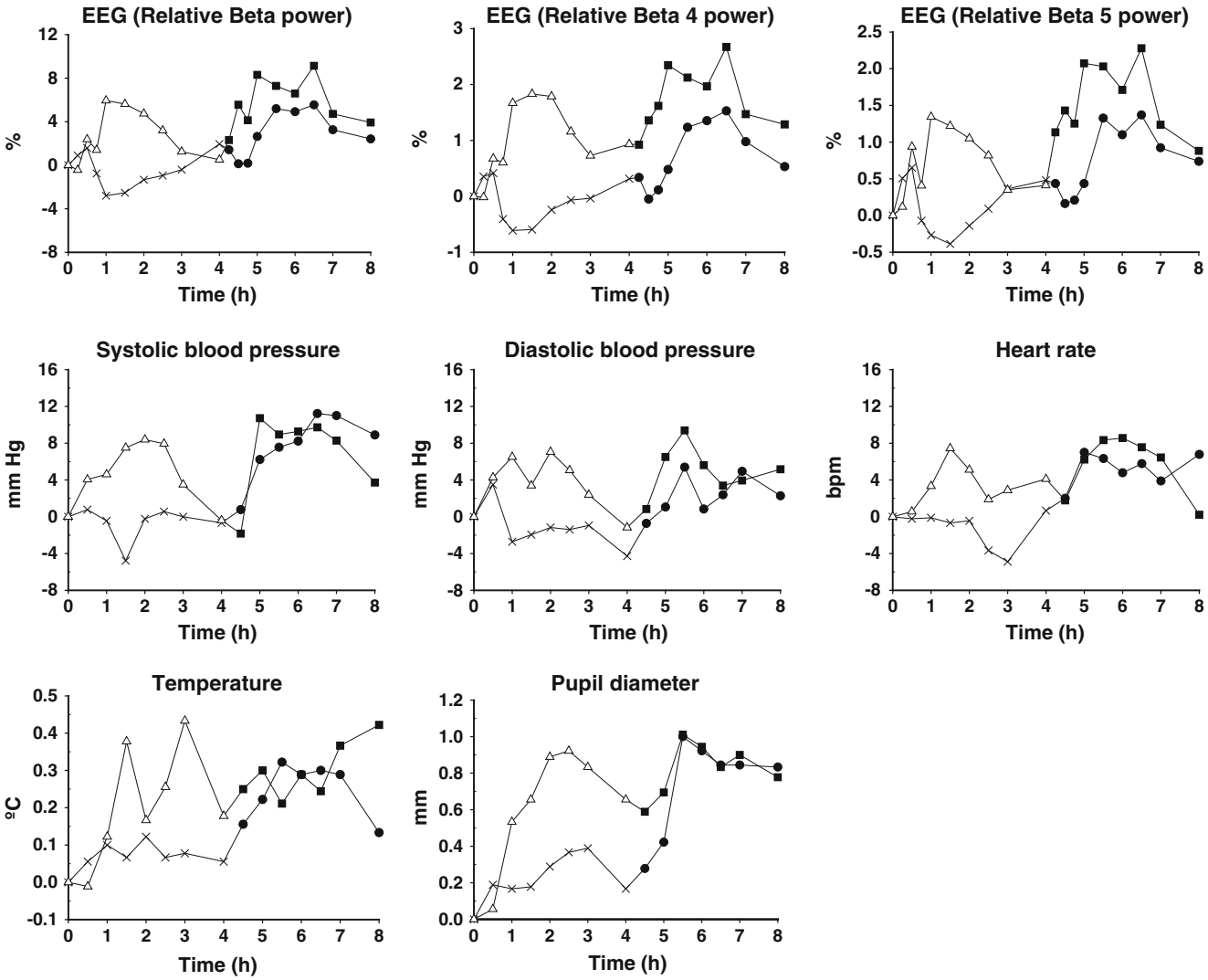

Fig. 2 Time course of electroencephalographic (EEG), cardiovascular and autonomic variables (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*filled square*). *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

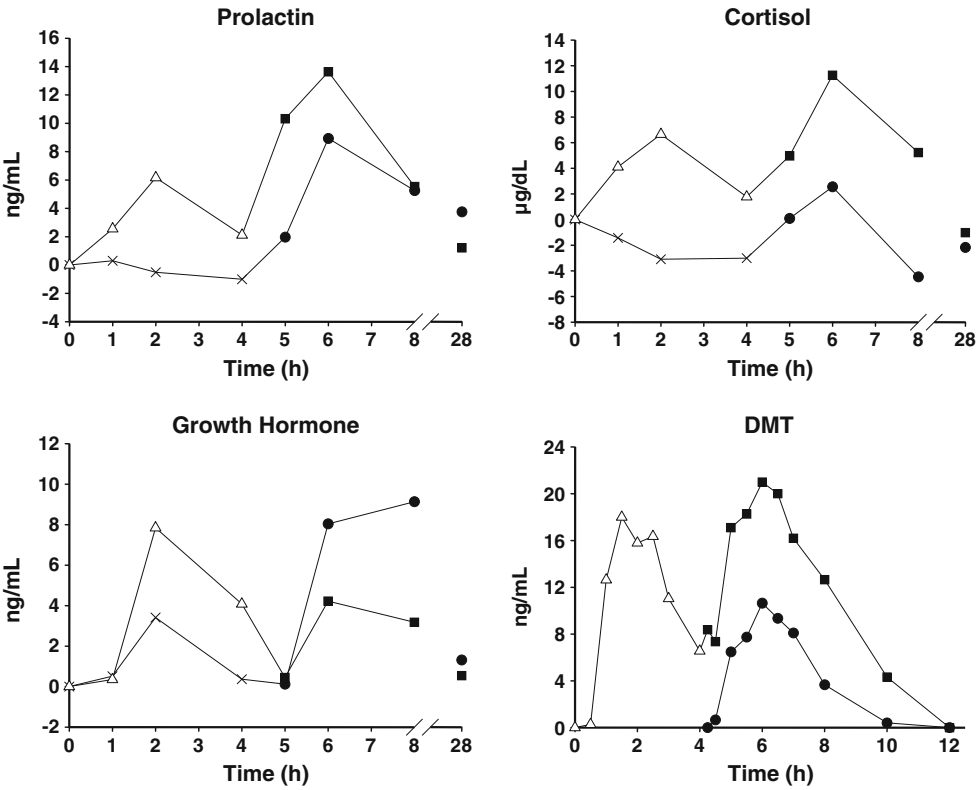

Fig. 3 Time course of neuroendocrine measures (means from nine volunteers) and DMT plasma concentrations (means from eight volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*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

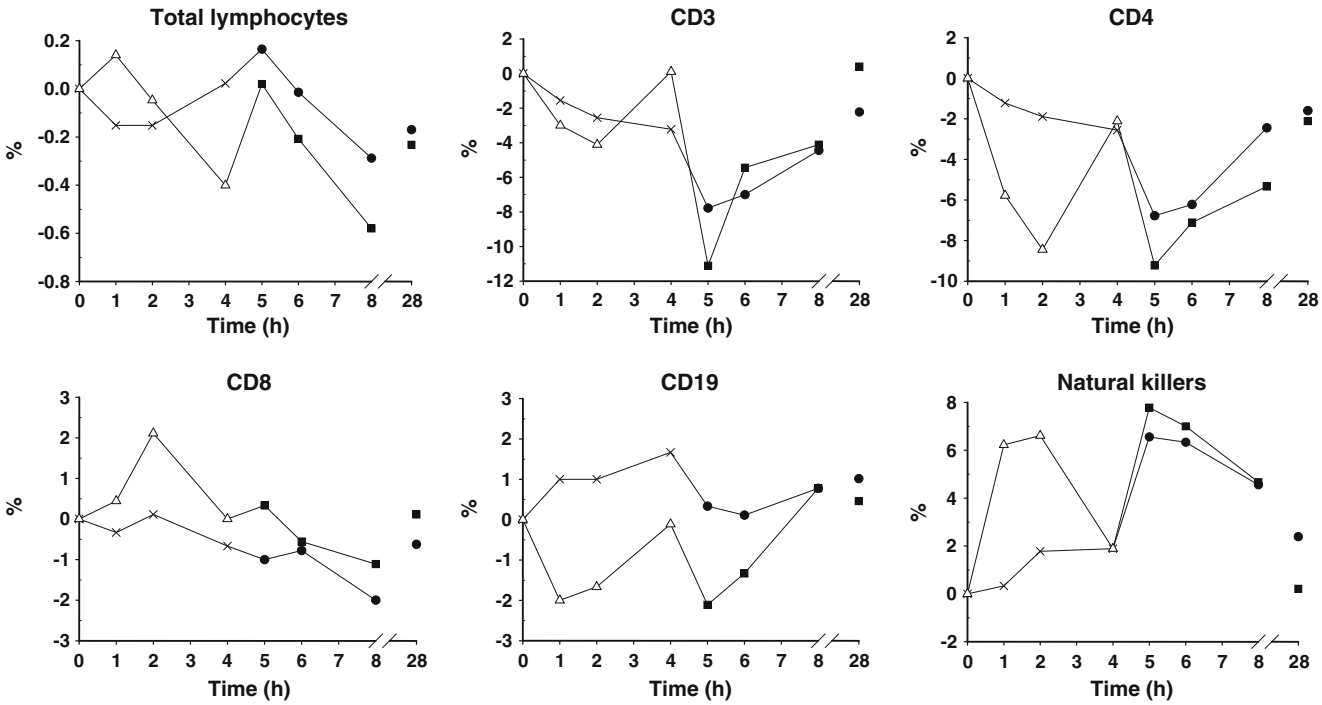

Fig. 4 Time course of effects on lymphocyte subpopulations (means from nine volunteers) after administration of placebo (*star*) and each of the three 0.75 mg DMT/kg body weight ayahuasca doses: *Aya1* (*open triangle*), *Aya0* (*filled circle*), and *Aya2* (*filled square*). *Aya0* was preceded 4 h 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.

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