Appendix I

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)

[In preparation]

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)

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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. The spatial distribution of *ayahuasca*-induced changes in brain electrical activity was investigated by means of low resolution electromagnetic tomography (LORETA). Electroencephalography (EEG) recordings were obtained from eighteen volunteers with prior experience in the use of psychedelics after the administration of a 0.85 mg DMT/kg body weight dose of encapsulated freeze-dried *ayahuasca* and placebo. The intracerebral power density distribution was computed with LORETA from spectrally analyzed data. Statistically significant differences with placebo were observed at 60 and 90 minutes after dosing. *Ayahuasca* decreased power density in the alpha-2, delta, theta and beta-1 frequency bands. This pattern of effects is analogous to that of the classical psychedelics and point out the involvement of 5-HT_{2A} receptor agonism in the neurochemical effects of *ayahuasca*. 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*.

Key words: Ayahuasca, DMT, EEG, Psychedelics, Brain electrical sources, LORETA, Human.

1. Introduction

The psychoactive plant tea known as *ayahuasca*, a Quechuan 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 (Schultes and Hofmann, 1982). In the second half of the last century, the use of *ayahuasca* reached the urban areas of Amazonian countries, where it is used by *ayahuasqueros* for healing and divination. However, modern non-indigenous use of *ayahuasca* mainly takes place within the context of syncretic religious groups, particularly the Brazilian *Santo Daime* and *União do Vegetal*, that have combined Old World religious beliefs with the sacramental use of the beverage (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 (Anonymous, 2000).

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 (THH), and to a lesser extent harmaline and traces of harmol and harmalol (Rivier and Lindgren, 1972; McKenna et al., 1984). *P. viridis* and *D. cabrerana* also contain indole alkaloids, mainly the potent short-acting psychedelic agent DMT (River and Lindgren, 1972). 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 (Smith et al., 1998).

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 (Ott, 1999), probably due to metabolism by the enzyme monoamine oxidase (MAO) (Suzuki et al., 1981). On the other hand, the β-carbolines present in *ayahuasca*, particularly harmine and harmaline, are potent natural MAO inhibitors (McKenna et al., 1984), apparently preventing the extensive gut and liver first-pass effect on DMT, which is subsequently able to reach unaltered systemic circulation and the CNS.

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 (Riba et al., 2001a).

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 (Pascual-Marqui et al., 1994). 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 (Pascual-Marqui et al., 1999). The technique has previously been used in the evaluation of acute effects of psychoactive drugs (Anderer et al., 2000; Frei et al., 2001).

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 a-priori hypothesis regarding the brain areas involved in the effects of *ayahuasca* on the EEG. However, PET and SPECT investigations on blood flow and glucose metabolism after acute psilocybin (an indoleamine structurally similar to DMT) and mescaline administration have evidenced increased activation in prefrontal regions (Hermle et al., 1992; Vollenweider et al., 1997a; Gouzoulis-Mayfrank et al., 1999). Consequently, we postulated that changes in electrical activity would be identified at least in the prefrontal cortex.

2. Materials and methods

2.1. Volunteers

Eighteen healthy volunteers (fifteen males and three females) participated in the study. Eligibility criteria in-

cluded prior experience with psychedelic drugs at least on five occasions without sequelae derived thereof, no current or previous history of neurological or psychiatric disorder, and no family history of 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 (STAI). Exclusion criteria included alcohol or other substance dependence, and high scores on trait anxiety (over 1 standard deviation above 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), 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 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.

2.2. 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 and coworkers (1996), and the β-carboline constituents following a modification of the method described therein. The 9.6 liter batch yielded 611 grams of freeze-dried powder, containing 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg THH per gram. Based on tolerability and subjective effects assessed previously (Riba et al., 2001a), 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 g, 0.25 g or 0.125 g of freeze-dried *ayahuasca.* Placebo capsules were 00 gelatin capsules containing 0.75 g lactose. These were administered on 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.

2.3. 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 hours prior to each experimental day. Urine was screened for illicit drug use on each experimental day. Experimental days were at least one week apart.

On each experimental day, volunteers remained in the clinical research unit for a period of approximately ten hours. 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 am with 250 ml tap water. EEG recordings were obtained at baseline and at regular intervals after treatment administration. For the first four hours, 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 eight hours and volunteers were discharged approximately nine hours after administration

3. Measurements

3.1. 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 (V-EEG) with eyes closed was recorded at baseline, prior to drug administration, and at different time points after dosing. 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 band-pass filtered at 0.3-30 Hz, and digitized online with a sampling frequency of 100 Hz. EEG recordings were obtained prior to drug administration (-15 min and baseline), and at 30, 60, 90, 120, 180, 360 and 480 minutes after dosing.

A two-step artifact processing procedure was used

(Anderer et al., 1992). It included ocular artifact minimization based on regression analysis in the time domain, as described by Semlitsch et al. (1986), and automatic artifact rejection based on a time and frequency domain approach as described by Anderer et al. (1987). Validity of the artifact processing procedure was visually assessed. After recomputation to average reference, spectral analysis was performed for artifact-free 5-s epochs. For each recording, spectral power of six 5-s epochs of artifact-free, vigilance-controlled EEG were averaged. Data were digitally filtered into seven frequency bands according to Kubicki et al. (1979): delta [1.5-6 Hz], theta [6-8Hz], 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 (Ary et al., 1981) registered to the Talairach human brain atlas (Talairach and Tournoux, 1988) available as a digitized MRI from the Brain Imaging Centre, Montréal Neurological Institute. The EEG electrode coordinates reported by Towle et al. (1993) were used for registration between spherical and realistic head geometry. The LORETA solution space was restricted to cortical gray matter and hippocampus, based on the Digitized Probability Atlas corresponding to the Talairach 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 2394 voxels with a spatial resolution of 0.343 cm^3 (Pascual-Marqui et al., 1999). The EEG lead field was computed numerically with the boundary element method (Pascual-Marqui, 1999). LORETA images represent the power (i.e., squared magnitude of computed intracerebral current density) in each of the 2394 voxels. "LORETA power", synonymous to "EEG power", refers to the spectral power of current density as estimated by 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 (for mathematical details see Frei et al., 2001).

3.2. Subjective effects

Volunteers were requested to answer the Hallucinogen Rating Scale (HRS), a self-report questionnaire measuring psychedelic-induced subjective effects (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 finally *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 HRS was administered at 240 minutes post-administration.

4. Statistical analysis

4.1. LORETA data

In a first step, in order to explore the time course of *ayahuasca* effects, paired-samples *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 non-parametric single-threshold test was applied on the basis of the theory for randomization and permutation tests developed by Holmes et al. (1996). 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 5000 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 (Lancaster et al., 1997), the number of significant voxels in each lobe (frontal, parietal, occipital, temporal, limbic and sub-lobar), gyrus, and Brodmann area (BA) of the left and the right hemisphere was computed separately for each suprathreshold region.

4.2. Subjective effects

Scores on the HRS questionnaire after *ayahuasca* were compared with placebo by means of paired-samples Student's *t*-tests.

5. Results

5.1. LORETA data

Fig. 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.

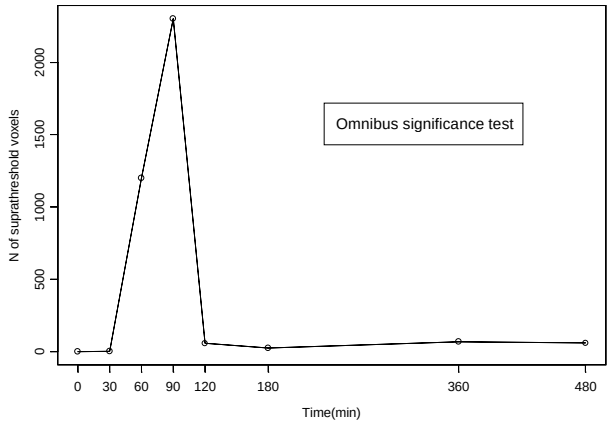

Fig. 1. Omnibus significance test. The total number of suprathreshold voxels at different time points after ayahuasca (0.85 mg DMT/kg body weight) administration are shown.

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

The voxel by voxel statistical comparison of *ayahuasca*-induced vs. placebo-induced effects at 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 (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.

Table 1
Ayahuasca- vs. placebo-induced decreases in Alpha-2 power (10-12 Hz) 60 min post-administration. 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).

Alpha-2 (10-12 Hz)	**Suprathreshold voxels**					
	Left hemisphere			**Right hemisphere**		
Gyrus	**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
Sub-gyral	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
Sub-lobar						
Insula	3	38	8	0	32	0

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

voxels, i.e., the pharmacodynamic peak, was observed at 90 min post-administration. At this time point statistically significant decreases were found for the delta, theta and beta-1 frequency bands. Alpha-2 power density was also

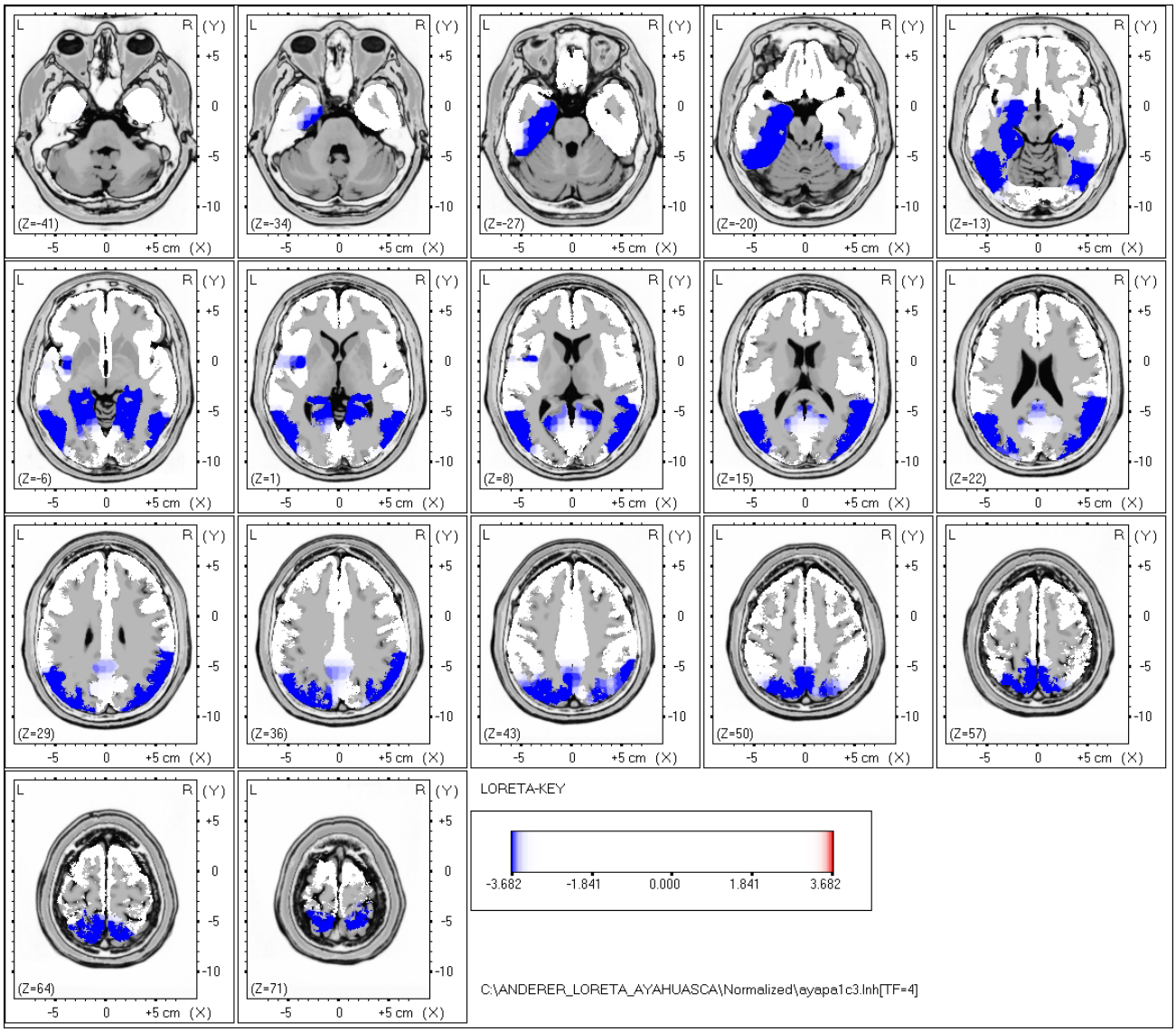

Fig. 2. Effects of ayahuasca (0.85 mg DMT/kg body weight) on regional cortical electrical activity at 60 min after administration (n=18): Statistical non-parametric maps based on t-values of differences between ayahuasca-induced and placebo-induced changes in the alpha-2 (10-12 Hz) frequency band. Blue 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).

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; cuttoff *t*-value =3.61). This local minimum was located in BA 24 in the medial frontal cortex.

As shown in Fig. 1, the largest number of suprathreshold

reduced relative to placebo, but contrary to what was observed at 60 min after administration these decreases were 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-occipi-

tal 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.

Fig. 3 shows LORETA axial brain slices as statistical non-parametric maps corresponding to the suprathreshold 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 non-confluent 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).

Fig. 4 shows LORETA orthogonal slices (axial, sagittal and coronal views) as statistical non-parametric maps corresponding to the three non-confluent 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 non-confluent 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).

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

Table 2
Ayahuasca- vs. placebo-induced decreases in Delta power (1.5-6 Hz) 90 min post-administration. 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).

Delta (1.5-6 Hz)	**Suprathreshold voxels**					
	Left hemisphere			**Right hemisphere**		
Gyrus	**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

5.2. 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 minutes, became more marked between 30 and 45, showed a steep rise at 60 minutes, reached a maximum

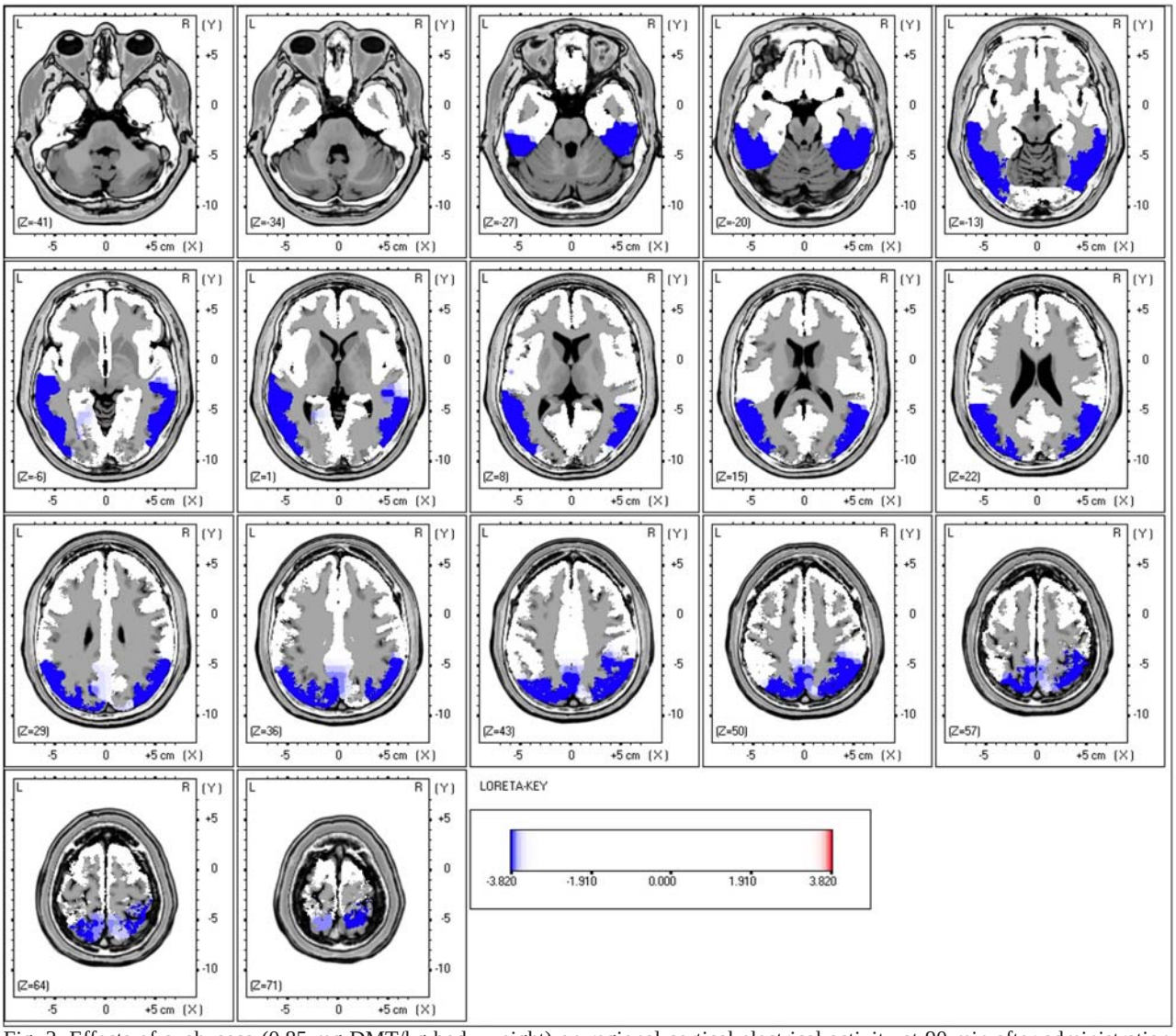

Fig. 3. Effects of ayahuasca (0.85 mg DMT/kg body weight) on regional cortical electrical activity at 90 min after administration (n=18): Statistical non-parametric maps based on t-values of differences between ayahuasca-induced and placebo-induced changes in the delta (1.5-6 Hz) frequency band. Blue 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).

Table 3
Ayahuasca- vs. placebo-induced decreases in Theta power (6-8 Hz) 90 min post-administration. 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).

Theta (6-8 Hz)	**Suprathreshold voxels**					
	Left hemisphere			**Right hemisphere**		
Gyrus	**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
Sub-gyral	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

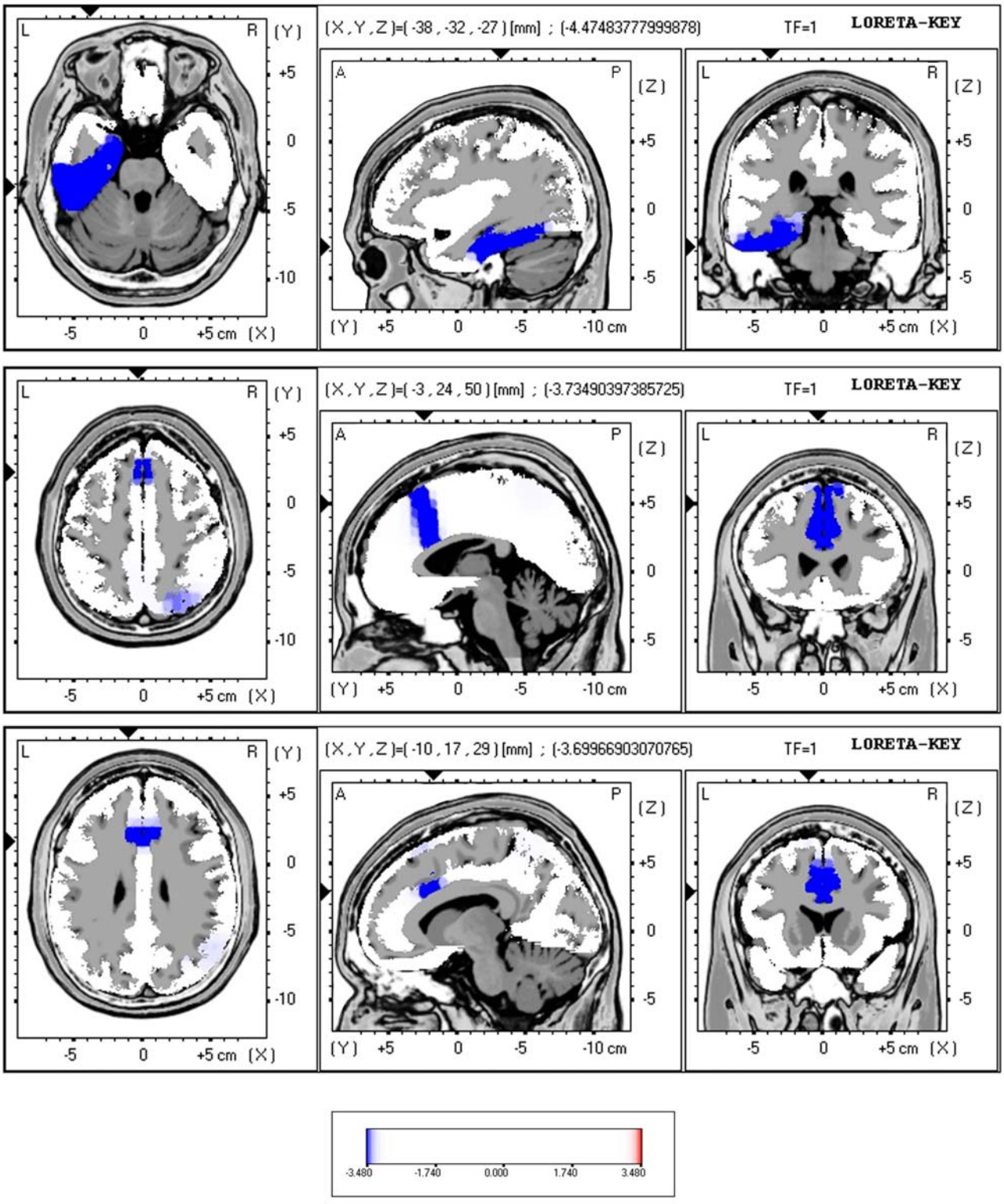

Fig. 4. Effects of ayahuasca (0.85 mg DMT/kg body weight) on regional cortical electrical activity at 90 min after administration (n=18): Statistical non-parametric maps based on t-values of differences between ayahuasca-induced and placebo-induced changes in the theta (6-8 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($P < 0.05$) as compared to placebo. Orthogonal (axial, sagittal, coronal) slices for each of the three non-confluent suprathreshold regions, i.e., temporomedial (upper row), frontomedial (middle row), and cingulate (lower row), through the voxel of the extreme t-value. 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.

Table 4
Ayahuasca- vs. placebo-induced decreases in Beta-1 power (12-18 Hz) 90 min post-administration. 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).

Beta-1 (12-18 Hz)	**Suprathreshold voxels**					
	Left hemisphere			**Right hemisphere**		
Gyrus	**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

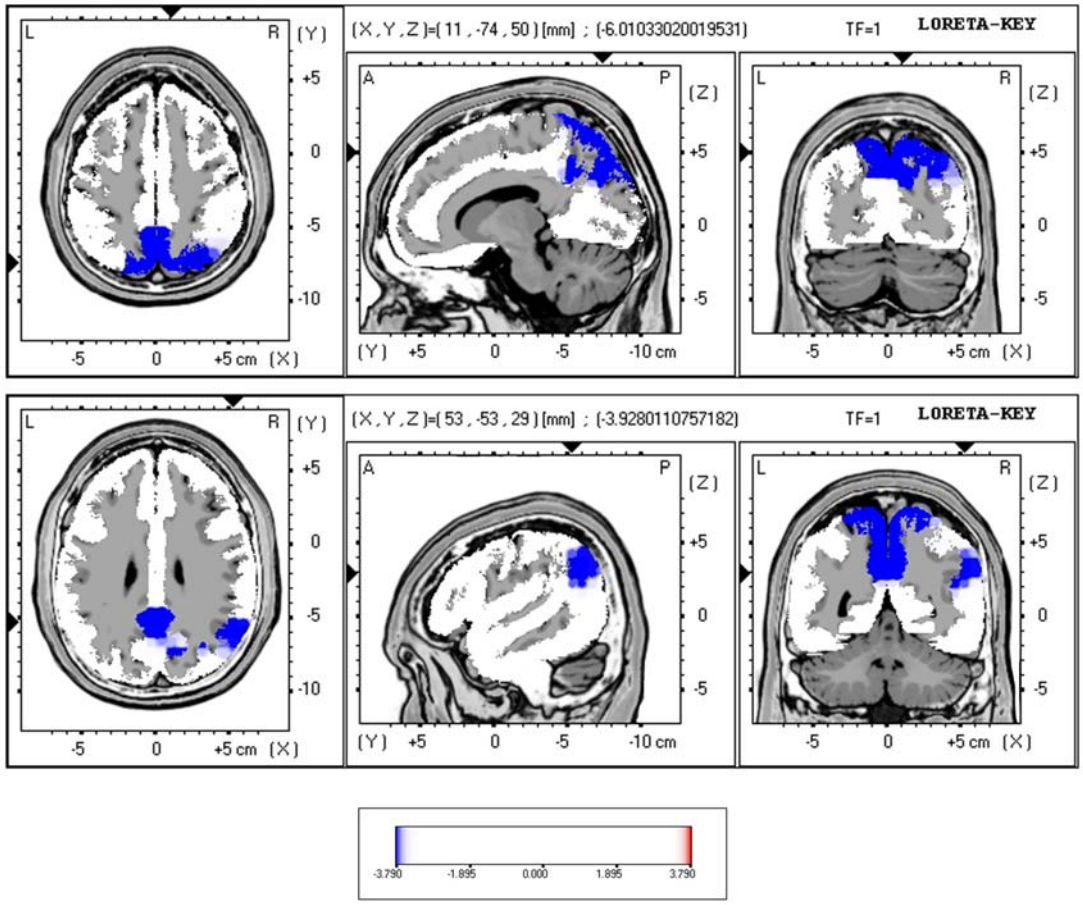

Fig. 5. Effects of ayahuasca (0.85 mg DMT/kg body weight) on regional cortical electrical activity at 90 min after administration (n=18): Statistical non-parametric maps based on t-values of differences between ayahuasca-induced and placebo-induced changes in the beta-1 (12-18 Hz) frequency band. Blue indicates significant decreases after Holmes correction ($P < 0.05$) as compared to placebo. Orthogonal (axial, sagittal, coronal) slices for each of the two non-confluent suprathreshold regions, i.e., parietomedial (upper row), and parietolateral (lower row), through the voxel of the extreme t-value. 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.

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

	HRS scores	
	Placebo	High dose
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) **

** = $P < 0.01$

between 90 and 120 minutes and decreased thereafter, disappearing entirely at 360 minutes. 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 greatly varied in quality and intensity between volunteers, ranging from distortions of objects and sounds, to elaborate visions with eyes closed and perception of non-existing 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.

6. Discussion

The analysis of brain electrical activity by means of LORETA identified significant drug-induced changes in the intracerebral power density distribution after 60 and 90 min following *ayahuasca* administration. At the peak of 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 statistical significance (theta), were also observed in analogous brain regions at 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 at 90 min post-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 in time course with previous results obtained in a smaller sample of volunteers (Riba et al., 2001a). 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 (Vollenweider et al., 1997a). The fact that 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 (Itil et al., 1966; Herrmann et al., 1986; Saletu et al., 1993). 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 (Itil et al., 1966). 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 (Saletu et al., 1993; Lee et al., 1999). An important difference between *ayahuasca* and psychostimulants is the alpha-2 decreasing properties found in the present study. In addition to slow wave dampening effects, amphetamine is known to enhance alpha, a feature not shared by *ayahuasca*. As mentioned above, Itil and Fink (1966) found 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 temporo-parietal heteromodal association cortex (Mesulam, 2000). Power decreases in the beta-1 frequency band were predominantly found in somatosensory and visual association cortex, and also in heteromodal association cortex. Areas showing a theta power decrease, however, did not predominate in association cortex, but in paralimbic structures with relevant roles in emotion and memory processes (Mesulam, 2000). Thus, it can be hypothesized that drug-induced bioelectrical changes on 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* (Riba et al., 2001a). 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 (Squire and Zola-Morgan, 1991; Devinsky et al., 1995; Nyberg et al., 1996).

The results obtained in the present study show interesting similarities, but also differences, with 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 (Vollenweider et al., 1997a). These areas largely coincide with those showing theta decreases after *ayahuasca*. Nevertheless, PET and SPECT studies following psilocybin and mescaline have emphasized a hyperfrontality pattern, i.e., increased blood perfusion or glucose metabolism in frontal regions (Hermle et al., 1992; Vollenweider et al., 1997a). 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 (Vollenweider et al., 1997a; Gouzoulis-Mayfrank et al., 1999) and after subanesthesic doses of ketamine (Vollenweider et al., 1997b). In the present study, however, only small areas within the frontal lobes were found to show drug-induced changes in power density distribution. Besides of 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, *ayahuasca* effects 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 unimodal association cortex in the somatosensory, auditory and visual perception modalities, 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 the complex psychological modifications elicited by *ayahuasca*.

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Appendix II

Estimation of the bioavailability of DMT in Ayahuasca

Estimation of the bioavailability of DMT in *ayahuasca*

Introduction

It is a widely accepted hypothesis that the psychotropic effects of *ayahuasca* are mainly caused by the DMT present in the tea. It can be postulated that in order to obtain psychoactivity, a sufficient amount of DMT must survive first-pass metabolism in the gastrointestinal tract and liver, and reach unaltered systemic circulation and the CNS. In vitro and animal studies have shown that DMT can be degraded by MAO and that the β-carbolines in the tea are potent MAO inhibitors. Oral psychoactivity of DMT has also been demonstrated when MAO inhibitors other than the harmala alkaloids are ingested concomitantly. Additional support has thus been given to the DMT-β-carboline interaction hypothesis in *ayahuasca*. However, no data are available regarding the efficacy of this mechanism in terms of how much of the DMT found in the tea is successfully absorbed undegraded when *ayahuasca* is ingested.

Aim

The calculations presented in this appendix of the present dissertation aimed to provide an estimate of the bioavailability of DMT, i.e., the absorbed fraction or percentage of the total administered amount of DMT in *ayahuasca* reaching systemic circulation.

Methods

Bioavailability of DMT in *ayahuasca* was estimated by combining pharmacokinetic data from the final study presented in the present dissertation, with pharmacokinetic data obtained in a previous study conducted by other researchers following the i.v. administration of DMT to a group of healthy volunteers (Strassman and Qualls, 1994).

Design of the i.v. DMT study

Twelve volunteers received doses of 0.05, 0.1, 0.2 and 0.4 mg of DMT hemifumarate per kg body weight in a 30 second i.v. bolus followed by a 15 second flush with saline. Volunteers received the DMT injections on different experimental days and according to a double-blind, randomized placebo-controlled design. In the course of the experiment, various pharmacodynamic variables were assessed and blood samples were

drawn at 2, 5, 10, 15, 30 and 60 min after completion of the bolus. Time "0" coincided with the end of the 45 second bolus (Strassman and Qualls, 1994).

Design of the *ayahuasca* study

The design of the final *ayahuasca* study has been presented in detail in a preceding chapter of the present dissertation. In summary, in a double-blind, placebo-controlled, randomized, crossover clinical trial, 18 volunteers received two different doses of *ayahuasca* corresponding to 0.6 and 0.85 mg DMT/kg body weight. The low and the high dose contained the following amounts of β-carbolines per kg body weight: 1.0/1.4 mg harmine, 0.07/0.09 mg harmaline and 0.82/1.16 mg THH. DMT plasma levels were quantified from blood samples drawn at baseline, 30, 60, 90, 120, and 150 min, and 3, 4, 6, 8 and 24 h after *ayahuasca* administration (Riba et al., 2003).

Pharmacokinetic parameters

Pharmacokinetic parameters for i.v. DMT were calculated based on plasma concentrations at the following time points: 2, 5, 10, 15 and 30 min measured after administration of the 0.1, 0.2 and 0.4 mg DMT/kg doses. Both the lower 0.05 mg DMT/kg dose, and the 60 min time point after each of the other three doses were not analyzed due to values being too close to the limit of detection of the analytical method. Parameters were calculated for the 8 volunteers for whom DMT plasma levels were successfully measured after the three selected doses. The following pharmacokinetic parameters were calculated using a non-compartmental approach by means of WinNonlin software (version 3.0, Pharsight Corporation, California, USA): maximum observed concentration (C_{max}, at t=2 min); area under the concentration-time curve from 0 to infinity ($AUC_{0-\infty}$); $AUC_{0-\infty}$ normalized by dose ($AUC_{0-\infty}/D$); terminal half-life ($t_{1/2\lambda z} = \ln 2/\lambda_z$), obtained by linear regression analysis of the terminal log-linear portion of the plasma-concentration curve; clearance (Cl) determined as dose/$AUC_{0-\infty}$; and finally volume of distribution (V_z) calculated as dose/($\lambda_z \bullet AUC_{0-\infty}$). All data presented in the results section are expressed as mean (SD). In order to examine possible differences in pharmacokinetic parameters between doses, comparisons between doses were performed by means of Student's t-test for $AUC_{0-\infty}/D$, $t_{1/2\lambda z}$, Cl and V_z.

Pharmacokinetic parameters for DMT in *ayahuasca* were calculated for 15 of the 18 volunteers as described in a previous section of the present dissertation. In the results section of this appendix only V_z values are presented.

Estimation of bioavailability

Bioavailability (*F*) of DMT in *ayahuasca* was calculated by dividing the V_z value obtained from the i.v. administration study by the apparent volume of distribution V_z/F obtained for DMT in *ayahuasca*: $F=V_z/(V_z/F)$. *F* was calculated for each of the two *ayahuasca* doses administered. The method employed for the calculation of *F* is based on the assumption that *V*z does not change for DMT whether the drug is administered alone (i.v. study) or in combination with the other alkaloids present in *ayahuasca*. We assumed that while the *β*-carbolines, whose main pharmacological action is the inhibition of MAO, would affect DMT absorption and elimination, they would no affect the distribution of the drug in the organism. On the other hand, the inhibition of MAO by the *β*-carbolines would most likely alter the clearance value of DMT in *ayahuasca*. Consequently, the expression $F=Cl/(Cl/F)$ would not be adequate to calculate bioavailability in this case; nor would $F=(AUC_{oral}/AUC_{iv})$x(i.v. dose/oral dose) since the AUC value is mainly affected by the elimination phase of the drug.

Results

The calculated pharmacokinetic parameters for i.v. DMT are shown in table 1. Values indicate mean (SD).

Table 1

i.v. DMT

Dose	C_{max} (ng/ml)	$AUC_{0-\infty}$ (ng/ml . min^{-1})	$AUC_{0-\infty}/D$	$t_{1/2\lambda z}$ (min)	Cl (l/min)	V_z (l)
0.1 mg/kg	16.78 (9.77)	215.51 (105.41)	0.042 (0.021)	9.03 (3.77)	31.12 (18.51)	363.89 (173.80)
0.2 mg/kg	58.70 (56.77)	577.83 (471.02)	0.054 (0.04)	11.01 (10.02)	26.57 (16.43)	398.32 (438.78)
0.4 mg/kg	81.38 (57.68)	952.53 (512.06)	0.045 (0.021)	8.39 (2.09)	27.61 (14.53)	325.72 (183.25)

No differences were found between doses for $AUC_{0-\infty}/D$, $t_{1/2\lambda z}$, *Cl* and V_z calculated for i.v. DMT.

Bioavailability values for DMT in *ayahuasca* are presented in Table 2. Values were calculated for each of the two administered *ayahuasca* doses and employing each of the three V_z values calculated for i.v. DMT (one V_z per dose).

Table 2

i.v. DMT	***Ayahuasca***			
	Low dose		High dose	
V_z *(l)*	V_z/F *(l)*	*F (%)*	V_z/F *(l)*	*F (%)*
363.89	3509.86	10.37	2505.97	14.52
398.32	3509.86	11.35	2505.97	15.89
325.72	3509.86	9.28	2505.97	13.00
Mean *F*		**10.33**		**14.47**

As shown in the table, mean *F* values were around 10% for the low *ayahuasca* dose and around 15% for the high *ayahuasca* dose.

Comment

The obtained *F* values indicate that a relatively small fraction of the ingested DMT in *ayahuasca* is actually absorbed. The approach used to calculate *F* is not ideal, since i.v. and oral data were obtained in different groups of volunteers. Calculations had to be performed with grand-mean values rather than on an individual basis. Results should consequently be considered an approximation to the real *F* value. In their study, Callaway and coworkers (1999) provide a V_{ss}/F value of 54.8 l/kg, which would result in 4,066 l in a 74.2 kg individual (reported as the mean weight of the volunteers in their study). Thus, applying the calculation method used in the present work, *F* values in the Callaway et al. study (1999) ranged from 8% to 10%, which is in line with our own findings. A 10-15% bioavailability appears to be sufficient to elicit psychotropic effects, as demonstrated in the previous sections of the present dissertation. However, these

values pose questions regarding the metabolism of DMT in vivo. First, not all DMT in *ayahuasca* reaches systemic circulation, and second, the fraction which does pass is eventually cleared from the bloodstream. Thus, MAO activity has either only partially been inhibited by the *β*-carbolines, or DMT is redirected to other metabolic routes. In actual fact, metabolites other than 3-indoleacetic acid have been detected when DMT is incubated in several biological matrices (see the introduction section of the present dissertation). Future studies should identify the metabolism products of oral DMT administered alone in order to better understand the fate of this compound in the organism.

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Summary of results

Main Results

1. Results on subjective effect measures in the pilot and final study demonstrated statistically significant psychotropic effects for *ayahuasca,* with dose-dependent increases in five of the six subscales of the HRS and in the LSD, MBG and A scales of the ARCI. Subjective 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). Psychological effects were first noted after 30-60 min, peaked between 60-120 min and were resolved by 4-6 h.

2. *Ayahuasca* induced dose-dependent modifications of brain electrical activity as compared with placebo. These effects consisted of an overall decrease in absolute power for all the frequency bands evaluated, and an acceleration of the center-of-gravity frequency. Absolute power decreases were most prominent in theta, delta and beta-1 bands at 90-120 min, while the alpha and fast beta rhythms were less intensely affected. However, the alpha-2 band showed a highly significant decrease at all leads at 60 min. 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*. Non-parametric analysis of all variables and leads over time showed the first changes relative to placebo between 15-30 min. These were followed by a steep rise at 45 min, reaching the maximum effects between 45-90 min. EEG measures gradually declined thereafter to reach baseline values around 4-6 h.

3. Intracerebral power density showed the maximum differences with placebo at 60 and 90 min. *Ayahuasca* decreased power density in the alpha-2, delta, theta and beta-1 bands. At 60 min, 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 min, 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.

4. *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 startle response, its habituation rate or on the percentage prepulse inhibition at any of the prepulse-to-pulse intervals studied.

5. The major *ayahuasca* alkaloids, DMT, harmaline and THH, could be measured in plasma following *ayahuasca* administration, while levels of harmine were negligible and only observed in a small subset of volunteers. Additionally, levels of harmol and harmalol, the *O*-demethylated analogues of harmine and harmaline, were detected in all volunteers. C_{max} and AUC values increased with dose for all measured compounds. 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 and coincided with the peak of subjective effects. The AUC normalized by dose increased with dose for DMT. Conversely, 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. The calculation of the bioavailability of DMT based on i.v. DMT data from another study by other researchers yielded a value around 10-15 %.

6. *Ayahuasca* induced a statistically significant increase in 24 h normetanephrine excretion. However, instead of the expected decreases in deaminated monoamine metabolites (VMA, HVA, 5-HIAA), drug administration did not significantly modify excretion of these compounds.

7. In both the pilot and the final study, mean SBP, DBP and HR values were found to be increased at specific time points following *ayahuasca* administration as compared with placebo. However, statistically significant results were only obtained for DBP, these being in the final study. This variable showed a moderate 9 mm Hg increase at 75 min after the 0.85 mg DMT/kg body weight dose. Somatic-dysphoric effects, particularly nausea, were frequently associated to drug intake. However, vomiting was only observed in 4 of 53 occassions in which *ayahuasca* was administered (pilot and final studies combined). In the course of the pilot study one volunteer experienced an intensely dysphoric reaction with

transient disorientation and anxiety which led to his voluntary withdrawal from the study. No clinically relevant alterations were observed in the hematological or biochemical parameters tested in any volunteer.

Secondary Results

1. The HRS questionnaire was translated into Spanish and evaluated in two groups of volunteers. Scores above zero (no effect) were observed both in the immediate and delayed retrospective assessment of drug effects. Reliability data indicated that four of the six scales, i.e., Affect, Cognition, Perception and Somaesthesia, show an acceptable level of internal consistency. Significant but limited correlations were found between the Perception and Somaesthesia scales and the ARCI LSD scale, indicating the construct validity of the questionnaire. The HRS was sensitive to psychedelic drug effects other than those elicited by intravenous DMT, for which it was originally designed, and showed reasonable reliability and convergent validity.

2. DMT was quantified by gas chromatography with nitrogen-phosphorus detection, following liquid–liquid extraction with *n*-pentane. Recovery was 74%, and precision and accuracy were better than 9.9%. The limit of quantification (LOQ) was 1.6 ng/ml. The three main *β*-carbolines present in *ayahuasca*, i.e., harmine, harmaline and THH, plus harmol and harmalol (*O*-demethylation metabolites of harmine and harmaline, respectively) were quantified by means of high performance liquid chromatography (HPLC) with fluorescence detection. Sample preparation was accomplished by solid-phase extraction. All five *β*-carbolines were measured using a single detector by switching wavelengths. Method validation demonstrated good recoveries, above 87%, and accuracy and precision better than 13.4%. The LOQ was 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. Good linearity was observed in the concentration ranges evaluated for DMT (2.5-50 ng/ml) and the *β*-carbolines (0.3-100 ng/ml).