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Inhibition of alpha oscillations through serotonin-2A receptor activation underlies the visual effects of ayahuasca in humans

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Abstract
Ayahuasca is an Amazonian psychotropic plant tea typically obtained from two plants, *Banisteriopsis caapi* and *Psychotria viridis*. It contains the psychedelic 5-HT$_{2A}$ and sigma-1

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Ketanserin
Subjective effects
Neurophysiological effects
Human

agonist *N*,*N*-dimethyltryptamine (DMT) plus β-carboline alkaloids with monoamine-oxidase (MAO)-inhibiting properties. Although the psychoactive effects of ayahuasca have commonly been attributed solely to agonism at the 5-HT_{2A} receptor, the molecular target of classical psychedelics, this has not been tested experimentally. Here we wished to study the contribution of the 5-HT_{2A} receptor to the neurophysiological and psychological effects of ayahuasca in humans. We measured drug-induced changes in spontaneous brain oscillations and subjective effects in a double-blind randomized placebo-controlled study involving the oral administration of ayahuasca (0.75 mg DMT/kg body weight) and the 5-HT_{2A} antagonist ketanserin (40 mg). Twelve healthy, experienced psychedelic users (5 females) participated in four experimental sessions in which they received the following drug combinations: placebo +placebo, placebo+ayahuasca, ketanserin+placebo and ketanserin+ayahuasca. Ayahuasca induced EEG power decreases in the delta, theta and alpha frequency bands. Current density in alpha-band oscillations in parietal and occipital cortex was inversely correlated with the intensity of visual imagery induced by ayahuasca. Pretreatment with ketanserin inhibited neurophysiological modifications, reduced the correlation between alpha and visual effects, and attenuated the intensity of the subjective experience. These findings suggest that despite the chemical complexity of ayahuasca, 5-HT_{2A} activation plays a key role in the neurophysiological and visual effects of ayahuasca in humans.

1. Introduction

Ayahuasca is a psychoactive plant tea used traditionally by the indigenous peoples of the Upper Amazon (Schultes, 1980) and in more recent times by healers and members of religious syncretic groups (Tupper, 2008). This tea is receiving increased attention from the general public and biomedical researchers (Frood, 2015). It has been used to help treat addiction (Fernández et al., 2014), and recent open-label studies have shown preliminary evidence of rapid and lasting antidepressant effects after a single dose (Osório et al., 2015; Sanches et al., 2016).

Although there are many variations in the preparation of the tea, the common ingredient is the malpighiaceous vine *Banisteriopsis caapi*. This plant is rich in β-carboline alkaloids, mainly harmine, harmaline and tetrahydroharmine (THH) (Riba, 2003). These alkaloids show monoamine-oxidase inhibiting properties (Buckholtz and Boggan, 1977a, 1977b), while THH is also a serotonin reuptake inhibitor (Buckholtz and Boggan, 1977a, 1977b). In addition to *B. caapi*, other admixture plants are frequently used in the preparation of ayahuasca. One of the most common in the context of modern use is *Psychotria viridis*. The leaves of this plant are rich in the psychedelic indole *N*,*N*-dimethyltryptamine or DMT (Riba, 2003).

DMT is structurally related to the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) and shows agonist activity at the 5-HT_{2A} and 5-HT_{1A} receptors. DMT also acts as an agonist at the trace amine associated receptor (TAAR) (Bunzow et al., 2001) and it is a substrate of the serotonin and the vesicle monoamine transporters (Cozzi et al., 2009). It has been suggested that using these uptake mechanisms, intracellular concentrations could reach higher values than in plasma and interact with the intracellular sigma-1 receptor (Fontanilla et al., 2009). This receptor modulates the activity of many other proteins, conferring stability against cellular stress, and promoting brain plasticity (Chu and Ruoho, 2016; Tsai et al., 2009).

When administered to humans parenterally, DMT induces intense modifications of the ordinary state of awareness with intense visual effects, but it is devoid of psychoactivity when taken orally (Riba et al., 2015) due to degradation by MAO (Suzuki et al., 1981), and cytochrome-dependent mechanisms (Riba et al., 2015). The presence of the MAO-inhibiting β-carbolines in ayahuasca prevents is enzymatic degradation and allows its oral bioavailability (Riba et al., 2003a).

In previous studies by our group, we found ayahuasca to induce a pattern of psychedelic effects with a slower onset and longer duration than those induced by DMT (Dos Santos et al., 2011; Riba et al., 2003a, 2001b). Neurophysiologically, ayahuasca induces broad-band power decreases in spontaneous electrical brain oscillations (Riba et al., 2002a) and associated reductions in intracerebral current source density (CSD) in certain brain areas (Riba et al., 2004). These reductions are particularly strong for oscillations in the alpha band of the EEG, with CSD reductions over the posterior visual cortex, an effect thought to reflect increased cortical excitability (Romei et al., 2008b). Analogous findings in the range of the alpha band have also been observed using magnetoencephalography and the psychedelic and serotonin-$_{2A}$ receptor agonist psilocybin (Muthukumaraswamy et al., 2013).

The aim of this study was to assess the contribution of serotonin-$_{2A}$ receptor to the neurophysiological and psychological effects of ayahuasca. We postulated that despite the combination of various pharmacological mechanisms in ayahuasca, the general psychedelic effects and decreases in current density depend on activation of the 5-HT_{2A} receptor. To test this hypothesis, we studied the interaction of a medium dose of ayahuasca (Riba et al., 2001b) and ketanserin, a 5-HT_{2A} receptor antagonist, in a group of experienced psychedelic users in a laboratory setting.

2. Experimental procedures

2.1. Participants

For ethical reasons, we only recruited individuals with prior experience with psychedelics. We wanted to avoid introducing

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drug-naive individuals to psychedelics, and to make sure that volunteers would be familiar with the modified state of consciousness induced by these drugs. We therefore contacted psychedelic drug users and informed them about the goals of the study, the nature of ayahuasca, its psychological effects, and the potential adverse effects described in the literature for psychedelics. We recruited a group of 12 healthy volunteers (5 females, 7 males) with previous experience with psychedelic drugs (10 times or more). Despite their experience with psychoactive substances, no participant had a current or previous DSM/ICD-10 diagnosis of drug dependence.

The volunteers had a mean age of 35 years (26–43). Their past experience with psychedelic drugs mainly involved LSD (11/12), Psilocybe mushrooms (11/12) and ayahuasca (8/12). Nine of the participants also had experience with ketamine, six had used 2C-B, five had smoked *Salvia divinorum*, four had taken mescaline-containing cacti such as *peyote* or *San Pedro*, and two had smoked dimethyltryptamine. At the time of the study, eight were using cannabis sporadically (1–2 cigarettes per week). Only four were currently tobacco smokers and eleven consumed alcohol in moderate amounts, from one or two beers or glasses of wine per day to one per month.

Prior to participation, all volunteers underwent a complete medical examination that included medical history, physical examination, ECG, and standard laboratory tests, to confirm good health. Exclusion criteria included a current or past history of psychiatric disorders, alcohol or other substance use disorders, evidence of significant illness, and pregnancy. The study was conducted in accordance with the Declaration of Helsinki and subsequent amendments concerning research in humans and was approved by the Sant Pau Hospital Ethics Committee and the Spanish Ministry of Health. All volunteers gave their written informed consent to participate.

2.2. Drugs

Ayahuasca was administered in freeze-dried encapsulated form. The ayahuasca batch used in the study was analyzed using a previously described method using liquid cromatography–electrospray ionization–tandem mass spectrometry (McIlhenny et al., 2009). The analysis showed that ayahuasca contained the following alkaloid concentrations in mg per gram of freeze-dried material: 6.51 DMT, 13.14 harmine, 1.35 harmaline and 11.55 THH. The final dose was calculated individually for each participant, so that they received the equivalent of 0.75 mg DMT/kg body weight. The dose chosen is of medium intensity and was selected based on data from previous studies where it showed robust psychological and physiological effects (Dos Santos et al., 2012; Riba et al., 2001b). Given the alkaloid proportions present in the freeze-dried material, at the 0.75 mg/kg DMT dose, participants also ingested 1.51 mg/kg of harmine, 0.16 mg/kg of harmaline and 1.33 mg/kg of THH.

Ketanserin was administered as the trademark drug Ketensin (ketanserin tartrate), at the dose of 40 mg, and placebo capsules contained lactose.

2.3. Study design and drug administration

The study was conducted according to a double-blind, randomized, balanced, crossover design. It involved four experimental sessions one week apart each. Two weeks prior to the first experimental session and throughout the study, participants abstained from any psychoactive drugs and medications. Urine was collected for drug analysis on each experimental day. Participants tested negative for alcohol, cannabis, amphetamines, benzodiazepines, opiates and cocaine. In each session, participants received an initial treatment that could be placebo (lactose capsule) or 40 mg ketanserin. One hour later, they were administered a second placebo or encapsulated freeze-dried ayahuasca. Thus, on each experimental day, participants received one of four different treatment combinations: placebo+placebo, placebo+ayahuasca, ketanserin+placebo and ketanserin+ayahuasca.

Participants remained in the laboratory for 8 h after which they were discharged home. During the first four hours they remained seated in a reclining chair in a sound-attenuated and dimly lit room. EEG recordings were conducted before the administration of the first treatment (placebo or ketanserin). Ninety minutes after administration of the second treatment (ayahuasca or placebo), when the peak ayahuasca effects were expected, a second EEG recording was obtained. Four hours after administration of the second treatment, when most of the subjective effects of ayahuasca had disappeared, the volunteers were allowed to leave the room and were asked to answer the subjective effects questionnaires.

3. Data collection

3.1. EEG recording and processing

Three-minute EEG recordings with eyes closed were obtained from 19 standard scalp locations (Fp1/2, F3/4, Fz, F7/8, C3/4, Cz, T3/4, T5/6, P3/4, Pz and O1/2). Recordings were obtained using a BrainAmp amplifier (Brain Products GmbH, Gilching, Germany) before the first treatment (baseline) and 90 min after administration of the second treatment. Signals were referenced to the averaged mastoid electrodes, and vertical and horizontal electrooculograms (EOG) were also obtained for artifact minimization and removal. Signals were analogically band-pass filtered between 0.1 and 45 Hz, digitized with a frequency of 250 Hz.

EEG artifact minimization and removal was performed according to a two-step procedure before calculating the parameters. First, an ocular artifact minimization step was implemented using a previously described method based on blind source separation or BSS (Alonso et al., 2010; Romero et al., 2008). The continuous EEG recording was then segmented into 5 s epochs. These segments were automatically analyzed for saturation, muscular and movement artifacts using the procedure described by Anderer and colleagues (Anderer et al., 1992).

After computing the two-step artifact preprocessing procedure, spectral analysis was performed for all EEG channels. Power spectral density (PSD) functions were calculated from artifact-free 5 s epochs by means of a periodogram using a Hanning window, and averaged. Averaged PSD functions for each experimental situation were quantified into absolute powers in the following frequency bands: delta (0.5–3.5 Hz), theta (3.5–7.5 Hz), alpha (7.5–13 Hz), and beta (13–35 Hz). Additionally, frequency variability was measured calculating the deviation of the center-of-gravity frequency or centroid of the total activity (0.5–35 Hz).

3.2. Intracerebral current density calculation

The Standardized LORETA (sLORETA) software (Pascual-Marqui et al., 1994) was used to estimate the three-dimensional intracerebral current density distribution from the voltage values recorded at the scalp. sLORETA estimates a particular solution of the non-unique EEG inverse solution restricted to 6239 cortical gray matter voxels with a spatial

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resolution of 0.125 cm^3 according to a digitized head model from Montreal Neurological Institute (Pascual-Marqui, 2002). The current density values were estimated based on the EEG cross-spectral matrix and then squared for each voxel in the classical frequency bands.

3.3. Subjective effect measures

The psychological effects elicited by the administered treatments were measured using a battery of questionnaires on subjective effects: the Hallucinogen Rating Scale (HRS), the Addiction Research Center Inventory (ARCI), the Altered States of Consciousness Questionnaire (APZ), and a battery of self-administered visual analog scales (VAS).

The Hallucinogen Rating Scale (HRS), developed by Strassman and colleagues (Strassman et al., 1994), includes 71 items grouped in six subscales: *Somaesthesia*, reflecting somatic effects; *Affect*, measuring emotional and affective responses; *Perception*, measuring visual, auditory, gustatory, and olfactory experiences; *Cognition*, describing modifications in thought processes or content; *Volition*, indicating the volunteer's capacity to willfully interact with his/her "self" and/or the environment; and *Intensity*, which reflects the strength of the overall experience. The range of scores for all scales is 0–4. A validated Spanish version was administered (Riba et al., 2001a).

The Addiction Research Center Inventory (ARCI) (Martin et al., 1971) includes 49 items distributed in five scales or groups: the morphine-benzedrine group (MBG) that measures euphoria; the pentobarbital-chlorpromazine-alcohol group (PCAG), that measures sedation; the lysergic acid diethylamide scale (LSD), that measures somatic-dysphoric effects; the benzedrine group (BG) that measures subjectively experienced intellectual efficiency; and the amphetamine scale (A), which is sensitive to stimulants. The range of scores is 0–16 for MBG, −4 to 11 for PCAG, −4 to 10 for LSD, −4 to 9 for BG, and 0–11 for A. A validated Spanish version was administered (Lamas et al., 1994).

The Altered States of Consciousness questionnaire ("Aussergewöhnliche Psychische Zustände", APZ) (Dittrich, 1998) is composed of 72 items distributed in three subscales: oceanic boundlessness ("Ozeanische Selbst-entgrenzung", OSE), to measure changes in the sense of time, derealization and depersonalization; Dread of Ego-Dissolution ("Angstvolle IchAuflösung", AIA), for measuring thought disorder and decreased body and thought control associated with arousal and anxiety; and Visionary Restructuralization ("Visionäre Umstrukturierung", VUS), referring to visual phenomena such as illusions, hallucinations and synesthesia and to changes in the significance of objects. The range of scores is 0–13 for OSE, 0–22 for AIA, and 0–14 for VUS. We administered a Spanish version of the questionnaire that had been used previously in clinical studies involving psychedelic drugs (Riba et al., 2002b).

A battery of self-administered visual analog scales (VAS) was used to retrospectively rate peak effects during the session. Volunteers indicated the intensity of the drug effects on a grid of (from 0=no effects to 100=extremely intense effects) 100-mm horizontal lines. There were 10 VAS lines labeled as follows: "Any effect" indicated any effect, either physical or psychological, that the volunteer attributed to the administered dosage; "Good effects" indicated any effect the volunteer assessed as good; "Bad effects" indicated any effect the volunteer assessed as bad; "Liking" indicated that the volunteer liked the effects of the administered substance; "Fear" indicated apprehension or psychological discomfort; "Time" indicated modifications on the perception of time; "Feeling high" indicated any psychological alteration the participant attributed to the administered drug; "Changes in external reality" indicated changes in perception of external reality; "Loss of contact with external reality" indicated separation from the surroundings; and "Visions" indicated visual modifications with eyes open or closed.

As mentioned above, despite their recreational drug use, participants had no current or past diagnosis of substance use disorders. Consequently, we did not study the effects on ayahuasca on specific measures of addiction.

4. Statistical analysis

4.1. EEG data

Statistical analysis of EEG recordings was performed following the IPEG (International Pharmaco-EEG Group) guidelines for statistical design and analysis of pharmacodynamic trials (Ferber et al., 1999). Paired *t*-tests were carried out for all variables and EEG electrodes for assessing drug-induced changes at the 90 min time point. Statistical results were displayed as topographic significance probability maps.

4.2. Intracerebral current density data

Statistical differences between treatments and placebo were evaluated by paired-sample *t*-tests computed for the baseline-corrected and log-transformed LORETA power values in each voxel and for each frequency band at the 90 min time point. To correct for multiple comparisons, a non-parametric permutations test (NPT) based on the theory of randomization was applied (Nichols and Holmes, 2002). Voxel intensity NPT calculates a critical *t*-value by means of a random sample of all the possible permutations to estimate the distribution of the maximum *t*-statistic.

4.3. Subjective effect measures

Scores on the HRS, ARCI, APZ, and ARCI questionnaires were analyzed using repeated-measures ANOVAs with treatment as factor (placebo+placebo, placebo+ayahuasca, ketanserin+placebo, ketanserin+ayahuasca). When significant effects were found in the ANOVA, post-hoc pair-wise comparisons between treatments were conducted using Student's *t*-tests followed by Bonferroni correction. Results were considered significant for p values <0.05.

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5. Results

5.1. Neurophysiological effects

5.1.1. Topography

Figure 1 shows the topographical scalp maps of drug-induced changes in power in the different frequency bands following the three active treatments vs. placebo. Ayahuasca induced widespread absolute power decreases in the delta, theta and alpha frequency bands but did not induce any effects in absolute power the beta frequency range. Desynchronization of the EEG was evidenced by the reduction in the alpha rhythm and the increase in frequency variability indicated by the deviation of the center-of-gravity frequency. Ketanserin on its own showed effects opposed to those of ayahuasca. It increased absolute power in the delta band at the frontal leads and induced more widespread increases in the theta band. No effects were observed in the topographical scalp maps in the alpha and beta bands and only marginal decreases were seen in the centroid deviation. The administration of ketanserin prior to ayahuasca not only counteracted the delta and theta decreases but led to more marked and widespread increases in power throughout the scalp in these two frequency bands than those elicited by ketanserin alone. Ketanserin completely blocked the alpha decreases induced by ayahuasca and reverted the effects on the deviation of the center-of-gravity frequency.

5.2. Intracerebral current density data

Figure 2 shows the intracerebral analysis of the topographical changes and Table 1 shows the associated data. The most intense effects observed for ayahuasca were current density decreases in the alpha band in posterior brain regions involving the occipital, parietal, and temporal lobes. The highest decrease was located in the primary visual cortex in Brodmann area (BA) 18. Smaller significant areas were found in the frontal lobe. Theta decreases mainly involved the lateral and medial aspects of the frontal lobes. The highest decrease was located in the superior frontal gyrus in BA 10. Decreases in the delta band were found in the temporal lobe, with the highest decrease in the inferior temporal gyrus in BA 20.

Ketanserin on its own did not induce any changes in current source density in the alpha and delta bands. However, it induced increases in theta in the frontal lobe, specifically in the middle frontal gyrus in BA 6.

The administration of ketanserin as pretreatment abolished the alpha decreases induced by ayahuasca. Ketanserin also reverted the decreases in theta and led to increases in

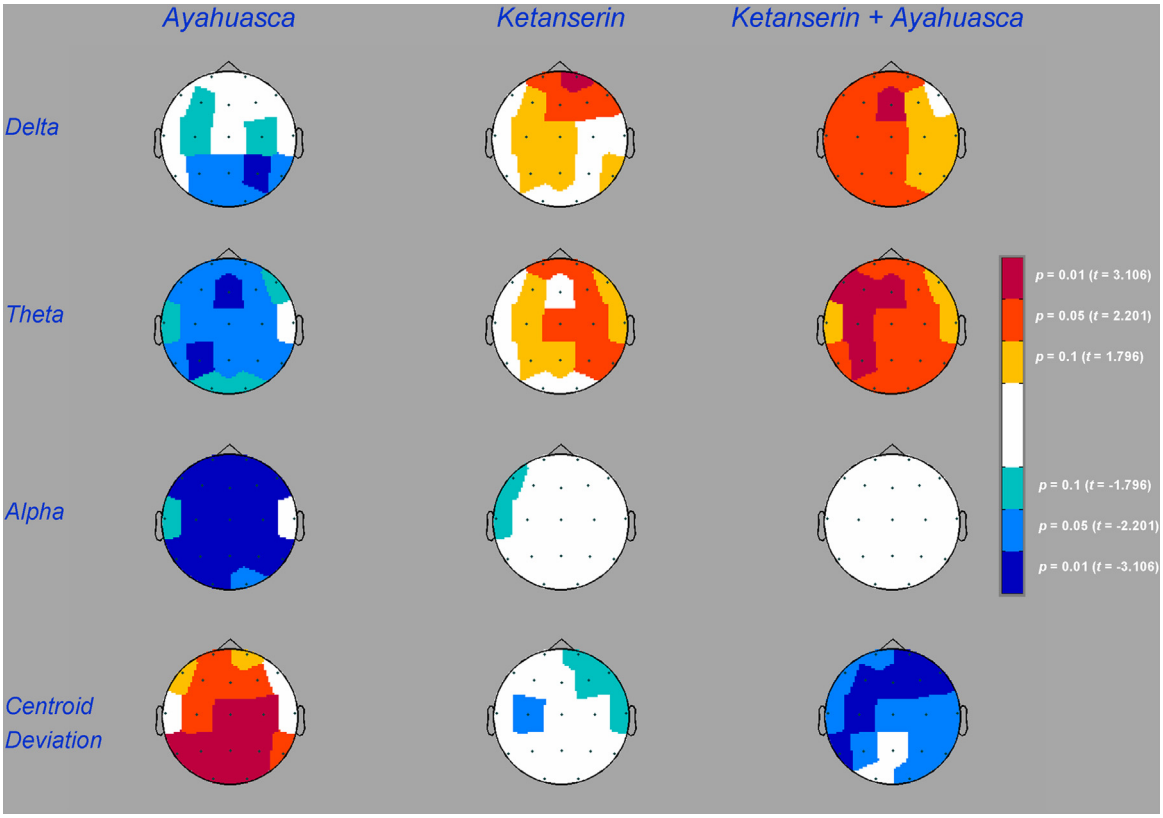

Figure 1 Significance probability maps showing differences between the three administered active treatments and placebo. These maps show changes at the 19 leads placed over the scalp. They depict modifications in absolute power in three different frequency bands and in the deviation of the center-of-gravity frequency (centroid deviation). The four rows from top to bottom show: (1) changes in delta (0.5–3.5 Hz) power; (2) changes in theta (3.5–7.5 Hz) power; (3) changes in alpha (7.5–13 Hz) power; and (4) changes in centroid deviation. The vertex view shows the nose at the top, the left ear to the left, the right ear to the right. Electrode positions are indicated by white dots. Warm colors denote increases and cold colors decreases at the significance level indicated by the color bar.

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power in this band over extensive areas (see Table 1). The highest increases were observed in the frontal lobe, over the precentral gyrus in BA 44. Finally, ketanserin inhibited the delta decreases and led to increases in the occipital lobe and, to a lower extent, in the parietal lobe. The highest increase was observed in the cuneus in BA 30.

5.3. Subjective effects

5.3.1. HRS

Figure 3 shows mean scores on the six subscales of the HRS after the four different treatments and Table 2 shows the results of the statistical analyses. Ayahuasca significantly increased scores on all subscales except Volition. Ketanserin on its own did not induce any significant changes relative to placebo. When ayahuasca was preceded by ketanserin (ketanserin+ayahuasca combination), the 5-HT_{2A} antagonist significantly reduced scores on Affect (62% reduction), Perception (56% reduction) and Intensity (36% reduction) subcales. An interesting deviant effect was observed in the Volition subscale. The ketanserin+ayahuasca combination yielded scores that were significantly higher than those after placebo. As shown in the figure, the combination had an additive effect, increasing incapacitation further than ayahuasca or ketanserin alone (76% increase relative to ayahuasca alone). Ketanserin reduced the scores on the somatic and cognitive effects induced by ayahuasca, but these reductions did not reach statistical significance.

5.3.2. ARCI

Figure 3 shows the mean scores on the five subscales of the ARCI, and Table 2 shows the results of the statistical

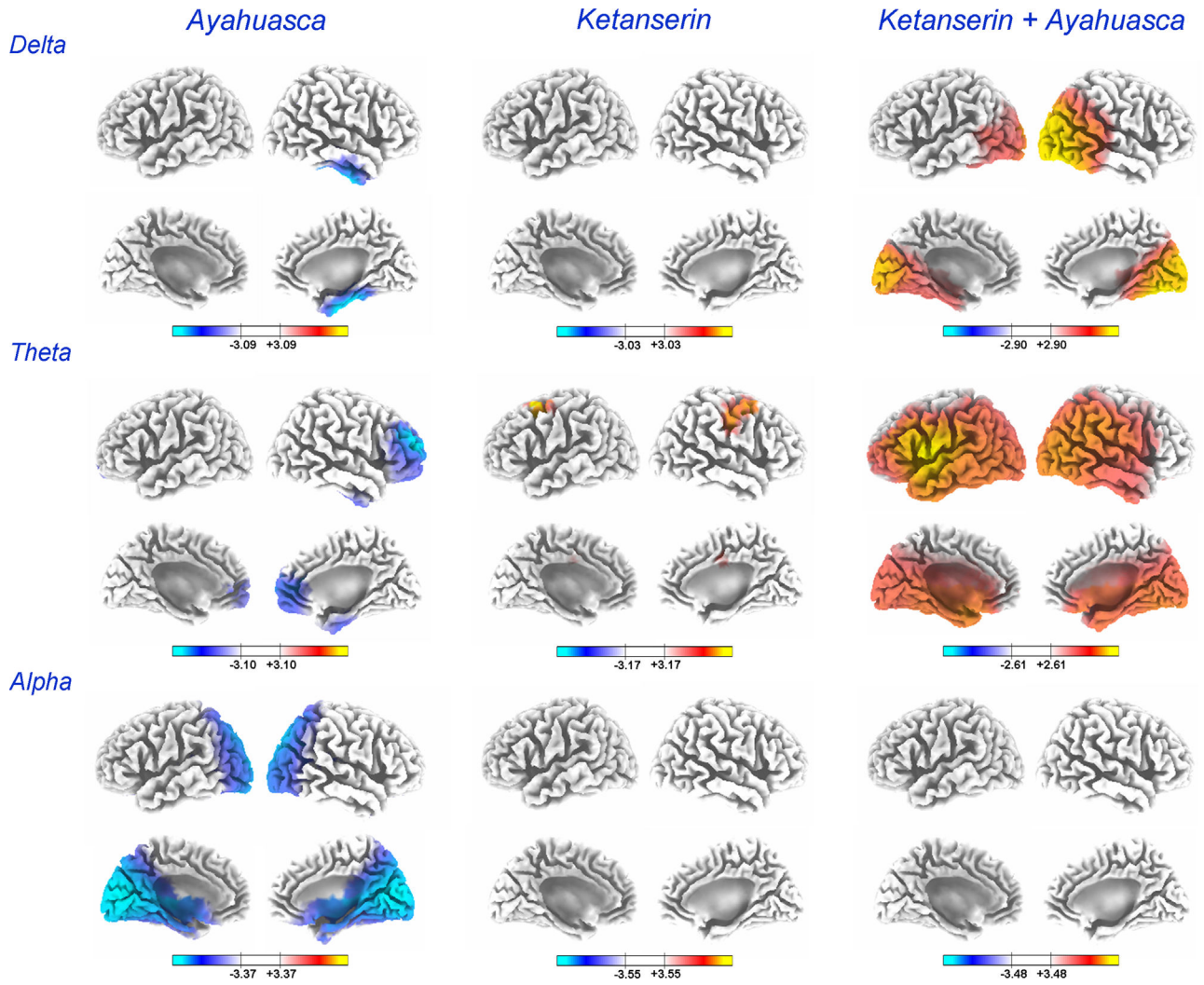

Figure 2 Effects of the three administered active treatments vs. placebo on current source density. These maps show the intracerebral current density distribution associated with energy changes recorded at the scalp. The color coding represents statistical significance above a critical value calculated using the non-parametric Holmes permutation test to correct for multiple comparisons. Results are shown for the delta band (0.5–3.5 Hz) in the top rows, the theta band (3.5–7.5 Hz) in the middle rows, and the alpha band (7.5–13 Hz) in the bottom rows. Cold colors denote significant decreases and warm colors significant increases compared to placebo (placebo+placebo) after Holmes correction ($p<0.05$). The color bars indicate the critical *t*-value for each frequency band and statistical comparison.

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Table 1 Areas showing significant current density differences between active treatments and placebo (placebo+placebo). The number of significant voxels, percentage of significant voxels in a lobe, maximum *t* value in a lobe and MNI coordinates of the voxel with the maximum *t* value are reported.

Lobe	Placebo+ayahuasca				Ketanserina+placebo				Ketanserina+ayahuasca			
	N° voxels	% voxels	Max *t* value	MNI *x,y,z*	N° voxels	% voxels	Max *t* value	MNI *x,y,z*	N° voxels	% voxels	Max *t* value	MNI *x,y,z*
Alpha												
Occipital	761	100	−5.86	0,−80,15	-	-	-	-	-	-	-	-
Parietal	691	60	-	-	-	-	-	-	-	-	-	-
Limbic	412	54	-	-	-	-	-	-	-	-	-	-
Temporal	265	23	-	-	-	-	-	-	-	-	-	-
Frontal	144	7	-	-	-	-	-	-	-	-	-	-
Sub_lobar	33	14	-	-	-	-	-	-	-	-	-	-
Theta												
Frontal	435	20	−4.17	35,50,25	196	9	−3.45	−35,10,60	1090	50	4.08	−60,0,10
Limbic	46	6	-	-	-	-	-	-	651	86	-	-
Sub_lobar	8	3	-	-	-	-	-	-	227	99	-	-
Temporal	-	-	-	-	-	-	-	-	1154	99	-	-
Parietal	-	-	-	-	31	3	-	-	1091	95	-	-
Occipital	-	-	-	-	-	-	-	-	761	100	-	-
Delta												
Temporal	187	16	−3.65	40,−10,−40	-	-	-	-	544	47	4.38	25,−75,10
Limbic	92	12	-	-	-	-	-	-	279	37	-	-
Occipital	4	1	-	-	-	-	-	-	761	100	-	-
Frontal	-	-	-	-	-	-	-	-	3	0.1	-	-
Parietal	-	-	-	-	-	-	-	-	303	26	-	-
Sub-lobar	-	-	-	-	-	-	-	-	34	14	-	-

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analyses. Ayahuasca significantly increased scores on the MBG, LSD and A subscales. As observed for HRS, ketanserin on its own did not induce any significant changes on any subscale.

When ketanserin preceded ayahuasca, the effects on the MBG subscale were significantly blocked (80% reduction), and no statistically significant differences were found between the combination and placebo. Analogously, ketanserin significantly reduced scores on the A subscale (53% reduction). Again, results after ketanserin+ayahuasca did not differ from placebo. Scores on the LSD subscale were not blocked by ketanserin. Scores after the combination were significantly higher than those after placebo and were not significantly different from those after ayahuasca alone.

Ketanserin not only blocked but reversed the effects of ayahuasca on BG, with values falling clearly below those after placebo. This effect of a reduction in subjectively perceived intellectual efficiency is consistent with the effects induced by the ketanserin+ayahuasca combination on the PCAG scale. Scores on this sedation scale were not modified by ayahuasca alone but were markedly increased after the combination ($\approx$ 20 times higher). As shown in the figure, mean values after ketanserin+ayahuasca were higher than the mere addition of the effects induced by ayahuasca plus those after ketanserin.

In summary, ketanserin effectively blocked the euphoriant (MBG) and stimulant effects of ayahuasca (BG, A) but not its somato-dysphoric effects (LSD). Furthermore, it caused marked sedative effects when combined with ayahuasca (PCAG).

5.3.3. APZ

Mean scores on all subscales of the APZ are shown in Figure 3, and the results of the statistical analyses in Table 2.

Ayahuasca significantly increased scores on all three subscales, whereas ketanserin alone did not induce any effects. Scores after ketanserin+ayahuasca were significantly lower

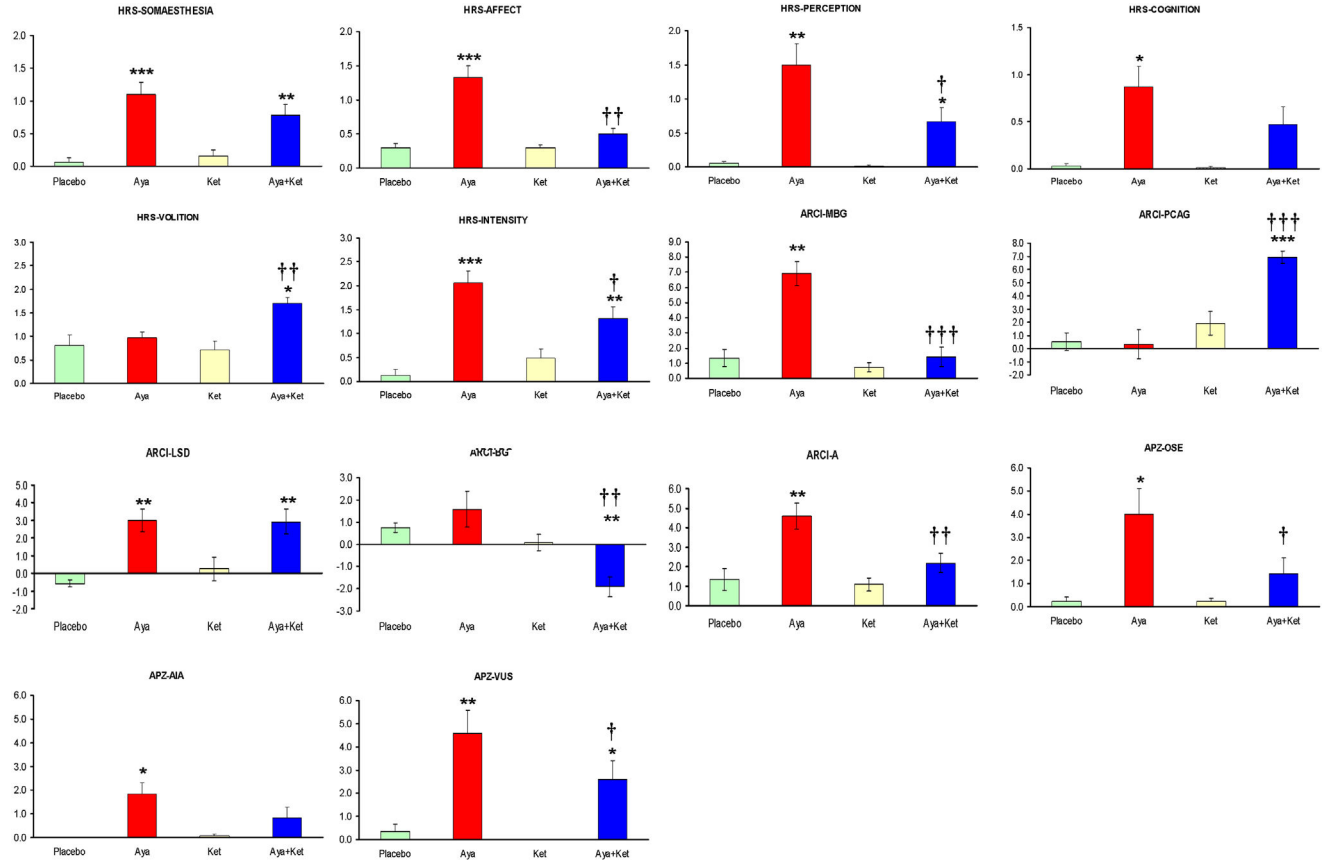

Figure 3 Mean scores on the Hallucinogen Rating Scale (HRS), Addiction Research Center Inventory (ARCI) and Altered States of Consciousness (Aussergewöhnliche Psychische Zustände, APZ) subscales for the three active treatments administered and placebo. Error bars denote 1 standard error of mean ($n=12$). Significant differences from placebo are denoted as * at $p<0.05$, ** at $p<0.01$, and *** at $p<0.001$. Significant differences between ayahuasca alone and ayahuasca after pretreatment with ketanserin are denoted as † at $p<0.05$, †† at $p<0.01$, and ††† at $p<0.001$. All pair-wise comparisons are shown after Bonferroni correction. Green: placebo, red: ayahuasca, yellow: ketanserin, blue: ketanserin+ayahuasca. The abbreviations indicate: Pla: placebo, Aya: Ayahuasca equivalent to 0.75 mg DMT/kg body weight, Ket: 40 mg ketanserin, Aya+Ket: combined treatment ayahuasca+ketanserin. ARCI−A: amphetamine scale; BG: benzedrine-group; MBG: morphine-benzedrine-group; PCAG: pentobarbital-chlorpromazine-alcohol-group; LSD: lysergic acid diethylamide scale. APZ-OSE: oceanic boundlessness, AIA: dread of ego dissolution, and VUS: visionary restructuralization.

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Table 2 Statistical analyses of subjective effects measures (HRS, ARCI, and APZ) induced after the four administered treatment combinations $n=12$. Pairwise comparisons following Bonferroni correction are shown.

HRS	ANOVA df = 3,33	Pairwise comparisons after Bonferroni correction[a]					
		Pla+Pla vs. Pla+Aya	Pla+Pla vs. Ket+Pla	Pla+Pla vs. Ket+Aya	Pla+Aya vs. Ket+Pla	Pla+Aya vs. Ket+Aya	Ket+Pla vs. Ket+Aya
Somaesthesia	$F=21$, $p<0.001$	<0.001*	1.000	0.006*	0.003*	0.293	0.003*
Affect	$F=31$, $p<0.001$	<0.001*	1.000	0.088	0.001*	0.001*	0.145
Perception	$F=19$, $p<0.001$	0.003*	1.000	0.043*	0.003*	0.013*	0.049*
Cognition	$F=10$, $p=0.001$	0.012*	1.000	0.194	0.016*	0.251	0.216
Volition	$F=8$, $p=0.001$	1.000	1.000	0.014*	1.000	0.001*	0.008*
Intensity	$F=24$, $p<0.001$	<0.001*	1.000	0.003*	<0.001*	0.031*	0.095
ARCI							
MBG	$F=32$, $p<0.001$	0.001*	1.000	1.000	<0.001*	<0.001*	1.000
PCAG	$F=14$, $p<0.001$	1.000	1.000	<0.001*	1.000	<0.001*	0.007*
LSD	$F=13$, $p<0.001$	0.002*	1.000	0.009*	0.017*	1.000	0.002*
BG	$F=10$, $p=0.001$	1.000	0.623	0.001*	0.413	0.007*	0.028*
A	$F=16$, $p<0.001$	0.004*	1.000	0.385	0.003*	0.003*	0.212
APZ							
OSE	$F=11$, $p=0.002$	0.017*	1.000	0.405	0.027*	0.034*	0.535
AIA	$F=7$, $p=0.005$	0.019*	1.000	0.576	0.028*	0.710	0.873
VUS	$F=16$, $p<0.001$	0.003*	1.000	0.049*	0.004*	0.036*	0.063

Pla: placebo, Aya: ayahuasca equivalent to 0.75 mg/kg body weight, Ket: 40 mg ketanserin. ARCI – A: amphetamine scale; BG: benzedrine-group; MBG: morphine-benzedrine-group; PCAG: pentobarbital-chlorpromazine-alcohol-group; LSD: lysergic acid diethylamide scale. APZ-OSE: oceanic boundlessness, AIA: dread of ego dissolution, VUS: Visionary restructuralization.

[a]Exact p values.

*Statistically significant.

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than those after ayahuasca alone. However, values in the VUS subscale measuring visual phenomena were still different from placebo. Thus, although ayahuasca effects on the APZ were not completely abolished by ketanserin, they were markedly reduced by this drug: 65% reduction for OSE, 55% for AIA and 44% for VUS.

5.3.4. VAS

Mean scores on all VAS items are shown in Fig. 4, and the results of the statistical analyses are shown in Table 3.

Ayahuasca significantly increased scores on all items except "bad effects", "fear" and "loss of contact with external reality". Again, ketanserin alone did not induce changes in any item. Scores after ketanserin+ayahuasca were significantly lower than after ayahuasca alone in the "any effect" (38%), "good effects" (62%), "liking" (61%), "time" (13%), "feeling high" (55%), "changes in external reality" (59%), and "visions" (38%). However, scores on "any effect", "time", "feeling high", and "visions" were still significantly higher than after placebo. Thus, results on the VAS also suggest ketanserin partially blocked the subjective effects induced by ayahuasca.

6. Correlation analysis

A correlation analysis was conducted between the ayahuasca-induced current density and subjective effects changes. Decreases in alpha correlated with the intensity of "visual effects" ($r=-0.593$, $p=0.042$) and showed a trend toward significance with the APZ-VUS subscale ($r=-0.512$, $p=0.059$). These correlations were reduced and significance was lost in the ketanserin+ayahuasca combination: VAS "visual effects" ($r=-0.412$, $p=0.183$) and APZ-VUS ($r=-0.321$, $p=0.310$). Decreases in theta correlated with changes in VAS "contact with external reality" ($r=-0.591$, $p=0.043$). Again, correlation values were reduced and significance was lost after the ketanserin pretreatment ($r=-0.384$, $p=0.217$). Decreases in delta also correlated with changes in contact with external reality ($r=-0.724$, $p=0.008$). This correlation was also reduced and significance was lost in the ketanserin+ayahuasca combination ($r=-0.179$, $p=0.578$). Scatter plots for several statistically significant correlations are shown in Fig. 5.

7. Discussion

The present study assessed the contribution of 5-HT_{2A} receptors to the neurophysiological and subjective effects of ayahuasca in humans. Results showed that at the administered dose ayahuasca induced significant psychedelic effects and power decreases in the delta-alpha frequency range. Decreases in alpha-band oscillations had their source in posterior brain regions and correlated with the intensity of the visual modifications induced by ayahuasca.

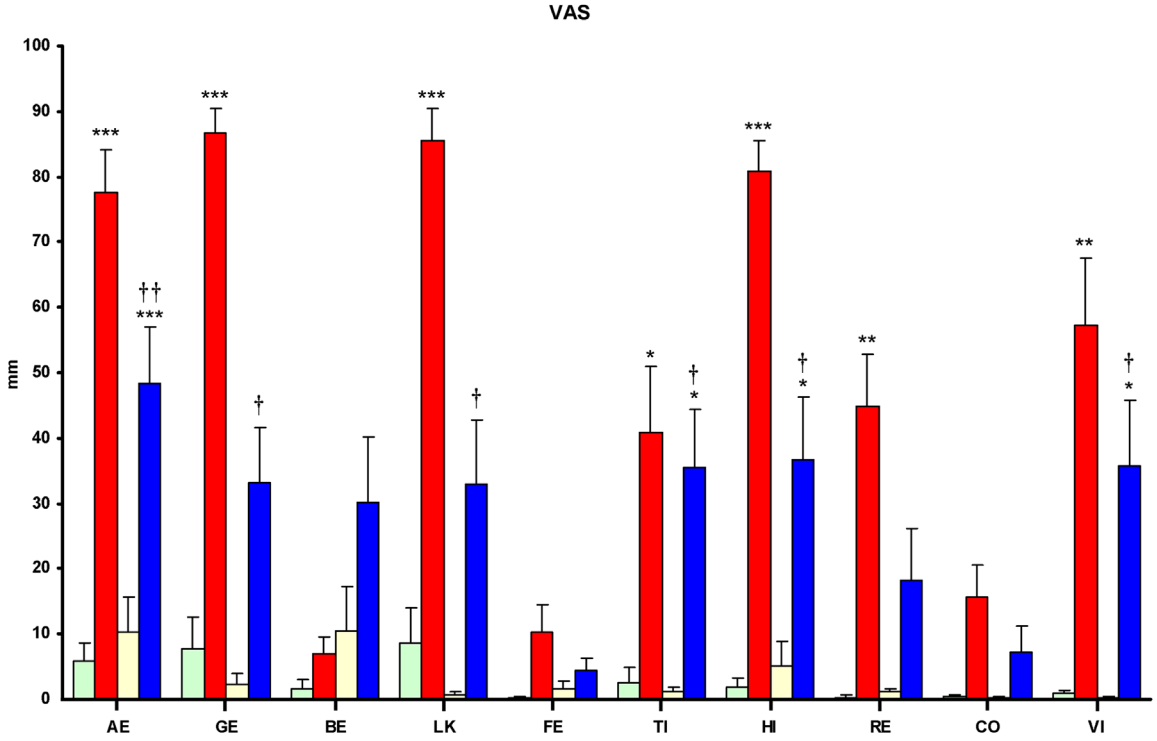

Figure 4 Mean scores on the visual analog scales (VAS) items for the three active treatments administered and placebo. Error bars denote 1 standard error of mean ($n=12$). AE: any effect; GE: good effects; BE: bad effects; LK: liking; FE: fear; TI: modifications of time perception; HI: feeling "high"; RE: changes in external reality; CO: loss of contact with external reality; VI: visions. Significant differences from placebo are denoted as * at $p<0.05$, ** at $p<0.01$, and *** at $p<0.001$. Significant differences between ayahuasca alone and ayahuasca after pretreatment with ketanserin are denoted as † at $p<0.05$, †† at $p<0.01$, and ††† at $p<0.001$. All pairwise comparisons are shown after Bonferroni correction. Green: placebo, red: ayahuasca, yellow: ketanserin, blue: ketanserin +ayahuasca.

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Table 3 Statistical analyses of subjective effects measures (Visual Analog Scales or VAS) induced after the four administered treatment combinations $n=12$. Pairwise comparisons are shown following Bonferroni correction.

VAS	ANOVA df = 3,33	Pairwise comparisons after Bonferroni correction[a]					
		Pla+Pla vs. Pla+Aya	Pla+Pla vs. Ket+Pla	Pla+Pla vs. Ket+Aya	Pla+Aya: Ket +Pla 2:3	Pla+Aya vs. Ket+Pla	Pla+Aya vs. Ket+Aya
AE	$F=48$, $p<0.001$	<0.001*	1.000	<0.001*	<0.001*	0.010*	0.005*
GE	$F=62$, $p<0.001$	<0.001*	1.000	0.087	<0.001*	<0.001*	0.023*
BE	$F=4$, $p=0.029$	0.099	1.000	0.079	1.000	0.257	0.607
LK	$F=53$, $p<0.001$	<0.001*	1.000	0.069	<0.001*	0.001*	0.046*
FE	$F=4$, $p=0.059$	0.237	1.000	0.239	0.479	1.000	0.988
TI	$F=11$, $p<0.001$	0.015*	1.000	0.018*	0.016*	1.000	0.018*
HI	$F=54$, $p<0.001$	<0.001*	1.000	0.026*	<0.001*	0.002*	0.016*
RE	$F=18$, $p<0.001$	0.001*	0.800	0.269	0.001*	0.004*	0.338
CO	$F=6$, $p=0.011$	0.067	1.000	0.788	0.063	0.297	0.733
VI	$F=18$, $p<0.001$	0.001*	1.000	0.030*	0.001*	0.388	0.029*

Pla: placebo, Aya: Ayahuasca equivalent to 0.75 mg/kg body weight, Ket: 40 mg ketanserin. VAS, Visual analog scales – AE: any effect; GE: good effects; BE: bad effects; LK: liking; FE: fear; TI: time; HI: feeling "high"; RE: changes in external reality; CO: loss of contact with external reality; and VI: visions.

[a]Exact p values.

*Statistically significant.

Ketanserin effectively blocked these decreases and reduced this correlation, suggesting a prominent role of the 5-HT_{2A} receptor in the neurophysiological and visionary effects of ayahuasca.

The pattern of subjective effects is also in agreement with prior studies with ayahuasca. The 0.75 mg DMT/kg body weight ayahuasca dose chosen for the present experiment was an intermediate dose used in prior experiments by our group (Dos Santos et al., 2012; Riba et al., 2001b). It effectively induced a transient modified state of awareness characterized by introspection, increased affect and visual phenomena with eyes closed.

The broadband power reductions observed in oscillatory activity replicate results in the literature and are consistent with the previous study by our group involving ayahuasca at the dose of 0.85 mg/kg body weight and scalp topography analysis (Riba et al., 2002a). Our results concerning the source location analysis also agree with a previous study in which broad-band power reductions were localized to parieto-temporo-occipital regions and to a lesser extent to the medial prefrontal/anterior cingulate cortex (Riba et al., 2004). This pattern of neurophysiological effects has been demonstrated for other psychedelics. Kometer et al. (2013) also using EEG, have found that psilocybin-induces reductions in spontaneous alpha oscillations . Muthukumaraswamy and coworkers using magenetoencephalography, a technique that measures the magnetic component of brain oscillations, have also described that psilocybin decreases power of the magnetic signal in a wide range of frequencies, from delta to high gamma (Muthukumaraswamy et al., 2013).

Energy decreases in brain oscillations suggest an excitatory effect of ayahuasca and psychedelics on the cortex. Studies in animals show that psychedelics induce excitatory postsynaptic potentials and currents (Kłodzinska et al., 2002). Further, the spontaneous alpha rhythm exerts an inhibitory role on the visual cortex in the occipital and parietal lobes (Romei et al., 2010, 2008a). Decreases in alpha rhythm are coupled with increased regional metabolism (Moosmann et al., 2003). This inverse relationship between EEG alpha power and increased cortical activity has also been seen in PET studies of blood flow (Buchsbaum et al., 1984), and more recently in studies using fMRI. A negative correlation has been found between alpha and BOLD in the anterior cingulate and in the parieto-occipital cortex (de Munck et al., 2007; Goldman et al., 2002; Laufs et al., 2003), a negative relationship that has been extended to the theta and beta bands of the EEG (de Munck et al., 2009).

Our results support an excitatory effect of ayahuasca on cortical regions involved in the processing of visual sensory information (alpha-occipital), memory-affect (delta-medial temporal lobe or MTL), and cognition-affect (theta-frontolateral and frontomedial cortex). Suppression of inhibitory alpha in the visual network, an area rich in 5-HT_{2A} receptors (Savli et al., 2012), potentially led to the visionary phenomena experienced by participants in the absence of external cues (eyes closed). Analogous to our current findings, Kometer et al. (2013) found that

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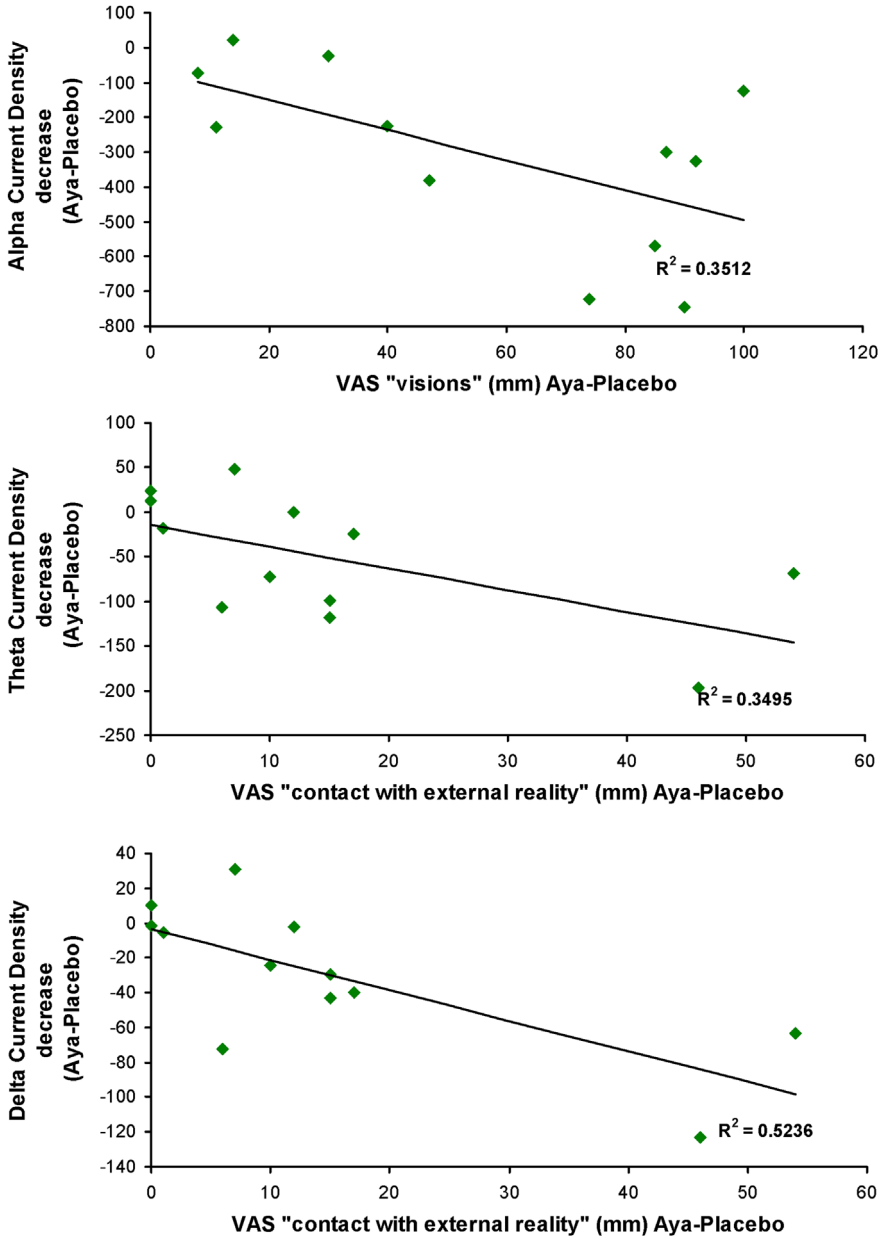

Figure. 5 Scatter plots showing the relationship between current density changes and the intensity of subjective effects as measured by visual analog scales (VAS) and expressed in millimeters from 0 to 100. Top panel: decreases in alpha and the VAS "visual effects" ($r=-0.593$, $p=0.042$); middle panel: decreases in theta and the VAS "contact with external reality" ($r=-0.591$, $p=0.043$); and bottom panel: decreases in delta and the VAS "contact with external reality" ($r=-0.724$, $p=0.008$).

ketanserin blocked psilocybin-induced reductions in alpha oscillations. The marked attenuation of alpha decrease and the reduction of visual effects obtained for the ketanserin +ayahuasca pretreatment suggests that both effects are mediated by 5-HT_{2A} receptor activation.

Increased sensory excitability in posterior visual areas fits the findings of a recent study assessing ayahuasca effects on directed functional connectivity (Alonso et al., 2015) well. Using Transfer Entropy and Granger Causality, the authors found that, under ayahuasca, oscillations in posterior regions increased their influence over signals measured at anterior locations. Conversely, oscillatory activity at frontal sources decreased its influence over activity at occipital sites. The authors interpreted their findings in terms of increased feed-forward or bottom-up information transfer and decreased feed-back or top-down control. Our findings would support 5-HT_{2A} agonism as a key mechanism in the modification of brain dynamics induced by ayahuasca (McKenna and Riba, 2015).

Ayahuasca administration in the present study led to significant increases in measures of affective modifications, in agreement with previous results (Dos Santos et al., 2011; Riba et al., 2006, 2003b). We also found EEG power reductions in areas associated with affective processing, i.e., the MTL and the medial frontal lobe/anterior cingulate cortex (ACC). Power decreases and increased blood flow in these regions had been reported previously using LORETA

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(Riba et al., 2004) and SPECT (Riba et al., 2006), respectively. The amygdala in the MTL and the ACC are key hubs of affective processing. The amygdala is associated with fear and emotional arousal, whereas the ACC and neighboring medial frontal areas integrate emotion and cognition. Effects at these levels have been proposed to underlie the therapeutic potential of ayahuasca (Soler et al., 2015). Ketanserin led to a 62% reduction in the HRS-Affect, subscale, an 80% reduction in the ARCI-MBG (positive mood/euphoria), and antagonized the delta and theta decreases in these regions. These results indicate a prominent role of 5-HT_{2A} activation in the affective effects of ayahuasca and warrant future studies of the cognitive aspects of emotional processing during the ayahuasca experience.

An unexpected finding was that while ketanserin decreased the overall intensity of the subjective experience, blockade was only partial. This finding contrasts with more intense inhibition found in studies involving psilocybin. Vollenweider and colleagues, for example, administered the same ketanserin dose (40 mg) prior to a high oral dose of 25 mg psilocybin and obtained reductions between 75% and 98% in the intensity of visual effects (Vollenweider et al., 1998). Examining the individual data in our study we found that two participants had a deviant pattern in their scores, with higher scores (60 and 100 mm, respectively) on the VAS-Visions item after the ketanserin+ayahuasca combination than after ayahuasca alone (14 and 74 mm, respectively). If these two participants are removed from the sample, the global mean for this item rises to 60 mm after ayahuasca and drops to 27 mm after the combination. However, even if we removed these two outliers, the inhibition of visual effects in our study would still be far from the suppression levels attained by Vollenweider.

One possible explanation for these unexpected results is that DMT interacts with other receptors in addition to 5-HT_{2A}. Indolealkylamines such as DMT and psilocybin also show agonist effects at the 5-HT_{1A} sites. These predominantly pre-synaptic receptors are present in large amounts in the raphe nuclei and their activation suppresses serotonin release. They are also present in the visual cortex, but they exert an inhibitory effect (Gerstl et al., 2008; Moreau et al., 2010). It is therefore doubtful that increased 5-HT_{1A} activation by DMT in the presence of ketanserin increases visual phenomena. However, 5-HT_{1A} agonism may have been responsible for the marked sedation experienced by participants when given the combination (indicated for instance by the high score on the ARCI-PCAG subscale obtained for the combination). Reductions in vigilance and attentional performance have been observed for the combination of ketanserin plus psilocybin (Carter et al., 2005). Theta power increases, as seen in our experiment for ayahuasca+ketanserin, are the characteristic effects of the anxiolytic-HT_{1A} agonist buspirone (Barbanoj et al., 1994).

DMT shows additional mechanistic differences from other psychedelics, which might account for the partial blockade of the observed effects. For instance, in contrast to the prototypical 5-HT_{2A} agonist 2,5-dimethoxy-4-iodoamphetamine (DOI), DMT induces a weak behavioral head twitch response in animals (Carbonaro et al., 2015) and unlike LSD, it does not seem to lead to acute tolerance (Dos Santos et al., 2012; Strassman et al., 1996). Molecular mechanisms specific to DMT are agonism at the trace amine associated receptor (TAAR) (Bunzow et al., 2001); the fact that DMT is a substrate of both the serotonin and the vesicle monoamine transporters (Cozzi et al., 2009); and that it modulates the intracellular sigma-1 receptor (Fontanilla et al., 2009).

The sigma-1 receptor is a chaperone protein localized in the endoplasmic reticulum. It is involved in the regulation of many other proteins and signaling pathways and it has a protective role in cell survival (Chu and Ruoho, 2016; Hayashi and Su, 2007). It is also involved in neural plasticity, as it promotes dendritic spine and synapse formation (Tsai et al., 2009). Abnormal function of the sigma-1 receptor has been associated with various neurodegenerative disorders (Tsai et al., 2014). It is noteworthy that several antidepressants, such as fluvoxamine and sertraline show high affinity for this receptor (Rousseaux and Greene, 2015). It can be speculated that sigma-1 activation by DMT could play a role mediating the antidepressant effects found for ayahuasca (Osório et al., 2015; Sanches et al., 2016). These mechanisms should be addressed in future studies.

As a limitation of the study we would like to mention that volunteer recruitment emphasized previous experience with psychedelic drugs. Although participants had no present or previous diagnosis of drug dependence, detailed information on the dose, frequency and duration of consumption of other drugs was not collected.

To sum up, the present study assessed the contribution of the 5-HT_{2A} receptor to the neurophysiological and psychological effects of ayahuasca. Ayahuasca induced an intense inhibitory effect on alpha oscillations in the parieto-occipito-temporal cortex that correlated with visual phenomena, a hallmark of the ayahuasca experience. The selective 5-HT_{2A} receptor antagonist ketanserin effectively inhibited neurophysiological modifications, reduced the correlation between alpha and the visual effects, and attenuated the intensity of the subjective experience. These findings suggest that despite the chemical complexity of ayahuasca, 5-HT_{2A} activation may especially contribute to its visual perceptual effects. Future studies should assess the role of other molecular mechanisms, such as sigma-1 agonism, in the perceptual, affective and cognitive effects of DMT and ayahuasca.

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Contributors

MV, PF, AF and JR conceived the study. AEM, MR and AR-P conducted the experimental sessions. RMA provided logistical support. SR, JFA, MAM and JR analyzed the neurophysiological data. MV, AEM, MR, AR-P and JR analyzed the subjective effects data. SB analyzed the ayahuasca used in the study. All authors participated in data interpretation, contributed to and have approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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