

Behavioural and neurotoxic effects of ayahuasca infusion (*Banisteriopsis caapi* and *Psychotria viridis*) in female Wistar rat



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ABSTRACT

Ayahuasca, a psychoactive beverage used by indigenous and religious groups, is generally prepared by the coction of *Psychotria viridis* and *Banisteriopsis caapi* plants containing *N,N*-dimethyltryptamine (DMT) and β -carboline alkaloids, respectively. To investigate the acute toxicity of ayahuasca, the infusion was administered by gavage to female Wistar rats at doses of 30X and 50X the dose taken during a religious ritual, and the animals observed for 14 days. Behavioural functions were investigated one hour after dosing at 15X and 30X using the open field, elevated plus maze, and forced swimming tests. Neuronal activation (*c-fos* marked neurons) and toxicity (Fluoro-Jade B and Nissl/Cresyl staining) were investigated in the dorsal raphe nuclei (DRN), amygdaloid nucleus, and hippocampal formation brain areas of rats treated with a 30X ayahuasca dose. The actual lethal oral dose in female Wistar rats could not be determined in this study, but was shown to be higher than the 50X (which corresponds to 15.1 mg/kg bw DMT). The ayahuasca and fluoxetine treated groups showed a significant decrease in locomotion in the open field and elevated plus-maze tests compared to controls. In the forced swimming test, ayahuasca treated animals swam more than controls, a behaviour that was not significant in the fluoxetine group. Treated animals showed higher neuronal activation in all brain areas involved in serotonergic neurotransmission. Although this led to some brain injury, no permanent damage was detected. These results suggest that ayahuasca has antidepressant properties in Wistar female at high doses, an effect that should be further investigated.

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1. Introduction

Ayahuasca (or hoasca) is a psychoactive beverage traditionally used in shamanic rituals by various indigenous populations of the Amazon (McKenna, 2004). It was introduced to non-indigenous Brazilians in the 1930s, and its use by religious groups, including *Santo Daimé* and *União do Vegetal* (UDV), spread beyond the Amazon borders in the 1970s (MacRae, 2004). Religious use of ayahuasca has been regulated in Brazil since 1986 in response to concerns raised by society and health authorities regarding its inadequate use, while ensuring the freedom of religious practices (CONAD,

2010). Its use has also been regulated in other countries, including the USA, Belgium and the Netherlands (Labate and Feeney, 2012; Blainey, 2014). However, ayahuasca consumption has extended beyond religious practices, and may occur in recreational contexts by people seeking the psychedelic effects of the infusion. This non-religious use is illegal in Brazil, and the material can be seized by Brazilian government authorities (Alvarenga et al., 2014).

The psychoactive properties of ayahuasca are produced by the substances present in the plants normally used to prepare the infusion: *N,N*-dimethyltryptamine (DMT) present in the leaves of *Psychotria viridis*, and β -carboline alkaloids such as harmine, harmaline and tetrahydroharmine, present in the *Banisteriopsis caapi* vine (MacKeena, 2004) (Fig. 1). DMT, a non-selective serotonin (5-hydroxytryptamine, 5-HT; Fig. 1) receptor agonist, elicits its effect through stimulation of the 5-HT_{2A} serotonin receptor (Smith et al., 1998), an action that may be attenuated by its interaction with 5-HT_{1A} receptors (Halberstadt and Geyer, 2011). However, unlike

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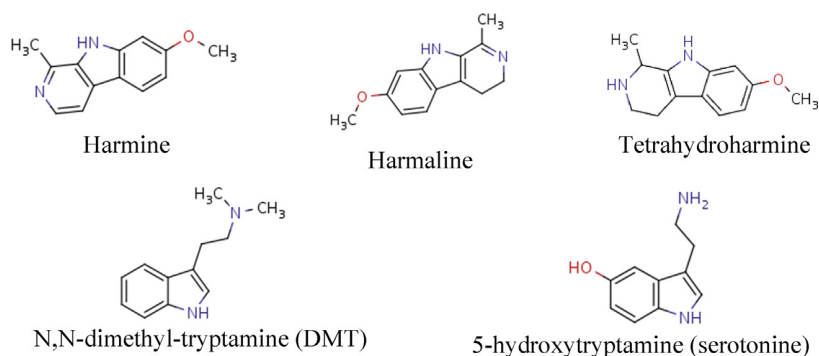


Fig. 1. Chemical structures of the main components of ayahuasca infusion (harmine, harmaline, tetrahydroharmine and DMT), and of serotonin.

other hallucinogens, DMT is inactive when administered orally, as it is readily metabolized by monoamine oxidases (MAO) (Suzuki et al., 1981; Riba et al., 2014). β -carbolines, mainly harmine and harmaline, inhibit MAO activity (Wang et al., 2010), and therefore block the metabolic breakdown of DMT in the liver and gut. Thus, when DMT is absorbed in the gastrointestinal tract, the psychoactive properties of the ayahuasca infusion can occur (Ott, 1999; Riba et al., 2003). Furthermore, as MAO inhibitors, β -carbolines can increase the level of serotonin in the brain (McKenna et al., 1984), and are capable of inducing direct psychoactive effects (Freedland and Mansbach, 1999; Brierley and Davidson, 2012). Brierley and Davidson (2013) also suggested that harmine augments dopamine efflux via a novel shell-specific, presynaptic 5-HT_{2A} receptor-dependent mechanism, independent of MAO inhibitory activity.

The effects and pharmacokinetics of ayahuasca in healthy volunteers after a single or two-repeated ritual doses (about 0.5–1 mg/kg bw of DMT) have been thoroughly described in the literature (Callaway et al., 1999; Riba et al., 2003, 2012; Barbanoj et al., 2008; Bouso et al., 2012; Santos et al., 2012). Other studies have investigated the therapeutic properties of ayahuasca, primarily to treat drug addiction (Brierley and Davidson, 2012; Thomas et al., 2013; Loizaga-Velder and Verres, 2014).

Although the ritualistic use of ayahuasca is considered to be safe (Bouso et al., 2012), a small number of case reports suggest that some individuals may be more prone to experience significant side-effects from ayahuasca intake (Santos, 2013a,b). However, experimental data from studies with animals exposed to high doses of the infusion remain scarce in the literature. The present study aimed at investigating the lethal dose, the impact on behaviour, and the neurotoxic potential in female Wistar rats after acute exposure to high-dose levels of an ayahuasca infusion.

2. Materials and methods

2.1. Animals

This study was conducted with female rats, which are normally more sensitive to acute tests than males (OECD, 2001). Healthy nulliparous female Wistar rats aged between 9 and 12 weeks were acquired from Granja RG (São Paulo, Brazil) and allowed to acclimatize for a 15-day period in the Faculty of Health Sciences of the University of Brasilia (UnB) animal house prior to study initiation. Subjects were kept individually in polypropylene cages under controlled conditions: 12 h/12 h, light/dark; 22–25 °C; 45–60% humidity, and received water and the commercial feed Purina® *ad libitum*. Animals were fasted for 12 h prior to gavage, but with free access to water, and food was re-introduced 4 h after dosing (OECD, 2001). The experimental protocol was approved by the Ethics Committee on Animal Use of the UnB Institute of Biological Sciences (No. 107766/2010).

2.2. Ayahuasca infusion

The ayahuasca infusion was provided by a *União do Vegetal* (UDV) group in the Federal District, Brazil. Plants were collected and the infusion prepared in April 2011. *B. caapi* (vine) was collected in Águas Lindas de Goiás (15° 46' 17"S; 48° 14' 56"W) and *P. viridis* (leaves) were collected in Sobradinho, Federal District (15° 75' 23"S; 47° 72' 92"W). Specimens of *B. caapi* vine and *P. viridis* leaves used to prepare the infusion were deposited in the University of Brasilia (UnB) Herbarium under the reference numbers Azevedo EP 149880 BRAHMS and Trieto B 149879 BRAHMS, respectively. The ayahuasca infusion prepared by the UDV was kept at –20 °C until lyophilization (Liotop L101) to be used throughout the experiment. Dry matter corresponded to 16% (w/v) of the infusion. As most studies conducted with ayahuasca, the doses given to the animals were related to the dose taken during a religious ritual (1X), which in this study corresponds to 150 mL for a 70 kg person (taken in a UDV ritual). Appropriately, weighed lyophilized materials were resuspended in 2 or 3 mL of filtered water prior to treatment, and administered by oral gavage to the rats.

2.2.1. Chemical characterization

Harmaline (99.2% purity) and harmine (98% purity) standards were obtained from Sigma–Aldrich. DMT was synthesized according to Qu et al. (2011), and its identity and purity confirmed by GC–MS/MS (Trace GC Ultra coupled with a TSQ Quantum XLS Triple Quadrupole; Thermo Scientific), ¹H and ¹³C NMR (Varian Mercury Plus spectrometer 7.05 T operating at 300 MHz for ¹H and at 75.46 MHz for ¹³C), and LC–MSD TOF (Agilent 1100 Series) for exact mass determination.

A 100 μ L aliquot of the infusion was dissolved in 10 mL acetonitrile, 1 mL filtered in Millex LCR PTFE 0.45 μ m membrane, and 1 μ L injected in the GC–MS/MS for quantification against standard curves of harmaline, harmine and DMT prepared in acetonitrile. For DMT (MW of 187.3), the quantification and confirmation ions were *m/z* of 77 and 58 (β -cleavage), respectively. The results were confirmed by LC–MS/MS (LC Shimadzu coupled to a triple quadrupole mass spectrometer 4000QTRAP, Applied Biosystems/MDS Sciex). The analysis showed that the ayahuasca infusion contained 0.141 mg/mL DMT, 1.56 mg/mL harmine and 0.122 mg/mL harmaline. A ritual dose of this infusion (150 mL) corresponds to 0.302 mg/kg bw DMT, 3.34 mg/kg bw harmine and 0.261 mg/kg bw harmaline. Tetrahydroharmine was not analyzed in this study.

2.3. Acute oral toxicity test

An attempt to determine the acute oral toxicity of ayahuasca in female Wistar rats was made based on the OECD Guide protocol 423/2001 (Acute Oral Toxicity – Acute Toxic Class Method;

OECD, 2001), designed to be used for single substances. This protocol begins with three animals exposed to an initial dose, and the test repeated with an additional three animals to confirm the results. Depending on the mortality and/or moribund status of the animals, the experiment is repeated at a lower dose (at least 2 moribund or dead animals), or a higher dose (a maximum of one moribund or dead animal). Hence, six animals are tested at each dose to determine the classification in a toxicological ranking. As ayahuasca is an infusion containing a mixture of substances, the initial test was conducted using an empirical dose of 30 times the ritual dose (30X), and 3 mL total volume.

Animals were weighed prior to administration (276.4 ± 9.0 g), and at three-day intervals following administration. Daily observations were made for clinical effects, including posture, tremor, piloerection, vocalization and convulsion. On day 14, all surviving animals were euthanized by CO₂ exposure, and the liver, spleen, heart, brain and kidneys were macroscopically analyzed and weighed. Organ fragments were fixed in 4% formalin for 24 h and embedded in paraffin; 5–6 μ m sections were obtained using a microtome (Leica), and stained with haematoxylin and eosin (H&E). Alterations from the normal structure were investigated under a light microscope (Olympus BX41 with a SCANSCOPE).

2.4. Behavioural tests

Female rats (223 ± 16 g) were treated once at 15X (4.5 mg/kg bw DMT) and 30X (9 mg/kg bw DMT) doses ($n = 10$ at each dose), to investigate motor and sensory behavioral functions using the open field (Hall, 1934), elevated plus maze (Pellow et al., 1985), and forced swimming tests (Porliss et al., 1978; Detke et al., 1995; Slattery and Cryan, 2012). The tests were performed one hour after dosing. The forced swimming test protocol (Detke et al., 1995; Detke et al., 1995) requires the inclusion of a fluoxetine positive control group ($n = 10$) at an intraperitoneal (ip) dose of 20 mg/kg bw (fluoxetine hydrochloride in 0.9% saline). This protocol also requires that the rats are subjected to a 15 min swimming adaptation phase 24 h prior to test initiation. Each test was performed by a trained person, blind to the treatment doses.

The open field equipment consisted of a 96 cm diameter circular white wooden arena, with a 34 cm wall. The floor was divided into 18 squares and a central area. One hour after ayahuasca administration, the animals were placed in the central area and their behaviour observed for 5 min for locomotion (number of quadrants crossed), number of entries in the central square, and number of rearing, grooming, defecation (number of faecal boli), and urination.

Immediately after the open field test, the animal was placed in the central platform of the elevated plus-maze, facing a closed arm. The equipment consisted of a plus-shaped wooden apparatus painted black with two open and two closed arms (each 50 cm long), with an open roof, and elevated 38 cm from the floor with a central platform (10 \times 10 cm). The closed arms had 43-cm-high walls. Animal behavior was observed for 5 min for number of entries in the open and closed arms, time spent in the open and closed arms and in the central area, and number of rearing, grooming, defecation and urination.

Following the elevated plus maze test, each rodent was submitted to the forced swimming test in a transparent glass tank (50.5 cm tall by 39 cm in diameter), filled with water (approximately 25 °C) to a depth of 30 cm. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) antidepressant, was administered 23 h, 5 h and 1 h prior to the test. The test was scored every 5 s for 5 min for immobility, swimming and climbing (Detke et al., 1995).

2.5. Neuronal activation and neurotoxicity

Animals treated at 30X and controls ($n = 6$ for each group) were placed in an open field arena for a 2-h period prior to euthana-

Table 1

Food consumption and organ weight of the control group and ayahuasca treated rats. Data are the mean \pm standard error.

	Control ($n = 7$)	30X ($n = 5$)	50X ($n = 5$)
Food consumption, g			
Day 0–3	$62.5 \pm 1.5^{a†}$	72.6 ± 12.4^a	$27.6 \pm 10.5^{b†}$
Day 3–7	$45.4 \pm 2.6^{\ddagger}$	59.6 ± 10.7	$65.6 \pm 8.6^{\ddagger}$
Day 7–10	$65.2 \pm 3.5^{\ddagger}$	58.9 ± 4.3	$66.4 \pm 11.0^{\ddagger}$
Day 10–14	$37.4 \pm 2.6^{a†}$	49.5 ± 1.3^b	$51.7 \pm 5.3^{b†}$
Organ weight, g			
Liver	11.3 ± 0.56	10.9 ± 0.77	11.2 ± 0.72
Spleen	0.94 ± 0.05	0.80 ± 0.03	0.78 ± 0.07
Left kidney	0.91 ± 0.08	1.0 ± 0.05	1.1 ± 0.06
Right kidney	0.99 ± 0.04	1.1 ± 0.05	1.1 ± 0.07
Stomach	2.8 ± 0.20^a	4.3 ± 0.40^b	3.8 ± 0.23^b
Heart	0.94 ± 0.05	0.90 ± 0.05	1.0 ± 0.06
Brain	2.0 ± 0.16	2.0 ± 0.03	2.1 ± 0.14

Letters: comparison among groups at the same day; symbols (‡ or ‡): comparison within the same group during the study period. Significant differences were found for means with different letters or symbols ($P < 0.05$).

sia *via* intraperitoneal thiopental overdose (240 mg/kg bw). Using a peristaltic pump (AVS Projects), transcatheter perfusion was conducted in each animal with a 0.9% saline solution (at 8 mL/min for 5 min), followed by a 4% formalin solution (10 mL/min for 15 min). The brain was removed, fixed in 4% formalin solution for 48 h, and stored in 30% sucrose solution. Each brain was sliced (coronal 50 μ m thick) using a KD-400 Vibrating Microtome, and the slices kept in an anti-freezing solution at 4 °C for a minimum of 48 h. Slices from the following regions of the brain were selected for investigation: dorsal raphe nuclei (dorsal part; DRN), amygdaloid nucleus (basolateral posterior nucleus: BLP), and hippocampal formation (dentate gyrus, GD, and the *Cornu Ammonis* areas CA1, CA2, CA3). These areas are important structures of the serotonergic pathways that participate in the processing of emotional information, and are involved in the modulation of behavioural responses in animal models of anxiety or depression. The regions were identified based on the Rat Brain Atlas (Paxino and Watson, 2006).

The immediate-early gene product *c-fos* is a well-known marker of neuronal activation in the central nervous system (Kobelt et al., 2004). Three slices of each selected brain region from each animal were pre-treated with 40 mL H₂O₂:methanol (3%) solution (9:1), followed by two washes with 0.3% Triton X-100 in phosphate buffered saline (PBS) solution. Non-specific binding was blocked by incubation of the brain slices at room temperature for 30 min in 3% goat serum in PBS. Following the non-specific blockage, the primary polyclonal rabbit anti-*c-fos* antibody (Sigma-Aldrich), diluted 1:1000 with PBS, was added and incubated at 4 °C for 48 h. The slices were washed with PBS and incubated with goat anti-rabbit IgG-biotinylated secondary antibody (Sinapse Biotecnologia) in a 1:100 PBS dilution for 2 h. Sections were treated with avidin biotin complex (ABC) reagent (Thermo Scientific) for 30 min. Immunoreactions were visualized by exposing the slices to a 0.06% 3,3'-diaminobenzidine solution containing 10 μ L of 30% H₂O₂ for 8–10 min. Finally, the slices were washed with PBS, placed on gelatinized slides, dehydrated in graded alcohol, cleaned in xylene and covered by entellan (Merck). The number of *fos*-positive neurons was counted using the Leica Application Suite (LAS 4.1.0). The full areas were counted in the dorsal raphe nuclei, and hippocampal formations and selected areas were counted for BLP.

To evaluate neuron degeneration, three slices of each brain region were stained with Fluoro-Jade B using the protocols described by Schmued and Hopkins (2000). The analysis of this staining was performed according to the following classification: (0) no stained cells, (+) weakly stained, less than 20% of the analyzed region; (++) mildly stained, 30–70% of the region; and (+++) strongly stained, more than 70% of the analyzed region. Neuron death and

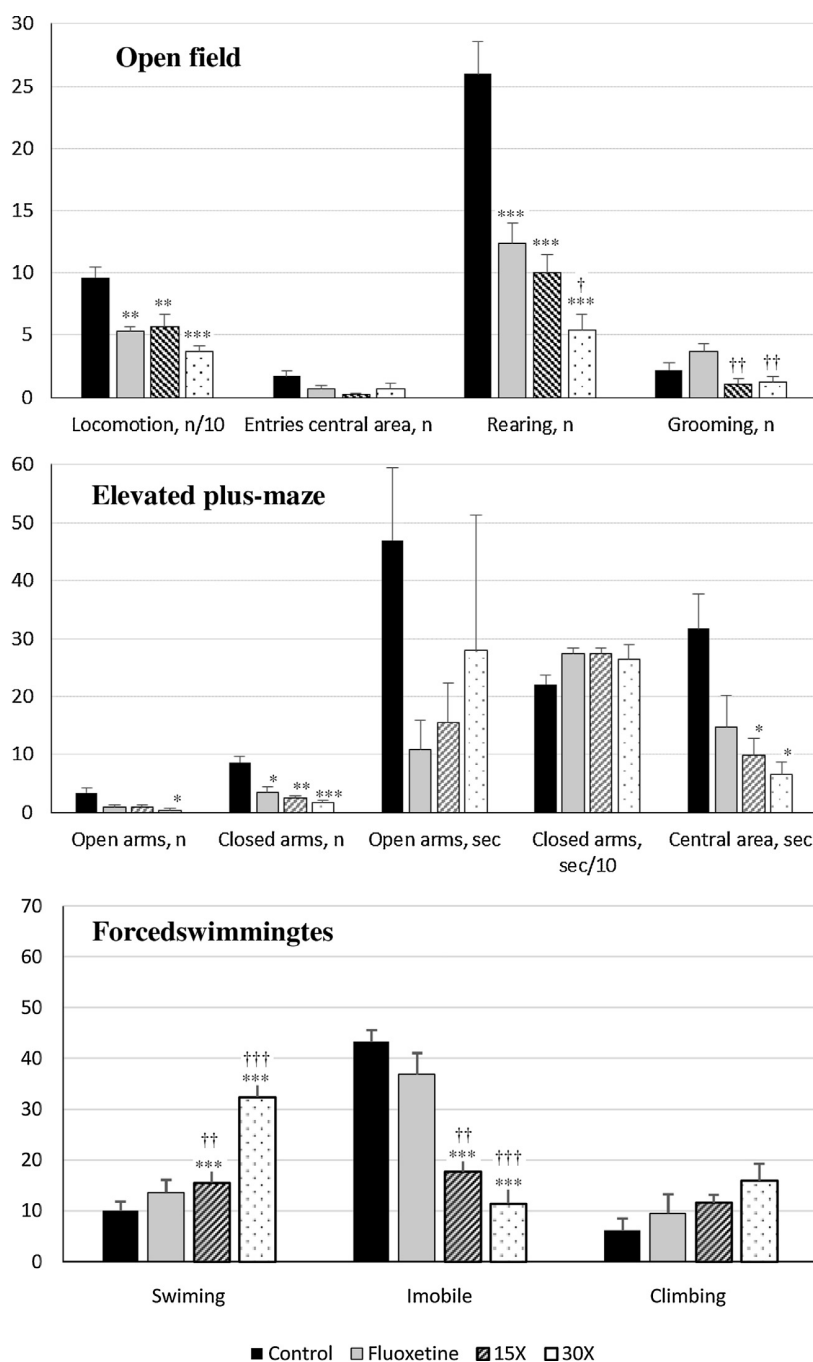


Fig. 2. Results of the open field, elevated plus maze and forced swimming tests for female Wistar rats exposed to fluoxetine or ayahuasca (15X or 30X dose; 4.5 and 9 mg/kg bw DMT, respectively). Each value represent the mean \pm SEM of 10 animals; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with the control group; † $P < 0.05$, †† $P < 0.01$ and ††† $P < 0.001$ compared with the fluoxetine group. Values for locomotion and time spent in closed arms are divided by 10 to fit the graphic scales.

loss were evaluated after Nissl/Cresyl violet staining. Three slices of each selected brain region from each animal were stained and analyzed.

2.6. Statistical analysis

Statistical analyses of the behavioral test data were performed using IBM® SPSS® Statistics version 20, using one-way ANOVA. *Post hoc* comparisons between groups were conducted using Tukey (homogeneous variance) or Dunnett T3 (non-homogenous variance) tests. Statistical analyses of the neurotoxicity assay data were

performed with the GraphPad Prism Software version 6.0 for Windows (GraphPad Software, San Diego, USA), using the Student's *t* test. In all cases, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Acute toxicity

None of the first three animals treated with 30X dose (corresponding to 9 mg/kg bw DMT) died or had any morbidity signs over the 14-day study. When this test was repeated, one animal

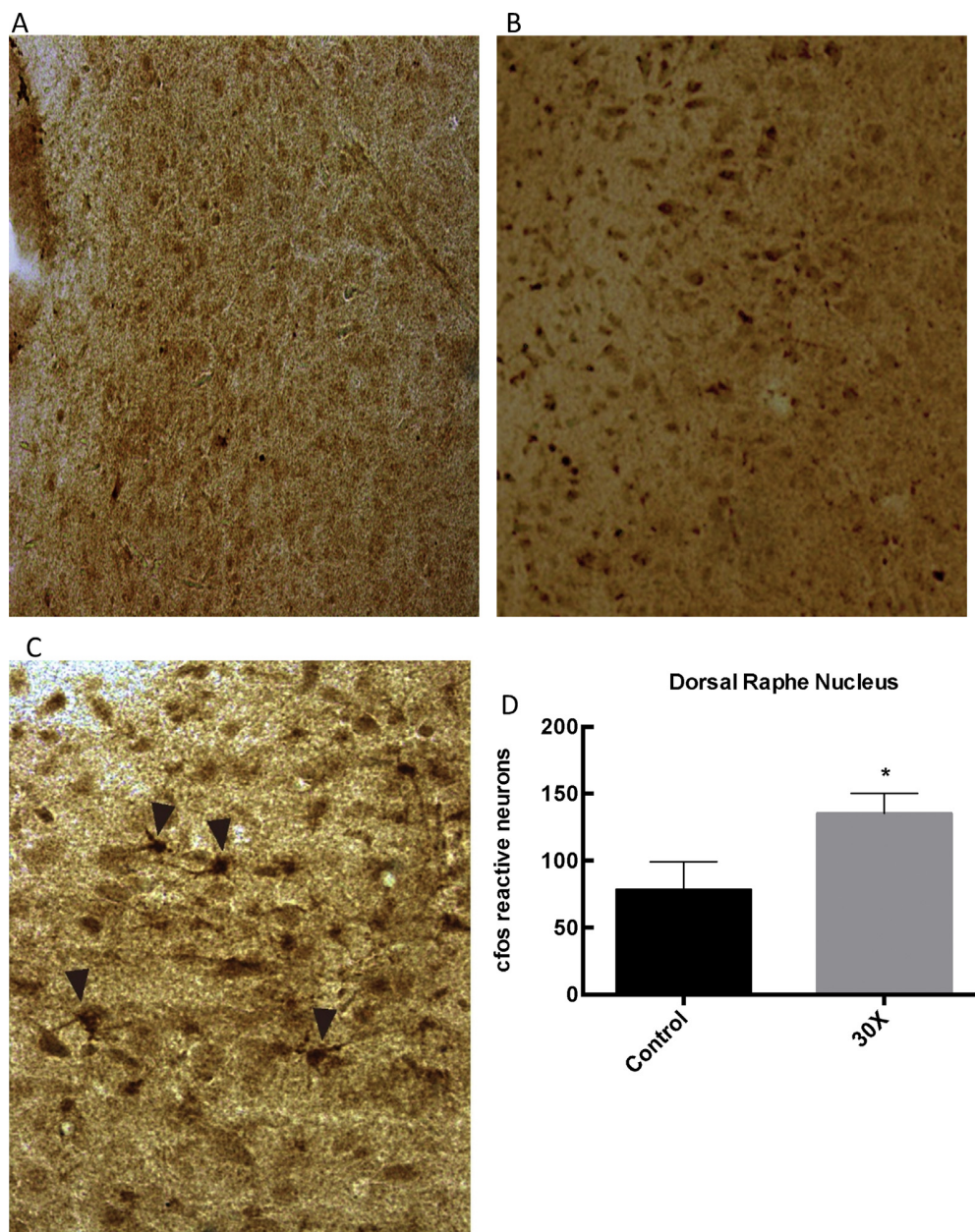


Fig. 3. *C-fos* reactive neurons from dorsal raphe nuclei region of female Wistar rats exposed to 30X dose of ayahuasca (9 mg/kg bw DMT). Coronal section from midbrain region, showing the dorsal raphe nuclei of the control (A) and ayahuasca (B) groups; (C) *c-fos* reactive neurons in the dorsal part (arrow heads) of a treated rat (20× magnification); only cells that had distinctive staining in the nucleus were considered; (D) number of *c-fos* reactive neurons for control and treated rats. Each value represent the mean \pm SEM of 6 animals. * $P < 0.05$ compared with the control group.

died in the first 2 h after oral gavage. Following the OECD 423/2001 protocol, the experiment was repeated at a higher dose, which in this study was the highest possible dose that could be given to the rat by gavage, 50X dose (15 mg/kg bw DMT). One of the six animals tested with 50X died (5 h and 40 min after gavage). The maximum dose limitation of this study was due to the difficulty of dissolving the lyophilized material in water using a maximum of 3 mL dose volume. Furthermore, a previous test had shown that a 70X dose dissolved in 5 mL caused the death of the animal in a few minutes, apparently as a result of stomach expansion leading to compression of the diaphragm and asphyxia. These results indicate that the lethal oral dose of ayahuasca infusion for female

Wistar rats was higher than the 50X dose, or higher than 15 mg/kg DMT. Piloerection and tremors were observed during the first 24 h in all dosed animals, and both animals that died showed flat body posture, reciprocal forepaw treading, hindlimb abduction, and lateral head weaving, characteristic symptoms of 5-HT behavioural syndrome (Halberstadt and Geyer, 2011).

All treated animal stomachs were dilated at the time of necropsy 14 days after treatment, with a significant increase in weight compared with the controls (Table 1). No other macroscopic alterations were observed in the animal organs, and no histological alterations were observed in liver, spleen, heart, brain and kidneys at any dose level.

3.2. Behavior tests

In the open field test, there was a significant decrease in locomotion [$F(3,36) = 13.3, P = 0.000$] and rearing [$F(3,36) = 23.8, P = 0.000$] performed by animals from the fluoxetine and ayahuasca treated groups compared with the controls (Fig. 2). There was also a significant decrease in grooming in the ayahuasca treated groups compared with the control and fluoxetine groups [$F(3,36) = 5.52, P = 0.003$]. Although rats from the treated groups entered the central area less than the control, this effect was not significant.

Ayahuasca and fluoxetine treated rats had statistically fewer entries in the closed arms than the controls in the elevated plus-maze test [$F(3,36) = 16.1, P = 0.000$], and those from the 30X dose group also entered less in the open arms than the controls (Fig. 2; $P = 0.028$). Treatment with either fluoxetine or ayahuasca did not significantly affect the time spent in the open or closed arms, however ayahuasca-treated rats spent significantly less time in the centre of the platform than the controls ($P = 0.34$ and 0.12 for the 30X and 50X groups, respectively; Fig. 2). Treated rats had a significant decrease in the number of rearing than the controls [$F(3,36) = 17.1, P = 0.000$], but grooming was only significantly lower for the 30X group ($P = 0.12$).

Ayahuasca-treated rats from both groups showed significantly more swimming activity and less immobility than the control and fluoxetine groups ($P < 0.01$; Fig. 3). This effect was not significant for the animals from the fluoxetine group. No treatment had a significant effect on climbing activity compared to controls. No statistical differences were found between treated groups and the control for urination and/or defecation in all tests performed (data not shown).

3.3. Neuronal activation and neurotoxicity

Fig. 3A shows the area selected for *c-fos* reactive neuron counting of the dorsal part of the dorsal raphe nuclei (DRN), with strongly labelled neurons considered positive and indicating high neuron activation in the region (Fig. 3B and 3C). A significant increase in labelled neuron counting of the dorsal raphe nuclei was observed in comparison with the control (Fig. 3D). This increase was also identified in the basolateral posterior amygdaloid nucleus (Fig. 4A) and in all hippocampal formation regions investigated (Fig. 4B).

In the Fluoro-Jade B analysis, the control rats did not show any evidence of labelled neurons, whereas the treated rats showed labelled neurons in all areas analysed. The dorsal raphe nuclei

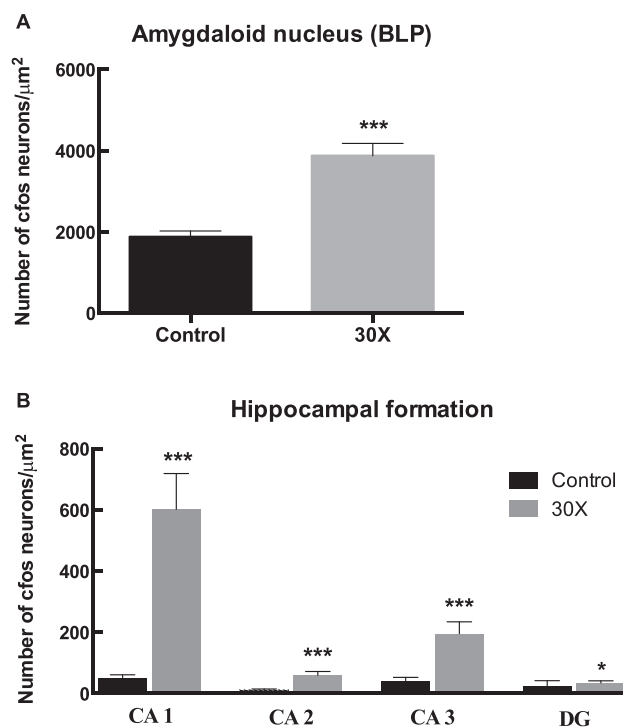


Fig. 4. Number of *c-fos* reactive neurons from basolateral posterior amygdaloid nucleus (A) and hippocampal formation (B) regions of female Wistar rats treated with 30X dose of ayahuasca (9 mg/kg bw DMT) and control. Each value represent the mean \pm SEM of 6 animals. * $P < 0.05$ and *** $P < 0.001$ compared with the control group.

region (DRN) was strongly labelled (+++), probably as a consequence of the strong activation of the *c-fos* reactive neurons, shown in Fig. 3C. Dentate gyrus (Fig. 5) and amygdaloid nucleus showed to be mildly marked (++), and the CA1 region of hippocampal formation was weakly-labelled (+). No marks were observed in the CA2 or CA3 neurons, probably because the experiment was conducted only 2 h after exposure, and changes in this region are expected to be seen later. Control animals showed no labelled neurons in any region investigated. Nissl substance staining did not show any significant difference between control and treated animals in viable neurons and composition of hippocampal formation layers.

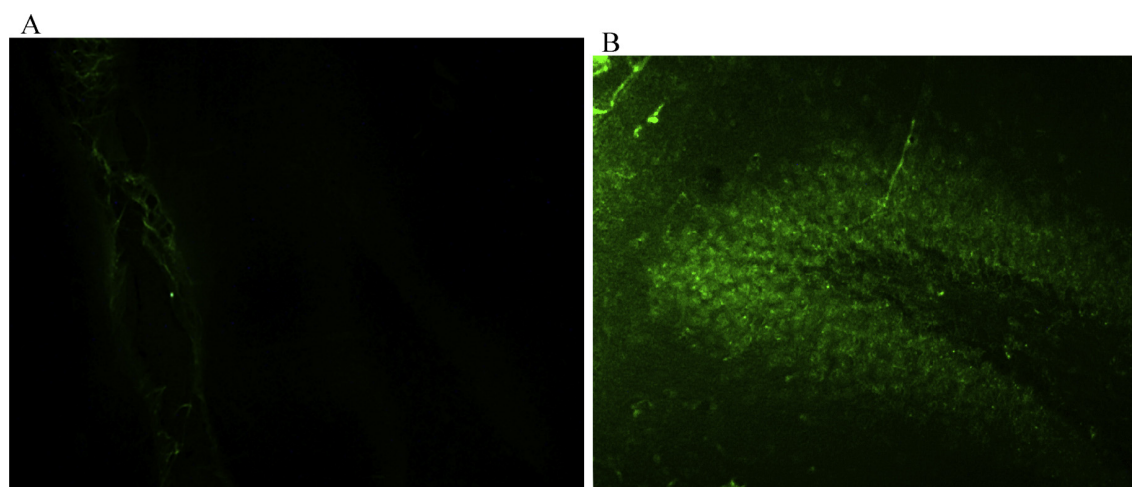


Fig. 5. Fluoro-Jade images of the dentate gyrus area from (A) control and (B) ayahuasca treated at 30X dose (9 mg/kg bw DMT), showing the fluorescent labeled neurons.

4. Discussion

Ayahuasca alkaloid profiles vary considerably, mainly due to the proportion of the plants and the method used to prepare the infusion, as well as the plant cultivars (McKenna et al., 1984; McKenna, 2004). The level of harmine present in the material used in this study (1.56 mg/mL) was similar to that reported by Callaway et al. (1999) (1.7 mg/mL) in a human pharmacokinetic study, and by Oliveira et al. (2010) (1.37 mg/mL) in toxicological studies. However, our infusion contained less DMT (0.141 mg/mL) than the material used by other authors (0.23–0.42 mg/mL).

The lethal dose of the ayahuasca infusion in female Wistar rats was found to be greater than 50X the usual dose in humans, which was the highest dose tested due to the limited water solubility of the lyophilized ayahuasca material. This dose corresponds to 15.1 mg/kg bw DMT, 13.1 mg/kg bw harmaline, and 167 mg/kg bw harmine. To the best of our knowledge, oral lethal dose values for these substances have not been directly determined. Based on an intravenous LD₅₀ (dose that killed 50% of the animals in the group) of 32 mg/kg bw and an intravenous to oral conversion factor of 1:5, Gable (2007) estimated the oral LD₅₀ of 160 mg/kg bw for DMT in mice, which is over ten times the highest dose that could be investigated in our study. Using a safety factor of 20 (accounting for interspecies variability, assuming humans to be more sensitive), the authors estimated a LD₅₀ for DMT in humans as 8 mg/kg bw, which is at least 20 times higher than the usual dose taken by a 70 kg person (an ayahuasca infusion containing 27 mg DMT).

Serotonin behavioural syndrome was observed in the two rats that died at the 30 or 50X dose in this study (9 and 15 mg/kg bw DMT). In humans, serotonin syndrome may occur after an antidepressant overdose, or a combination of several serotonergic drugs; clinical signs include changes in mental status, restlessness, myoclonus, hyperreflexia, diaphoresis, shivering, and tremor (Kalueff et al., 2008). With the wide use of pro-serotonergic drugs, such as SSRI, and the increasing popularity of ayahuasca, the syndrome may occur after ayahuasca consumption concomitantly with the drugs (Callaway and Grob, 1998). Sklerov et al. (2005) reported a fatal case involving the recreational use of an ayahuasca-like preparation followed by the ingestion of 5-MeO-DMT. Although DMT, harmine, harmaline and 5-MeO-DMT were found in the gastric content of this individual, hallucinogenic amine intoxication was ruled out as the cause of death, which could not be determined. The media have reported fatalities involving ayahuasca consumption in Brazil and other South American countries in the last decade. However, no direct implication with the infusion could be made due to lack of forensic analyses and information on the actual dose taken, and the previous health conditions of the individual (Santos, 2013). Although the actual lethal dose could not be determined in the acute toxicity test, we could use its results to identify a high, but still safe dose to female rats (30X the usual dose) that could be used in the subsequent tests performed in this study.

The behavioral effects of ayahuasca infusion on female Wistar rats after a single exposure were evaluated in this study using the open field, elevated plus-maze, and forced swimming tests. The open field test is a common measure of exploratory behaviour and general activity in rodents, and was originally used to investigate the emotionality of rats (fear), indicated by increased defecation and urination (Hall, 1934). In this study, no significant differences were found in these parameters between the control and treated animals (15 and 30X). In addition, the open field model is based on rodent aversion to open spaces and a tendency to walk close to walls, a behaviour known as thigmotaxis (Lamprea et al., 2008), allowing the assessment of the potential stimulant, depressant or anxiolytic effects of a given compound (Prut and Belzung, 2003). Parenteral administration of 5HT_{1A} agonists generally induces anxiolytic-like effects in rats subject to the open field

tests, although non-specific 5-HT agonists (such as DMT) were anxiogenic or had no effect in most studies (Prut and Belzung, 2003). In our study, fluoxetine and ayahuasca-treated rats clearly showed a decrease in locomotion activity, and less exploring behaviour than controls, shown by decreasing rearing and grooming (only ayahuasca groups). Fluoxetine is one of the most widely prescribed antidepressant drugs worldwide, and is also approved for other mood disorders, including generalized anxiety (Zou et al., 2013).

The elevated plus-maze test is based on the spontaneous exploratory behaviour of rodents, and their natural aversion to the open arms caused by fear and anxiety (Pellow et al., 1985; Walf and Frye, 2007). Thus, an increase in the number of entries added to the lengthy time spent in the open arm apparatus indicates a lower level of anxiety (Pellow et al., 1985; Hogg, 1996). Animals treated with fluoxetine or ayahuasca entered the closed arms significantly less than the controls, the 30X treated group entered the open arms less, and both ayahuasca groups entered the central area less, meaning that the animals moved through the apparatus less. These results confirm the open field test findings of a decreased mobility effect on rats exposed to fluoxetine and ayahuasca at the doses tested. Furthermore, as in the open field, the treated animals showed significantly less rearing than the control group, reflecting less intention to explore.

These results confirm previous studies showing that serotonergic hallucinogens produce significant effects on mobility and exploratory behaviours in rodents. In mice, moderate doses of phenyl alkylamines (such as mescaline) were shown to increase locomotion activity, which are mediated by the 5-HT_{2A} receptor. However, indoleamines, such as psilocin and 5-MeO-DMT, decrease locomotor activity, which is mediated by the 5-HT₁ receptor (Halberstadt et al., 2009; Halberstadt and Geyer, 2011). In addition, rats exposed to 5-MeO-DMT (indoleamine with a longer acting profile when compared to DMT) at 0.1 mg/kg bw, either alone or in combination with a MAO inhibitor, showed decreased locomotor activity in the behavioural pattern (Halberstadt et al., 2008).

The forced swimming test (also known as behavioural despair or the Porsolt test) is one of the most widely used tools for the screening of antidepressants in rodent models. When rats are forced to swim in a cylinder from which they cannot escape, after an initial period of vigorous activity, they will adopt a characteristic immobile posture. Immobility is reduced by various clinically effective antidepressant drugs at doses which would otherwise decrease spontaneous motor activity in an open field (Porsolt et al., 1978). The antidepressant effect of MAO inhibitors in this model was first described by Loomer et al. (1957). In our study, animals treated with ayahuasca showed significantly higher swimming behaviour and less immobility than the controls. Although fluoxetine (20 mg/kg bw ip) showed the same tendency, the difference in relation to the controls was not significant, contrary to what has been found in other studies (Dekte et al., 1995; subcutaneous injection, 5–20 mg/kg bw; Refaey and Amri, 2011, 10 mg/kg bw intraperitoneal). Taken together, the forced swimming test results strongly suggest that the ayahuasca infusion has an antidepressant effect. Indeed, harmine, the most concentrated β -carboline found in ayahuasca, has been shown to have an antidepressant-like effect in rodents submitted to the forced swimming tests (Fortunato et al., 2009, 2010). The swimming behavior in the forced swimming test is strongly related to the serotonergic activity (Micale et al., 2013), and the antidepressant properties of ayahuasca could be related to its activity on the 5-HTergic system. The animal studies seem to corroborate the results in humans, as reported by Santos et al. (2007), who showed that regular ayahuasca users scored lower on the scales for panic and hopelessness, which are indicators of a depressive state. More recently, Osório et al. (2015) suggested that ayahuasca has fast-acting anxiolytic and antidepressant effects in patients with a depressive disorder.

The psychoactive properties of ayahuasca are mainly due to the action of DMT as an agonist of the serotonergic 5-HT_{2A} receptor in the central nervous system (McKenna et al., 1984; Freedland and Mansbach, 1999; Ott, 1999; Riba et al., 2003; Wang et al., 2010; Brierley and Davidson, 2012). In this study, neuronal activation of ayahuasca, indicated by *c-fos* labelled neurons, was investigated in the dorsal raphe nuclei (DRN), amygdaloid nucleus and hippocampal formation brain regions of rats treated at 30X the usual dose. The raphe nuclei are a cluster of nuclei found in the brain stem whose main function is to release serotonin to correlated areas of the brain. The DRN plays an important role in facilitating anxiety-related physiological or behavioural responses to drugs or uncontrollable aversive stimuli, and has efferent projections on the amygdaloid complex, hippocampal formation and locus coeruleus (Peyron et al., 1998; Lowry et al., 2008). Indeed, we found that animals treated with a 30X dose of ayahuasca had higher neuronal activation in all brain areas investigated in comparison with the controls.

In regular ayahuasca users, neuroimaging studies of a single ayahuasca administration also showed activation of brain structures involved in emotional arousal, for example the amygdala and parahippocampal gyrus (Riba et al., 2006; for revision see Santos, 2013a). Moreover, ayahuasca increased neural activation in areas involved in the visual process, episodic and working memory, the processing of contextual associations, intentional prospective imagination, and the processing of information from internal sources (Araujo et al., 2012). Our results confirms that ayahuasca influences neural systems involved with interoception and emotional processing, indicating that serotonergic pathways can modulate these systems. Furthermore, Castro-Neto et al. (2013) showed that ayahuasca treated rats (at 5–16X the usual dose) had a higher level of 5-HT in the hippocampus and amygdala regions. However, alterations in brain activity induced by ayahuasca, as shown in this study, have not yet been demonstrated in animal models before.

Although neural activation led to some neurodegeneration (death or in the process of dying), as indicated by the Fluoro-Jade B staining, no permanent damage that could lead to alterations in the brain morphology and number of cells was detected. Figueroa (2012) showed neuron apoptosis in rats exposed to a 5X ayahuasca dose for 21 days through the TUNEL test (Terminal deoxy nucleotidyl transferase UTP nick end labeling), which could support the hypothesis that apoptosis occurs after neuron degeneration, as was detected in our study.

5. Conclusions

In this study, the actual lethal dose of an ayahuasca infusion could not be determined in female Wistar rats due to the limited solubility of the lyophilized material, but showed to be higher than the 50X dose (or 15 mg/kg bw DMT). Rats exposed to 15X and 30X doses presented decreased locomotor and exploratory activities in the open field and elevated plus-maze tests, similar to fluoxetine, a known antidepressant drug. Behavior of treated rats in the forced swimming test indicated a more pronounced antidepressant effect than what was observed for fluoxetine. Furthermore, *c-fos* expression activation observed in brain areas involved in serotonergic neurotransmission confirmed the role of ayahuasca components in interoception and emotional processing modulated by serotonergic pathways. Further studies conducted at lower and multiple doses are necessary to confirm the antidepressant effects of the ayahuasca infusion, and its potential therapeutic use. Additionally, it would be interesting to test the individual components of the ayahuasca infusion (DMT and β -carbolines) to elucidate the direct serotonin involvement in this process.

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