Journal of Ethnopharmacology 323 (2024) 117710

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Ayahuasca and its major component harmine promote antinociceptive effects in mouse models of acute and chronic pain

Pedro Santana Sales Lauria [a], Juliana de Medeiros Gomes [b], Lucas Silva Abreu [c], Rejane Conceição Santana [d], Victor Luiz Correia Nunes [e], Ricardo David Couto [a,f], Paulo Oliveira Colavolpe [g], Marcelo Sobral da Silva [b], Milena Botelho Pereira Soares [h,i], Cristiane Flora Villarreal [a,h,*]

[a] School of Pharmacy, Federal University of Bahia, 40.170-115, Salvador, BA, Brazil
[b] Department of Pharmaceutical Sciences, Federal University of Paraíba, 58.050-585, João Pessoa, PB, Brazil
[c] Chemistry Institute, Fluminense Federal University, 24.020-150, Niterói, RJ, Brazil
[d] Institute of Health Sciences, Federal University of Bahia, 40.231-300, Salvador, BA, Brazil
[e] School of Medicine, Federal University of Bahia, 40.231-300, Salvador, BA, Brazil
[f] School of Medicine, University Center of Technology and Science, 41.800-700, Salvador, BA, Brazil
[g] Hospital of Orthopedics and Traumatology - COT, 40.110-160, Salvador, BA, Brazil
[h] Gonçalo Moniz Institute, FIOCRUZ, 40.296-710, Salvador, BA, Brazil
[i] Institute of Advanced Systems in Health, SENAI CIMATEC, 41.650-010, Salvador, BA, Brazil

ARTICLE INFO

Handling Editor: dr. P Fernandes

Keywords:
Ayahuasca
Harmine
Neuropathic pain
Analgesic
Serotonin
GABA

ABSTRACT

Ethnopharmacological relevance: Ayahuasca (AYA) is a psychedelic brew used in religious ceremonies. It is broadly used as a sacred medicine for treating several ailments, including pain of various origins.
Aim of the study: To investigate the antinociceptive effects of AYA and its mechanisms in preclinical models of acute and chronic pain in mice, in particular during experimental neuropathy.
Materials and methods: The antinociceptive effects of AYA administered orally were assessed in the following models of pain: formalin test, Complete Freund's Adjuvant (CFA)-induced inflammation, tail flick test, and partial sciatic nerve ligation model of neuropathic pain. Antagonism assays and Fos immunohistochemistry in the brain were performed. AYA-induced toxicity was investigated. AYA was chemically characterized. The antinociceptive effect of harmine, the major component present in AYA, was investigated.
Results: AYA (24–3000 μL/kg) dose-dependently reduced formalin-induced pain-like behaviors and CFA-induced mechanical allodynia but did not affect CFA-induced paw edema or tail flick latency. During experimental neuropathy, single treatments with AYA (24–3000 μL/kg) reduced mechanical allodynia; daily treatments once or twice a day for 14 days promoted consistent and sustained antinociception. The antinociceptive effect of AYA (600 μL/kg) was reverted by bicuculline (1 mg/kg) and methysergide (5 mg/kg), but not by naloxone (5 mg/kg), phaclofen (2 mg/kg), and rimonabant (10 mg/kg), suggesting the roles of $GABA_A$ and serotonergic receptors. AYA increased Fos expression in the ventrolateral periaqueductal gray and nucleus raphe magnus after 1 h, but not after 6 h or 14 days of daily treatments. AYA (600 μL/kg) twice a day for 14 days did not alter mice's motor function, spontaneous locomotion, body weight, food and water intake, hematological, biochemical, and histopathological parameters. Harmine (3.5 mg/kg) promoted consistent antinociception during experimental neuropathy.
Conclusions: AYA promotes consistent antinociceptive effects in different mouse models of pain without inducing detectable toxic effects. Harmine is at least partially accountable for the antinociceptive properties of AYA.

* Corresponding author. School of Pharmacy, Federal University of Bahia, 40.170-115, Salvador, BA, Brazil.
E-mail addresses: pedrosslauria@gmail.com (P.S.S. Lauria), juliana@ltf.ufpb.br (J.M. Gomes), abreu_lucas@id.uff.br (L.S. Abreu), santana.rejane@ufba.br (R.C. Santana), victorluiz@hotmail.com (V.L.C. Nunes), rdc@ufba.br (R.D. Couto), pcolavolpe@yahoo.com.br (P.O. Colavolpe), marcelosobral.ufpb@gmail.com (M.S. Silva), milena@bahia.fiocruz.br (M.B.P. Soares), cfv@ufba.br (C.F. Villarreal).

https://doi.org/10.1016/j.jep.2024.117710
Received 14 November 2023; Received in revised form 20 December 2023; Accepted 2 January 2024
Available online 4 January 2024

1. Introduction

Chronic pain is a major cause of suffering and distress to patients and affects millions of people worldwide (Battaglia et al., 2023). Neuropathic pain is a highly prevalent type of chronic pain caused by diseases or injuries of the somatosensory system. (Attal et al., 2023; IASP, 2021). The pharmacological management of neuropathic pain is challenging, since the drugs currently available in the market induce important side effects and many patients respond poorly to treatments (Attal et al., 2023; Bernetti et al., 2021). To overcome these limitations, novel therapeutic approaches aiming to control refractory pain have been explored. Pioneer studies published in the 1960s and 1970s demonstrate the analgesic effects of psychedelics in patients suffering from cancer-related pain, phantom limb pain, cluster headache, and migraine (Kooijman et al., 2023). In recent years, a revived interest in the study of these drugs has grown, as shown by the increased number of publications on the topic – a phenomenon called the psychedelic renaissance (Hadar et al., 2023).

One of the most emblematic psychedelics is ayahuasca, a ritualistic brew originally used by native peoples of South America (Estrella-Parra et al., 2019). Ayahuasca is most commonly prepared as a decoction of the plants *Banisteriopsis caapi* and *Psychotria viridis*, which contain alkaloids that mediate the psychedelic effects of the brew, namely β-carbolines and N,N-dimethyltryptamine (DMT) (Jiménez and Bouso, 2022; McKenna et al., 1984). In addition to *B. caapi* and *P. viridis*, other 90 different plant species have been reported to compose ayahuasca samples, including *Banisteriopsis inebrians*, *Lophanthera lactescens*, *Diplopterys cabrerana*, *Tetrapterys methystica*, *Mascagnia psilophylla*, among others (Domínguez-Clavé et al., 2016; Ott, 1996). The use of ayahuasca began to expand in the first decades of the 20th century, when ayahuasca was incorporated into new religions that arose in Northern Brazil (Labate, 2012) and can now be found throughout the entire globe (Dos Santos and Hallak, 2021). The traditional use of ayahuasca is intimately related with healing practices. In the cult of Santo Daime, believers often resort to ayahuasca as a sacred medicine for healing ailments of the mind and the body, including pain of various origins (Moreira and MacRae, 2011). The traditional use of ayahuasca in the ritualistic context reduces bodily pain (Barbosa et al., 2009), pain associated with fibromyalgia (Orozco, 2020), and chronic pain (Labate and Cavnar, 2014; Maia et al., 2023).

Despite the reports that ayahuasca improves pain, it is not clear whether that feeling of improvement is due to an analgesic effect *per se* or rather a result of the subjective psychedelic experience, as patients state that ayahuasca helps them to "understand their bodies" and "find new ways to cope with pain" (Orozco, 2020). It has also been suggested that therapeutic outcomes of ayahuasca ceremonies can be influenced by psychological aspects affected by religiosity and group dynamics (Barbosa et al., 2009; Maia et al., 2023). On the other hand, a preclinical study has provided direct evidence of the antinociceptive effect of ayahuasca in mice (Pires et al., 2018), suggesting that ayahuasca may have intrinsic analgesic properties. However, this study is limited to the evaluation of single treatments in screening assays of acute antinociception and the mechanisms of action behind the effect were not addressed.

Most records of the analgesic effects of ayahuasca come from anecdotal evidence following the ritualistic use of the beverage. Therefore, the analgesic potential of ayahuasca should be investigated aside from the context of religious ceremonies. Considering the major gap in the pharmacological management of neuropathic pain, this work aimed to investigate the antinociceptive effects of ayahuasca and its mechanisms in preclinical models of acute and chronic pain in mice, in particular characterizing the effects and the safety of repeated administrations of ayahuasca during experimental neuropathy. The contribution of the major component harmine to the antinociceptive properties of ayahuasca was also assessed.

2. Material and methods

2.1. Animals

Male Swiss and C57BL/6 mice (*Mus musculus*), aged 3–6 months, weighing 20–25 g were obtained from the animal facilities of the Gonçalo Moniz Institute (FIOCRUZ, BA). Animals were kept in individually ventilated cages (Alesco ®) in a temperature-controlled room (22 °C) with food and water *ad libitum* under a 12-h light-dark cycle of artificial light. Swiss mice (n = 114) were used in the formalin test, in the CFA-induced model of inflammation, and in the tail flick test. C57BL/6 mice (n = 143) were used in all the other assays. At the end of behavioral assessments, mice were euthanized by an overdose of intraperitoneal sodium thiopental (50 mg/kg) followed by cervical dislocation to ensure death. The present study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Garber et al., 2011) and approved by the Animal Ethics Committee of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (CEUA/EMEVZ - 26/2020).

2.2. Plant material: ayahuasca

The ayahuasca used in this study was produced from the plant species *Banisteriopsis caapi* (local name: cipó-mariri) and *Psychotria viridis* (local name: chacrona). Ayahuasca was kindly donated by the Centro Espiritualista Universalista Irmão José (Brazilian National Registry of Legal Entities 21.390.934/0001-57), located in Cruzeiro do Sul (AC, Brazil). Plant material was harvested and ayahuasca was prepared in August 2020. Because the brew was made according to the religious protocols of that church, which were kept secret from the investigators, no voucher specimens were available. A chemical characterization of the brew was performed, as described in section 2.16. The use of ayahuasca in this research was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen register A7E742C). Doses used in the *in vivo* experiments are represented as microliters of ayahuasca per kilogram of mouse (μL/kg). Dilutions were made using drinking water as vehicle.

2.3. Formalin test

Mice were orally pre-treated with ayahuasca (24, 120, 600, and 3000 μL/kg), water (vehicle; control), or indomethacin (reference anti-inflammatory drug; 5 mg/kg), or subcutaneously pre-treated with morphine (reference opioid drug; 5 mg/kg). After 40 min, mice were inoculated by intraplantar route with 20 μL of 2.5% formaldehyde and then kept under glass cylinders in front of a mirror, where they were observed for 30 min. The time during which mice exhibited pain-like behaviors (licking, shaking, or protecting the inoculated paw) was registered with a stopwatch. The response was divided into first phase (0–10 min) and second phase (10–30 min) (Dubuisson and Dennis, 1977).

2.4. Complete Freund's adjuvant-induced persistent inflammation

Mice were orally pre-treated with ayahuasca (24, 120, 600, and 3000 μL/kg) or water (vehicle; control), or intraperitoneally pre-treated with dexamethasone (reference drug; 2 mg/kg) 40 min before the intraplantar injection of 20 μL of Complete Freund's Adjuvant (CFA) (Opretzka et al., 2019). CFA is a solution containing dead *Mycobacterium tuberculosis* (1 mg/mL) in 85% paraffin oil and 15% mannide monooleate that triggers persistent local inflammation. Mechanical nociceptive thresholds and paw edema were measured before the experiment (baseline) and at different times after CFA inoculation (1, 2, 4, 6, and 24 h), as described in the following sections.

2.5. Von frey test for assessment of mechanical nociceptive thresholds

The nociceptive thresholds to mechanical stimuli were measured with von Frey filaments, which consist of nylon thread segments with logarithmically incremental stiffness (0.008–0.6 g) previously determined by the manufacturer (Stoelting®). Mice were placed in transparent acrylic boxes upon a wired grid floor. After an adaptation period of 20 min, the right hind paw was touched vertically with a series of filaments with varying stiffness until they were slightly bent. The sudden withdrawal of the touched paw was considered a positive response (Espírito-Santo et al., 2017). Mechanical nociceptive thresholds were determined by the up-and-down method (Dixon, 1965).

2.6. Paw edema

To quantify paw edema, the thickness of the right hind paw was measured in millimeters, using a digital caliper (Mitutoyo®), immediately before (baseline) and at different times (1, 2, 4, 6, and 24 h) after intraplantar inoculation of CFA. Edema was calculated as the percentage increase in paw thickness over time compared to baseline (Van Arman et al., 1965).

2.7. Tail flick test

The tip of restrained mice's tail (approximately 3 cm) was immersed in hot water (48 °C) until a withdrawal reflex was observed (Santos et al., 2020). The time in seconds between tail immersion and withdrawal was registered with a stopwatch. A cut-off time of 10 s was established to avoid tissue damage. Mice were acclimatized in restriction tubes for 15 min and the withdrawal time was determined three times for each mouse with at least 1 min between assessments. Baselines were determined for three consecutive days before the experiment and mice whose average baseline latency was between 2 and 4 s were included in the test. Mice were orally treated with ayahuasca (24, 120, 600, and 3000 μL/kg) or water (vehicle; control), or subcutaneously treated with morphine (reference drug; 5 mg/kg). The tail flick test was then performed at 1, 2, 4, and 6 h following treatments.

2.8. Partial sciatic nerve ligation model of neuropathic pain

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) by intraperitoneal route and the partial ligation of the sciatic nerve was performed under aseptic conditions, as previously described (Gama et al., 2018). The right hind limb was epilated and disinfected with 70% ethanol. A small incision in the skin allowed the exposure of the biceps femoris muscle, whose fibers were separated using sterile forceps. The sciatic nerve was carefully exposed and crossed with a 7-0 polypropylene suture thread (Shalon®), which was then firmly tied, constricting 1/3 to 1/2 of the nerve diameter. In the *sham* group (false ligation), the nerve was exposed without constriction. The skin was apposed with two single sutures of 6-0 polypropylene (Shalon®). Mice were returned to their home cages and observed until full recovery from anesthesia.

2.8.1. Acute treatments in the experimental neuropathy

The antinociceptive effect of ayahuasca was tested in the partial sciatic nerve ligation model of neuropathic pain. During the week that followed surgery, nociceptive thresholds of mice were evaluated by the von Frey test. On the seventh day after surgery, mice were orally treated with ayahuasca (24, 120, 600, and 3000 μL/kg), harmine (0.035, 0.35, 3.5, and 35 mg/kg), water (vehicle; control), or gabapentin (reference drug; 70 mg/kg). *Naïve* mice (not submitted to surgical procedures or treatments) and *sham* mice (false ligation) were also evaluated. The mechanical nociceptive thresholds of mice were evaluated at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 24 h after treatments.

2.8.2. Daily treatments in the experimental neuropathy

The effect of repetitive treatments with ayahuasca was also evaluated in the partial sciatic nerve ligation model of neuropathic pain. Starting at the seventh day after surgery, mice received daily oral treatments with ayahuasca (24, 120, and 600 μL/kg), harmine (3.5 mg/kg), water (vehicle; control), or gabapentin (reference drug; 70 mg/kg) during fourteen days. Two treatment protocols were performed: once a day (every 24 h) and twice a day (every 12 h). *Naïve* and *sham* mice were also evaluated. In both protocols, the mechanical nociceptive threshold of mice was determined by the von Frey test. Thresholds were assessed daily in two moments: immediately before the first treatment of the day and at the time of maximum effect (7 h for ayahuasca, 3 h for gabapentin, and 1 h for harmine). Food and water intake as well as mice's body weight were monitored throughout the experiment. The integrity of motor coordination and spontaneous locomotion of mice after multiple exposures to ayahuasca were evaluated on the 7th and 14th days of treatments using the rotarod and the open field tests, respectively. At the end of the experimental protocol, blood and organs were collected to evaluate systemic toxicity, as described in following sections.

2.9. Pharmacological treatments

To investigate possible mechanisms by which ayahuasca (600 μL/kg) promotes antinociception, the effect of different antagonist drugs was tested against ayahuasca-induced antinociception in the partial sciatic nerve ligation model of neuropathic pain. The dose of ayahuasca was selected based on the dose-response curve built for the same experimental model. Two different protocols were conducted for each drug: either mice were pre-treated with antagonists and then received ayahuasca or mice were treated with ayahuasca and then given antagonists before ayahuasca's maximum effect (E_{max}, 7 h after administration). Neuropathic mice orally treated with ayahuasca (600 μL/kg) received naloxone (non-selective opioid antagonist, 5 mg/kg, i.p., 40 min before ayahuasca or before E_{max}) (Santos et al., 2020), bicuculline ($GABA_A$ receptor antagonist, 1 mg/kg, i.p., 15 min before ayahuasca or before E_{max}) (Hess et al., 2010), phaclofen ($GABA_B$ receptor antagonist, 2 mg/kg, i.p., 15 min before ayahuasca or before E_{max}) (Hess et al., 2010), methysergide (non-selective serotonergic antagonist, 5 mg/kg, i.p., 30 min before ayahuasca or before E_{max}) (Maia-Marques et al., 2021), or rimonabant (CB1 receptor inverse agonist, 10 mg/kg, i.p., 30 min before ayahuasca or before E_{max}) (Henderson-Redmond et al., 2021).

2.10. Fos immunohistochemistry

To investigate whether ayahuasca activates brain regions associated with endogenous analgesia, brain sections of mice were immunohistochemically marked to assess Fos expression. Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) by intraperitoneal route and then transcardially perfused with 20 mL of 0.01 M phosphate buffered saline (PBS) followed by 60 mL of 4% paraformaldehyde (PFA). Brains were surgically removed, stored in 4% PFA overnight, then submerged in 30% sucrose for at least two days, and coronally sectioned at 40 μm in a cryostat (Damon/IEC Division; IEC CTD Harris-Cryostat).

Free-floating brain sections were rinsed five times for 5 min in 0.1 M phosphate buffer (PB) – henceforth, this step will be referred to as a washing cycle. Then, sections were incubated with 0.3% hydrogen peroxide for 20 min to block endogenous peroxidase activity followed by a washing cycle. Brain sections were incubated with blocking solution (1.5% fetal bovine serum and 0.25% Triton X-100 in 0.1 M PB) for 1 h and then incubated with primary Fos antibody (Cell Signaling Technology®) diluted at 1:3200 in blocking solution for 18 h at 4 °C followed by a washing cycle. Sections were then incubated with 1.5% normal goat serum, 0.25% Triton X-100, and biotinylated rabbit IgG secondary antibody (Vectastain® Elite®) at 1:200 in 0.1 M PB for 2 h followed by a washing cycle. Then, brain sections were incubated with 0.25% Triton X-100 and avidin-biotinylated horseradish peroxidase complex

(Vectastain® Elite®) at 1:50 in 0.1 M PB for 1 h. After a final washing cycle, brain sections were incubated with 0.035% 3,3′-diaminobenzidine-tetrahydrochloride (DAB), 0.03% nickel sulfate, and 0.01% hydrogen peroxide in 0.1 M PB for 5 min. The peroxidase reaction was stopped by dipping the sections in 0.1 M PB. Brain sections were mounted on gelatin-coated glass slides and allowed to dry overnight, dipped for 10 min in 100% xylene and cover-slipped with Entellan mounting medium (Urzedo-Rodrigues et al., 2014).

Histological brain sections were photographed with a Moticam 5 camera (Mitutoyo) coupled to a light microscope (Nikon Eclipse TS100) and the number of Fos-positive nuclei was determined in the ventrolateral periaqueductal gray and in the nucleus raphe magnus with the program ImageJ (NIH). Two sections of each region of interest per mouse were assessed by two blind evaluators and then averaged. Regions of interest for nuclei quantification were delimited by overlapping micrographs with reference images from Paxinos and Franklin's mouse brain atlas (Paxinos and Franklin, 2001).

2.11. Rotarod test

The rotarod test was performed to verify the integrity of mice's motor function. The rotarod apparatus consists of a cylinder that rotates at a constant speed of 8 rpm. Mice must constantly move to remain on the cylinder; their performance was quantified by the average time spent on the device in three attempts of 120 s (Nascimento et al., 2016). Diazepam (10 mg/kg, intraperitoneally, 30 min before) was used as a reference drug.

2.12. Open field test

The spontaneous locomotion activity of mice was evaluated in the open field test. Mice were placed individually inside an acrylic arena (50 cm height x 45 cm width x 60 cm length) divided into twelve equal squares and allowed to freely explore the arena for 3 min. The number of squares crossed during the exploration period was recorded (Leite dos Santos et al., 2012). Diazepam (10 mg/kg, intraperitoneally, 30 min before) was used as a reference drug.

2.13. Body weight and food and water intake

Mice's body weight as well as their food and water intake were monitored throughout the protocol of daily treatments with ayahuasca twice a day for 14 days. Mice from the same experimental group were housed in pairs to allow nearly individualized consumption measurements while avoiding social isolation. Mice's body weight (g) was evaluated four times: (1) at the surgery day, (2) seven days after surgery, which was also the first day of treatments, (3) at the seventh day of treatments, and (4) at the fourteenth day of treatments. Food (g) and water (mL) intake were measured at the same time points and represent the total consumption per mouse during the previous seven days.

2.14. Hematological and biochemical profiles

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) by intraperitoneal route. Blood was collected by cardiac puncture and stored in vials containing heparin (Vieceli et al., 2021). This procedure was followed by cervical dislocation to ensure mice's death. Red blood cells, hemoglobin, hematocrit, platelets, white blood cell count, neutrophils, and lymphocytes were determined using an automated method in the BC-2800 VET/Mindray equipment. Alanine transaminase, aspartate transaminase, urea, and creatinine were quantified using standard commercial reagents (Labtest®) according to manufacturer's instructions in a semi-automatic spectrophotometer (Bio-Plus® biochemical analyzer).

2.15. Macroscopic and histological evaluation of organs

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) by intraperitoneal route. Following blood sampling and euthanasia, as described in the previous section, stomach, liver, and kidneys were collected and assessed for macro- and microscopic alterations. The weight of each organ was normalized according to mice's body weight and expressed as organ weight (g) per 10 g of body weight (Vieceli et al., 2021). Tissues were fixed in 10% formaldehyde, embedded in paraffin, cut in 4 μm sections, and stained with hematoxylin-eosin. Samples were then evaluated at the light microscope (Leica®, model ICC50 E) by a certified pathologist and assessed for morphological abnormalities and signs of toxicity.

2.16. Chemical characterization of ayahuasca

2.16.1. Preparation of samples

Ayahuasca was diluted following the proportion 1:5000. Briefly, 50 μL of ayahuasca were added to a 10 mL volumetric flask containing 5 mL of methanol and the volume was completed with ultrapure water acidified with 0.1% formic acid. Diluted samples were prepared in triplicate and filtered using nylon membranes with 0.45 μm pore size before injections (de Oliveira Silveira et al., 2020).

2.16.2. Liquid chromatography-mass spectrometry instrumentation and conditions

Aiming to obtain a phytochemical profile of ayahuasca, a Shimadzu® (Kyoto, Japan) HPLC system coupled with either Amazon X or microTOF II mass detectors (Bruker Daltonics, Billerica, MA, USA) with electrospray ion sources was used to perform electrospray ionization tandem mass spectrometry (ESI-MS/MS) and high-resolution electrospray ionization mass spectrometry (HRESIMS) analyses, respectively (Abreu et al., 2019). The HPLC system consisted of a LC-20AD solvent pump unit (flow rate of 0.6 mL/min), a DGU-20A_5 online degasser, a CBM-20A system controller, and an SPD-M20A (190–800 nm) diode array detector. HPLC separation was performed on a Kromasil C-18 5 μm 100 Å, 250 × 4.6 mm (Kromasil, Bohus, Sweden) analytical column. Injections (20 μL) were made using an autosampler (SIL-10AF). The mobile phase consisted of 0.1% formic acid in water (solvent A) and methanol (solvent B). An exploratory gradient (5 × 50% B) was carried out starting with 5% of solvent B, reaching 50% at 20 min and 100% at 30 min, remaining at 100% until 35 min, returning to 5% at 36 min and then remaining constant until the end of the run at 41 min. Analyses parameters were: capillary 4.5 kV, ESI in negative mode, final plate offset 500 V, 40 psi nebulizer, dry gas (N_2) with flow rate of 8 mL/min, and a temperature of 300 °C. CID fragmentation in Amazon X was achieved in auto MS/MS mode using enhanced resolution mode for MS and MS/MS mode. Spectra (m/z 50–1000) were recorded every 2 s.

2.16.3. Quantification of harmine by HPLC-DAD

Quantification of harmine in ayahuasca samples was made by a high-performance liquid chromatography with diode-array detection (HPLC-DAD) method (Lanaro et al., 2015). The calibration curve was built using harmine (Sigma®; 98% of purity) at the concentrations 6.25, 12.5, 25, 50, and 100 μg/mL. All samples were prepared in triplicate following the same steps described for ayahuasca. Elution was carried out using a Kromasil® C18 column using the conditions described in the previous section. Detection was performed at 247 nm.

2.17. Statistical analyses

Results are represented as mean ± standard deviation of 5–6 animals per group. Comparisons among three or more groups were performed by one-way analysis of variance (ANOVA) followed by Tukey's test. For repeated measures, two-way ANOVA followed by Bonferroni's test was used. The factors analyzed were treatments, time, and time-treatment

interaction. The maximum inhibition from ayahuasca in the formalin test was used to calculate its maximum effect; EC_{50} was then determined from the fitted curve. Data were analyzed using the program GraphPad Prism 9 (GraphPad, San Diego, CA, USA). Statistical differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Acute oral treatment with ayahuasca promotes antinociceptive effects

The effect of acute oral treatment with ayahuasca (24–3000 μL/kg) was initially evaluated in the formalin test, a screening assay for detecting antinociceptive activity. Mice pre-treated with water (vehicle) that received intraplantar injection of formalin exhibited intense nociceptive behaviors of biphasic nature (Fig. 1). Treatment with ayahuasca did not promote significant changes in mice's behavior in the first phase of the test (Fig. 1A). In the second phase, ayahuasca significantly reduced nociceptive behaviors at 120 μL/kg, 600 μL/kg, and 3000 μL/kg ($F(6, 34) = 22{,}88$; $p < 0.05$; Fig. 1B). Groups treated with ayahuasca at 120 μL/kg and 600 μL/kg were significantly different ($p < 0.05$), indicating dose-dependency. The ED_{50} of the antinociceptive effect of ayahuasca in the second phase of the formalin test was 118.1 μL/kg. Similarly, treatment with the reference anti-inflammatory drug indomethacin (5 mg/kg, p.o.) reduced nociceptive behaviors of mice in the second phase of the test ($p < 0.05$), but not in the first phase. On the other hand, the reference opioid drug morphine (5 mg/kg, s.c.) reduced nociceptive behaviors in both phases ($F(6, 35) = 20.26$ for the first phase; $F(6, 34) = 22.88$ for the second phase; $p < 0.05$). Ayahuasca, indomethacin, and morphine were equally effective in reducing nociception in the second phase of the formalin test.

Our results contrast with the findings from Pires et al. (2018), who also evaluated the effects of ayahuasca in the formalin test. The authors showed a more prominent antinociceptive effect of ayahuasca in the first phase of the test, while only at the highest dose the brew was effective in the second phase. The different effects can be attributed to the different chemical composition of the two ayahuasca samples, as discussed in section 3.9. Since the second phase of the formalin test is mediated by the release of inflammatory mediators (Carvalho et al., 2011; Damas and Liégeois, 1999), a possible anti-inflammatory activity of ayahuasca was next investigated.

3.2. Ayahuasca promotes antinociception but not antiedematogenic effect in the CFA model of persistent inflammation

The effect of oral treatment with ayahuasca was evaluated in the CFA-induced model of persistent inflammation. Vehicle-treated mice intraplantarly inoculated with CFA (20 μL) developed mechanical allodynia, shown by a decrease in mechanical nociceptive thresholds (Fig. 2A) as well as paw edema (Fig. 2B). There were significant main effects of time ($F(5, 145) = 125.7$; $p < 0.0001$), treatment ($F(5, 29) = 3.304$; $p = 0.0175$, and interaction time × treatment ($F(25, 145) = 2.878$; $p < 0.0001$) for nociceptive thresholds and time ($F(4, 116) = 60.63$; $p < 0.0001$), treatment ($F(5, 29) = 5.269$; $p = 0.0015$), and interaction time × treatment ($F(20, 116) = 4.236$; $p < 0.0001$) for paw edema. Oral treatments with ayahuasca reduced CFA-induced allodynia at 600 μL/kg and 3000 μL/kg ($p < 0.05$; Fig. 2A). These doses were equally effective, although at 3000 μL/kg an earlier onset of the antinociceptive effect was observed. Pre-treatments with ayahuasca had no influence on edema formation at the evaluated times (Fig. 2B). The reference drug dexamethasone (2 mg/kg, i.p.) partially prevented both mechanical allodynia and edema formation ($p < 0.05$) induced by CFA.

These results suggest that even though ayahuasca can reduce inflammatory nociception, it does not modulate other signs of inflammation. Divergently, previous studies suggest that other ayahuasca samples promote anti-inflammatory effects. Ayahuasca diminishes depressive and anxiety-like behaviors during neuroinflammation induced by LPS in rats (Goulart da Silva et al., 2022) and reduces plasma C-reactive protein in patients with depression (Galvão-Coelho et al., 2020). It has been suggested that DMT is partially responsible for the anti-inflammatory effects of ayahuasca, as it regulates inflammation and immune homeostasis after binding to both 5-HT_{2A} and sigma-1 receptors (da Silva et al., 2021; Flanagan and Nichols, 2018). Although the DMT content of the ayahuasca used in this study was not quantified, the phytochemical profile (Fig. 8) suggests that DMT is a minor component, which could be one of the reasons for the lack of antiedematogenic effects.

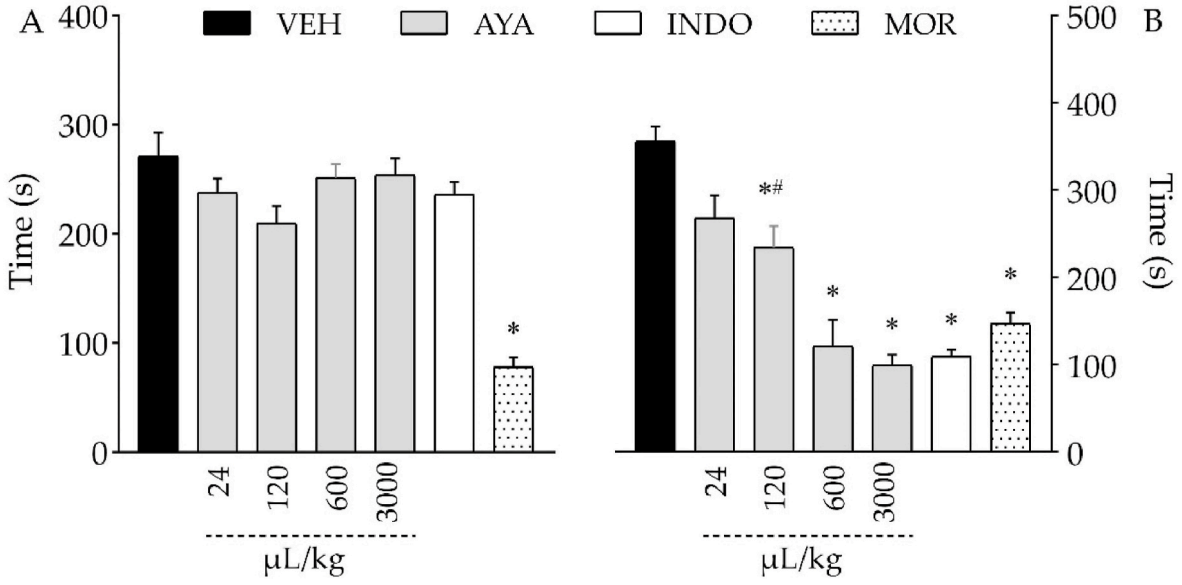

Fig. 1. Ayahuasca promotes antinociceptive effect in the formalin test. Mice were orally treated with ayahuasca (AYA; 24–3000 μL/kg), water (vehicle; VEH), or indomethacin (INDO; reference anti-inflammatory drug; 5 mg/kg), or subcutaneously treated with morphine (MOR; reference opioid drug; 5 mg/kg) 40 min before the intraplantar injection of 2.5% formalin. The time mice spent showing nociceptive behaviors (ordinate axis) was evaluated in the first phase (**Panel A**; 0–10 min) and in the second phase (**Panel B**; 10–30 min) of the test. Data are expressed as mean ± standard deviation (n = 6 mice per group). $^{*}p < 0.05$ compared to the VEH group; $^{\#}p < 0.05$ compared to groups 600 μL/kg and 3000 μL/kg, as determined by one-way ANOVA followed by Tukey's test.

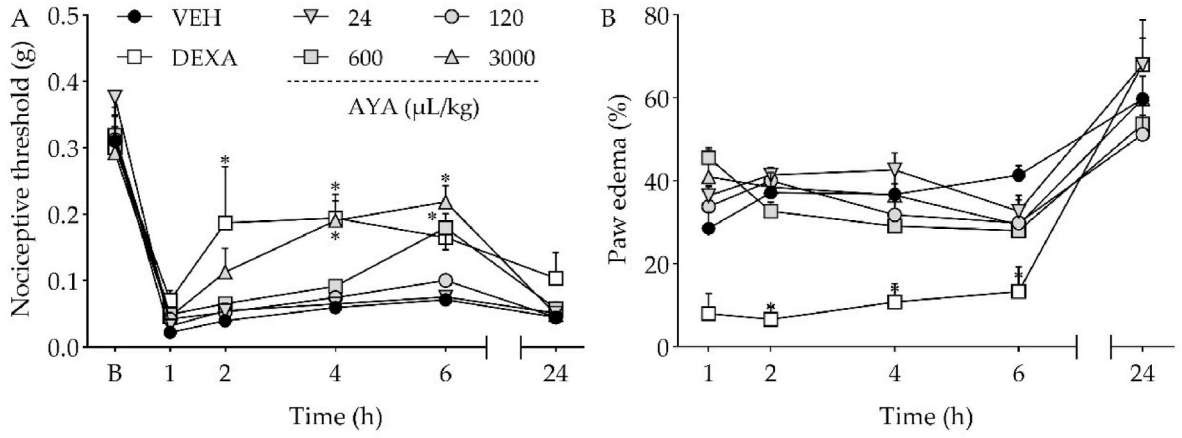

Fig. 2. Effects of ayahuasca in the CFA model of persistent inflammation. Mice were orally treated with ayahuasca (AYA; 24–3000 μL/kg) or water (vehicle; VEH), or intraperitoneally treated with dexamethasone (DEXA; reference drug; 2 mg/kg) 40 min before intraplantar injection of CFA (20 μL). **Panel A:** Mechanical nociceptive thresholds (ordinate axis) were assessed before the experiment (baseline; B) and at different times after CFA injection (abscissa axis) and represent the filament weight (g) to which mice respond in 50% of trials. **Panel B:** Paw edema (ordinate axis) was assessed at different times after CFA injection (abscissa axis) and represents the increase (%) in paw's thickness compared to baseline prior to CFA. Data are expressed as mean ± standard deviation (n = 6 mice per group). *$p < 0.05$ compared to the VEH group, as determined by two-way ANOVA followed by Bonferroni's test.

3.3. Ayahuasca does not promote antinociception in the tail flick test

To further investigate the antinociceptive properties of ayahuasca, mice orally treated with ayahuasca (24–3000 μL/kg) were submitted to the tail flick test (Fig. 3). There were significant main effects of time ($F(4, 116) = 125.7$; $p < 0.0001$), treatment ($F(5, 29) = 3.323$; $p = 0.0171$, and interaction time × treatment ($F(20, 116) = 12.82$; $p < 0.0001$). None of the tested doses of ayahuasca affected the latency time of mice, indicating that ayahuasca did not modify the nociceptive thresholds. As expected, the reference drug morphine (5 mg/kg, s.c.) increased the latency time until the tail flick reflex at 1 h and 2 h after treatments ($p < 0.05$).

The tail flick response is mediated by central mechanisms; the modulation of this response by a drug is suggestive of a morphine-like action (Irwin et al., 1951). The lack of effect of ayahuasca in this test agrees with Pires et al. (2018), who showed that ayahuasca is not antinociceptive in the hot plate test, another assay of thermal nociception responsive to opioids (Le Bars et al., 2001). Interestingly, the antinociceptive profile of ayahuasca resembles what has been described for duloxetine. This monoamine reuptake inhibitor reduces inflammatory allodynia induced by carrageenan or capsaicin but is not antinociceptive in the tail flick test and has limited effects in the hot plate test (Jones et al., 2005). Duloxetine, like other agents that modulate serotoninergic and noradrenergic neurotransmission, is a first-line drug in the treatment of neuropathic pain (Bates et al., 2019). Considering that ayahuasca reduced persistent nociception and is known to modulate serotonergic transmission, its effects were then evaluated in an experimental model of neuropathic pain.

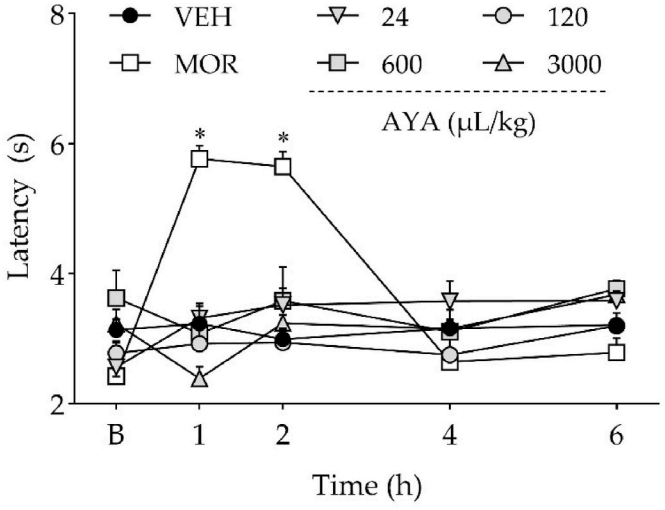

Fig. 3. Influence of ayahuasca in the tail flick test. Mice were orally treated with ayahuasca (AYA; 24–3000 μL/kg) or water (vehicle; VEH), or subcutaneously treated with morphine (MOR; reference drug; 5 mg/kg). The latency time in seconds until the tail flick reflex (ordinate axis) was evaluated before the experiment (baseline; B) and at different times after treatments (abscissa axis). Data are expressed as mean ± standard deviation (n = 6 mice per group). *$p < 0.05$ compared to the VEH group, as determined by two-way ANOVA followed by Bonferroni's test.

3.4. Ayahuasca promotes antinociception during experimental neuropathy

After establishing the antinociceptive potential of ayahuasca, the effect of the decoction was tested in a model of chronic neuropathic pain induced by partial ligation of the sciatic nerve (Fig. 4), which mimics a chronic painful condition that is refractory to conventional treatments. There were significant main effects of time ($F(14, 602) = 83.14$; $p < 0.0001$), treatment ($F(7, 43) = 434.2$; $p < 0.0001$, and interaction time × treatment ($F(98, 602) = 14.18$; $p < 0.0001$). Vehicle-treated ligated mice showed a gradual drop in nociceptive thresholds compared to *naïve* mice from the 3rd to the 7th day after surgery ($p < 0.05$), indicating that the model of neuropathic pain was successfully established. As a result of surgical manipulation, *sham* mice showed an initial reduction in nociceptive thresholds on days 3 and 5 compared to *naïve* mice ($p < 0.05$); the thresholds returned to baseline levels on the 7th day. Oral treatments with ayahuasca (24–3000 μL/kg) dose-dependently reduced mechanical allodynia compared to vehicle-treated neuropathic mice ($p < 0.05$). At 600 μL/kg, which was the maximum effective dose, the antinociceptive effect of ayahuasca was significant from the 5th to the 8th hour following treatments ($p < 0.05$); the peak of antinociceptive activity was the 7th hour post-treatment. At the supramaximal dose of 3000 μL/kg, the antinociceptive effect was longer-lasting, persisting up to 10 h after administration. Oral treatments with the reference drug gabapentin (70 mg/kg) significantly increased nociceptive thresholds at the 2nd and 3rd hours after administrations ($p < 0.05$); the peak of antinociceptive activity was the 3rd hour post-treatment.

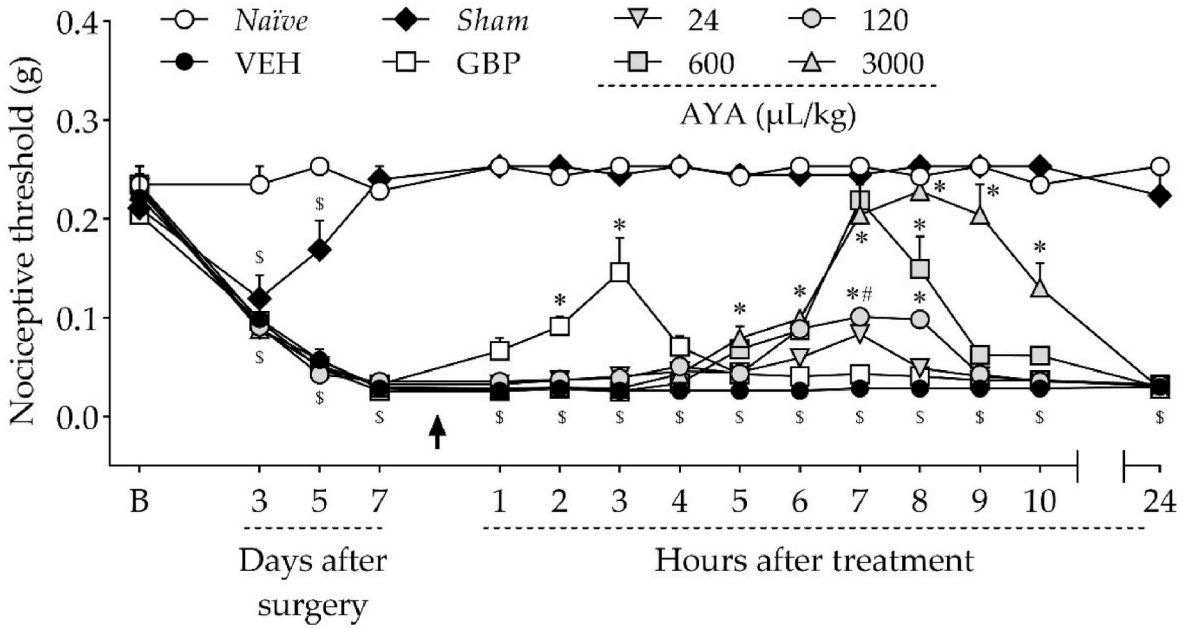

Fig. 4. Antinociceptive effect of single ayahuasca administration during experimental neuropathic pain. Neuropathic mice submitted to partial sciatic nerve ligation were orally treated with ayahuasca (AYA; 24–3000 μL/kg), water (vehicle; VEH), or gabapentin (GBP; reference drug; 70 mg/kg). *Naïve* mice were not submitted to surgical procedures or treatments. *Sham* mice underwent surgical manipulation without nerve ligation. Mechanical nociceptive thresholds (ordinate axis) represent the filament weight (g) to which mice respond in 50% of trials and were assessed before the experiment (baseline; B), after surgery (days 3–7), and at different times in hours after treatments. The black arrow indicates when treatments were performed. Data are expressed as mean ± standard deviation (n = 6 to 7 mice per group). $^{\$}$ $p < 0.05$ compared to the *naïve* group; $^{*}p < 0.05$ compared to the VEH group; $^{\#}p < 0.05$ compared to 600 μL/kg and 3000 μL/kg groups, as determined by two-way ANOVA followed by Bonferroni's test.

3.5. Daily doses of ayahuasca promote consistent and sustained antinociception during experimental neuropathy

After characterizing the effect of single oral doses of ayahuasca in the partial sciatic nerve ligation model of neuropathic pain, the effect of multiple oral administrations of ayahuasca (24–600 μL/kg) was assessed in the neuropathy model following two protocols: once a day for 14 days (Fig. 5A; time: F (31, 1178) = 50.37; $p < 0.0001$; treatment: F (6, 38) = 562.6; $p < 0.0001$; interaction time × treatment: F (186, 1178) = 9.449; $p < 0.0001$) and twice a day for 14 days (Fig. 5B; time: F (31, 1271) = 29.52; $p < 0.0001$; treatment: F (6, 41) = 612.3; $p < 0.0001$; interaction time × treatment: F (186, 1271) = 8.849; $p < 0.0001$). As in the single-dose experiment, the nociceptive thresholds of vehicle-treated neuropathic mice were significantly lower compared to *naïve* mice in the days that followed surgery ($p < 0.05$; Fig. 5A and B). Thresholds remained low throughout the 14 days of the experiment, attesting the chronicity of the model. *Sham* mice showed an initial reduction in nociceptive thresholds on days 3 and 5 compared to *naïve* mice ($p < 0.05$; Fig. 5A and B); the thresholds returned to baseline levels on the 7th day and remained unaltered throughout the experiment.

In both protocols, thresholds were assessed daily in two moments: immediately before the first treatment of the day and at the time of maximum effect, as determined in the previous experiment (7 h for ayahuasca and 3 h for gabapentin). Treatments with ayahuasca once a day for 14 days (Fig. 5A) at all tested doses consistently increased mechanical nociceptive thresholds ($p < 0.05$) after each treatment throughout the experiment timeframe, indicating that mice did not develop tolerance to ayahuasca's antinociceptive effect. However, in this protocol, mechanical thresholds would always decrease after 24 h, demonstrating that the antinociceptive effect was not sustained between treatments. On the other hand, treatments with ayahuasca twice a day for 14 days (Fig. 5B) promoted a plateau of antinociception at 120 μL/kg and 600 μL/kg from the fifth day onwards. The reference drug gabapentin (70 mg/kg, p.o.) reliably increased nociceptive thresholds ($p < 0.05$) when given once a day for 14 days. However, mice treated with gabapentin twice a day for 14 days showed inconsistent results, as the drug would not always increase nociceptive thresholds, suggesting the development of tolerance.

Our results suggest that ayahuasca could promote superior analgesia than first-line treatments used in neuropathic pain. The fact that two treatments a day was enough to promote a plateau of antinociception could translate to the clinical practice as better treatment adherence, considering that other drugs have a more inconvenient dose regimen without promoting the same benefits. For instance, gabapentin must be taken three times a day and is not always effective (Giovannini et al., 2021). This was the first experimental demonstration of the therapeutic potential of ayahuasca in the treatment of chronic neuropathic pain. Our findings agree with previous research on the benefits of using psychedelics in the management of chronic pain, whose mechanisms of analgesia are not yet fully understood (Hedau and Anjankar, 2022; Kooijman et al., 2023).

3.6. $GABA_A$ and serotonergic receptors contribute to the antinociceptive effect of ayahuasca in neuropathic mice

Putative mechanisms of action for the antinociceptive effect of ayahuasca in the partial sciatic nerve ligation model of neuropathic pain were investigated by pharmacological assays with antagonist drugs (Fig. 6). There were significant main effects of time (F (11, 440) = 206.3; $p < 0.0001$), treatment (F (7, 40) = 251.0; $p < 0.0001$, and interaction time × treatment (F (77, 440) = 9.513; $p < 0.0001$) when antagonists were given before ayahuasca (Fig. 6A), and time (F (11, 440) = 225.7; $p < 0.0001$), treatment (F (7, 40) = 527.3; $p < 0.0001$), and interaction time × treatment (F (77, 440) = 11.20; $p < 0.0001$) when antagonists were given before the maximum antinociceptive effect of ayahuasca (Fig. 6B). Pretreatment with bicuculline ($GABA_A$ receptor antagonist, 1 mg/kg, i.p.) prevented the antinociceptive action of ayahuasca ($p < 0.05$; Fig. 6A), suggesting that $GABA_A$ receptors are important for the establishment of the antinociceptive effect. Both bicuculline (1 mg/kg, i.p.) and methysergide (non-selective serotonergic antagonist, 5 mg/kg, i.

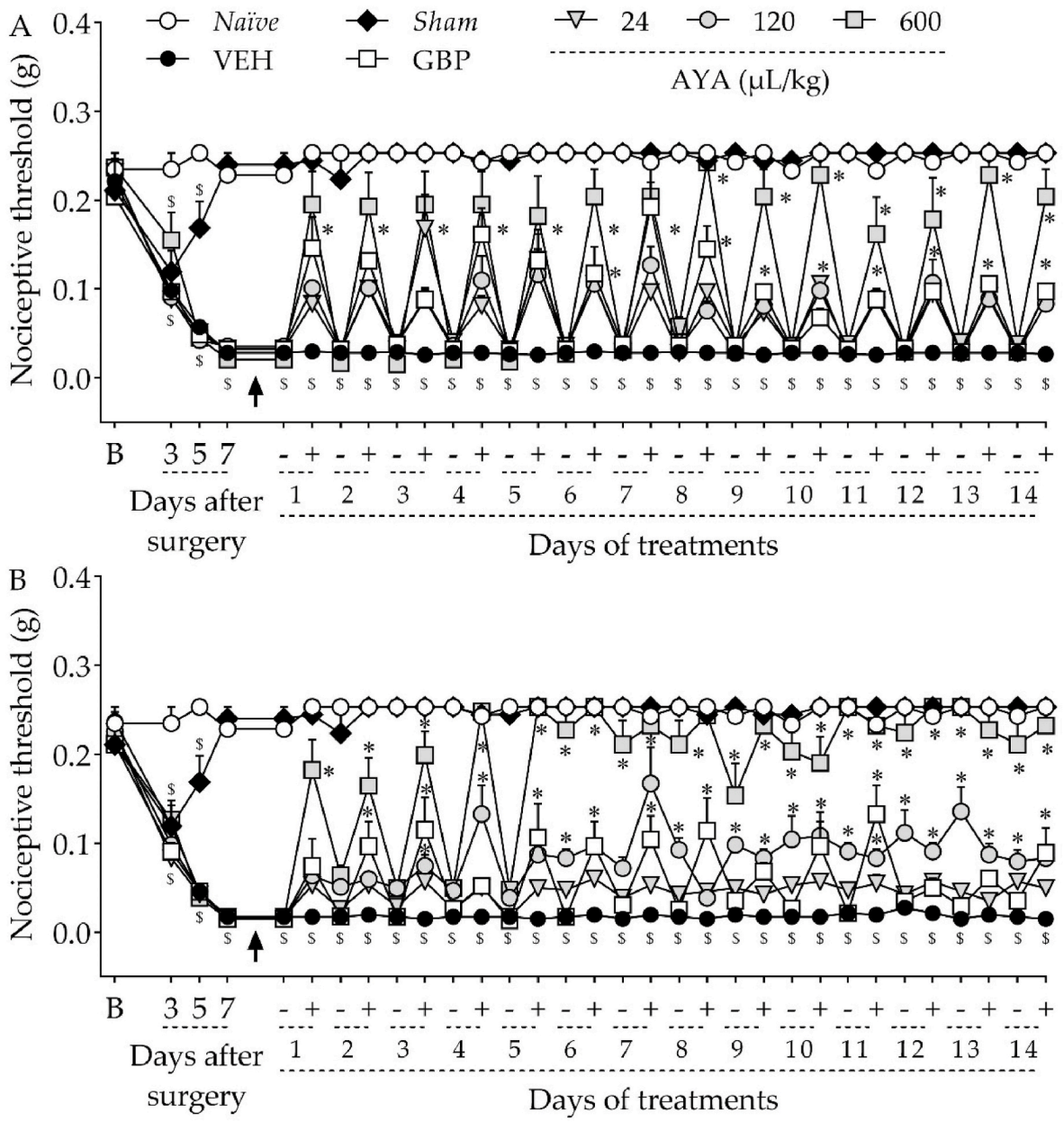

Fig. 5. Effect of repeated administrations of ayahuasca on mechanical nociceptive thresholds during experimental neuropathic pain. Neuropathic mice submitted to partial sciatic nerve ligation were orally treated with ayahuasca (AYA; 24–600 μL/kg), water (vehicle; VEH), or gabapentin (GBP; reference drug; 70 mg/kg) once a day for 14 days (**Panel A**) or twice a day for 14 days (**Panel B**). *Naïve* mice were not submitted to surgical procedures or treatments. *Sham* mice underwent surgical manipulation without nerve ligation. Mechanical nociceptive thresholds (ordinate axis) represent the filament weight (g) to which mice respond in 50% of trials and were assessed before the experiment (baseline; B), after surgery (days 3–7), and during the 14 days of treatments in two moments: before treatments (−) and at the time of maximum effect (+). The black arrow indicates when daily treatments started. Data are expressed as mean ± standard deviation (n = 6 to 7 mice per group). $^{\$}$ $p < 0.05$ compared to the *naïve* group; $^{*}p < 0.05$ compared to the VEH group, as determined by two-way ANOVA followed by Bonferroni's test.

p.) reverted ayahuasca's antinociceptive action when given prior to its maximum effect ($p < 0.05$; Fig. 6B), suggesting that $GABA_A$ and serotonergic receptors play a role in the maintenance of ayahuasca's antinociceptive effect. On the other hand, naloxone (non-selective opioid antagonist, 5 mg/kg, i.p.), phaclofen ($GABA_B$ receptor antagonist, 2 mg/kg, i.p.), and rimonabant (CB1 receptor inverse agonist, 10 mg/kg, i.p.) did not alter ayahuasca-induced antinociception in neuropathic mice, indicating that opioid, $GABA_B$, and CB1 receptors, respectively, do not contribute to ayahuasca's antinociceptive effects.

The participation of serotonergic mechanisms was expected, considering the well-described influence of ayahuasca on serotonergic transmission, either by directly activating 5-HT receptors or by increasing the levels of endogenous serotonin in the synaptic cleft (McKenna et al., 1984). Moreover, rats exposed to ayahuasca show increased brain levels of serotonin (Castro-Neto et al., 2013). Considering that the antinociceptive effects of ayahuasca during experimental neuropathy was at least partially mediated by serotonergic receptors, it is possible that descending inhibition pathways are being recruited. Hence, the next step was to evaluate the effect of ayahuasca on brain regions related to endogenous analgesia.

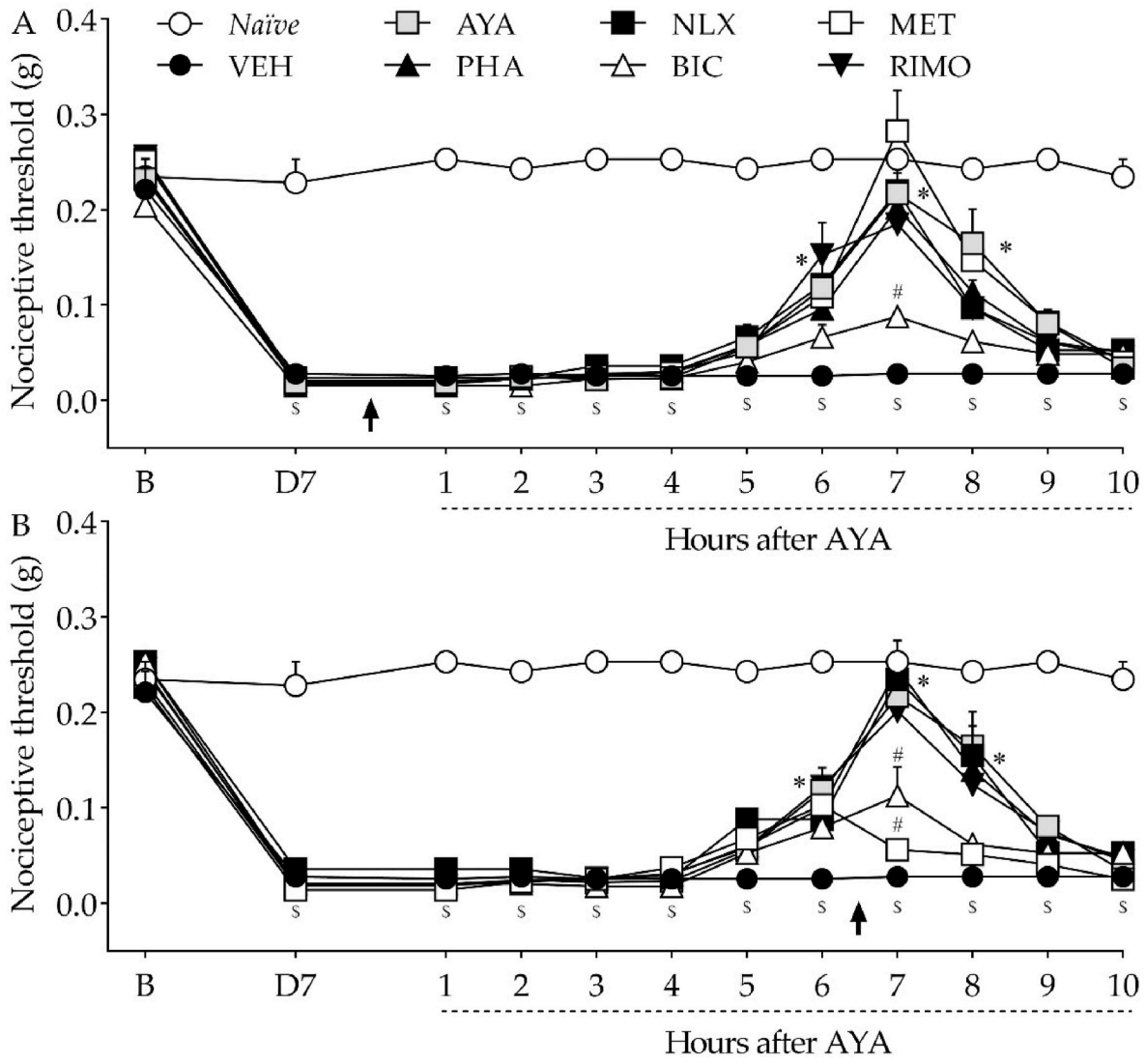

Fig. 6. Influence of pharmacological antagonists on the antinociceptive effect of ayahuasca during experimental neuropathic pain. Neuropathic mice submitted to partial sciatic nerve ligation were orally treated with ayahuasca (AYA; 600 μL/kg) or water (vehicle; VEH). *Naïve* mice were not submitted to surgical procedures or treatments. Systemic treatments with antagonists were made either before AYA (**Panel A**) or before the time of maximum effect at 7 h (**Panel B**). The black arrow indicates when the pharmacological treatments with antagonists were performed. Mice treated with AYA (600 μL/kg, p.o.) received naloxone (NLX; non-selective opioid antagonist, 5 mg/kg, i.p., 40 min before AYA or E_{max}), bicuculline (BIC; $GABA_A$ receptor antagonist, 1 mg/kg, i.p., 15 min before AYA or E_{max}), phaclofen (PHA; $GABA_B$ receptor antagonist, 2 mg/kg, i.p., 15 min before AYA or E_{max}), methysergide (MET; non-selective serotonergic antagonist, 5 mg/kg, i.p., 30 min before AYA or E_{max}), or rimonabant (RIMO; CB1 receptor inverse agonist, 10 mg/kg, i.p., 30 min before AYA or E_{max}). Mechanical nociceptive thresholds (ordinate axis) represent the filament weight (g) to which mice respond in 50% of trials and were assessed before the experiment (baseline; B), after surgery (day 7), and at different times in hours after AYA administration. Data are expressed as mean ± standard deviation (n = 6 to 7 mice per group). $^{\$}p < 0.05$ compared to *naïve* group; $^{*}p < 0.05$ compared to the VEH group; $^{\#}p < 0.05$ compared to the AYA group, as determined by two-way ANOVA followed by Bonferroni's test.

3.7. Ayahuasca acutely increases fos expression in brain areas that modulate nociception in neuropathic mice

The influence of oral treatments with ayahuasca (600 μL/kg) in both single and repeated dose regimens on Fos expression in the brain was assessed immunohistochemically (Fig. 7). A single administration of ayahuasca significantly increased Fos activity after 1 h, but not after 6 h, in the ventrolateral periaqueductal gray ($F(6, 27) = 11.39$; $p < 0.05$; Fig. 7A) and in the nucleus raphe magnus ($F(6, 24) = 31.83$; $p < 0.05$; Fig. 7B) compared to *naïve* mice. In the protocol of repeated administrations, no effects of ayahuasca on Fos expression were observed after 14 days. Vehicle-treated neuropathic mice showed increased Fos expression in the ventrolateral periaqueductal gray on the 14th day compared to *naïve* mice ($p < 0.05$; Fig. 7A). No significant differences were found between vehicle-treated and *naïve* mice when evaluating other timepoints or the nucleus raphe magnus. Representative images of all experimental groups can be seen in Fig. S1.

The results of Fos expression match the usual time course of the Fos protein levels, which peaks between 1 and 3 h following acute stimuli and return to baseline levels after 4–6 h (McReynolds et al., 2018). Fos expression in ayahuasca-treated mice was not different from the control group after daily exposures for 14 days, probably due to desensitization of c-*fos* mRNA induction after repetitive stimuli (Renthal et al., 2008), which does not mean absence of neuronal activity. The most relevant and well-studied circuit of descending pain modulation consists in projections from the PAG to the rostral ventromedial medulla (RVM), a region of the brainstem that includes the RMg and the adjacent reticular formation (Beitz, 1990; Ossipov et al., 2014). Stimulation of the VLPAG results in activation of the RMg, which is the main source of serotonin released in the spinal cord. Serotonin released from the RMg into the

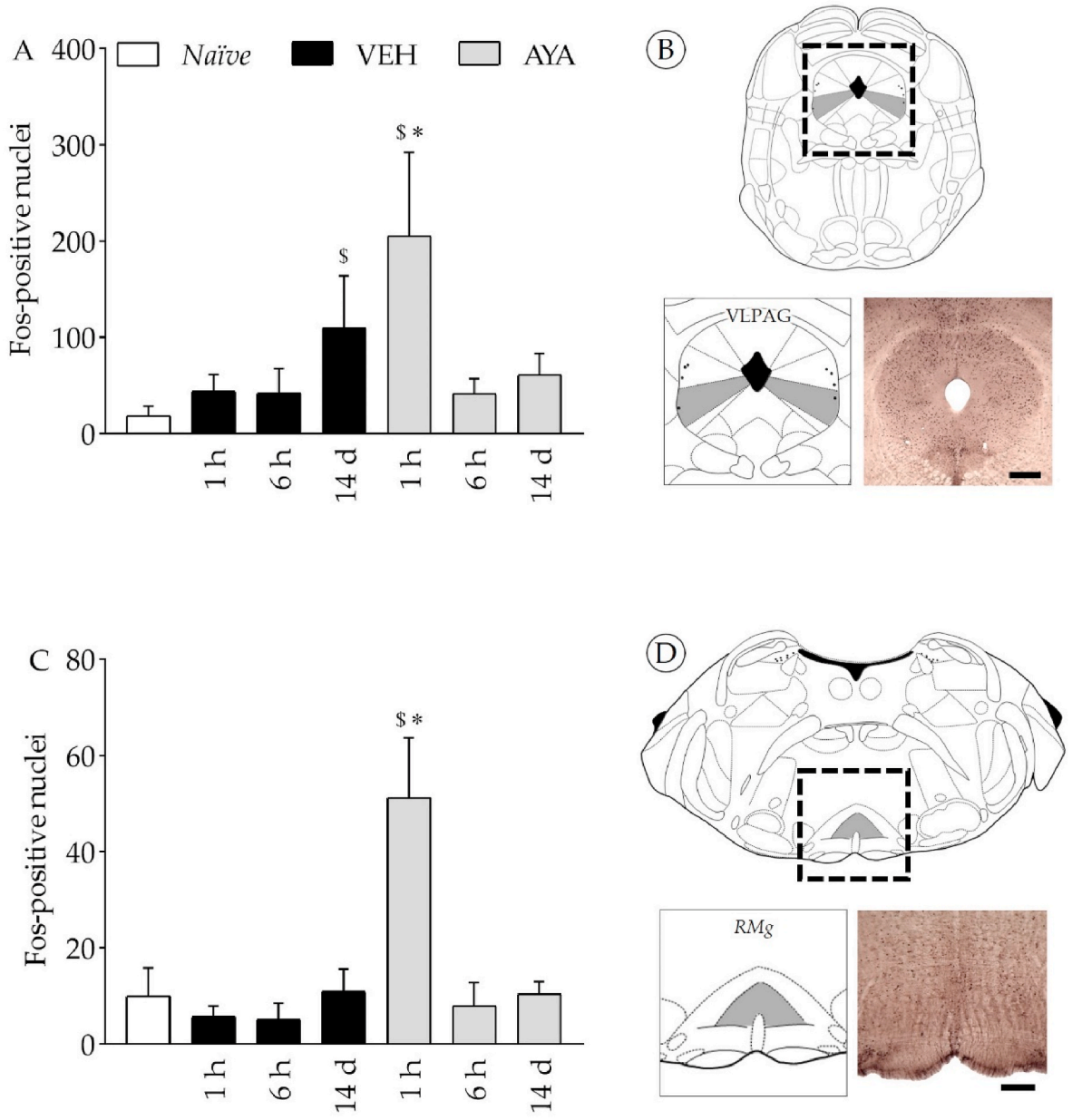

Fig. 7. Influence of ayahuasca on activation of brain regions involved in descending pain inhibitory pathways. Mice submitted to partial sciatic nerve ligation were orally treated with ayahuasca (AYA; 600 μL/kg) or water (vehicle; VEH) acutely or twice a day for 14 days. *Naïve* mice were not submitted to surgical procedures or treatments. Brains were removed 1 h or 6 h following a single treatment or on the 14th day of daily treatments. Brain sections were immunohistochemically marked to assess Fos expression in the ventrolateral periaqueductal gray (VLPAG; Bregma −4.60 mm; Panels A and B) and nucleus raphe magnus (RMg; Bregma −5.68 mm; Panels C and D). In Panels B and D, reference images from the Paxinos and Franklin's mouse brain atlas show regions of interest marked by a dashed black line. Representative micrographs are from the experimental group AYA 1 h. Scale bar: 300 μm. Data are expressed as mean ± standard deviation (n = 5 mice per group). $^{\$}$ $p < 0.05$ compared to the *naïve* group; $^{*}p < 0.05$ compared to the VEH group, as determined by one-way ANOVA followed by Tukey's test.

dorsal horn of the spinal cord negatively modulates pain through different mechanisms: (1) pre-synaptic inhibition of the primary afferent; (2) post-synaptic inhibition of the projection neuron; and (3) activation of inhibitory interneurons containing enkephalins and GABA (Cui et al., 1999; Millan, 2002). In agreement with our results, Pic--Taylor et al. (2015) have shown that ayahuasca increases Fos expression in the dorsal raphe nuclei of rats. Moreover, it has been suggested that one of the analgesic mechanisms of LSD is the activation of the dorsal raphe and hence the descending pain inhibitory system (Kooijman et al., 2023), which could be a shared mechanism with other psychedelics.

The VLPAG-RMg circuit is commonly associated with opioid-induced analgesia (Holden et al., 2005). Microinjections of morphine into either the PAG or the RVM induce antinociception (Morgan et al., 2005). Nevertheless, in the present study, treatments with naloxone did not prevent or reduce the antinociceptive effect of ayahuasca, suggesting that the opioid system is not important for the effect. Harasawa et al. (2016) have shown that neurons expressing opioid receptors in the RVM are not essential for the antinociceptive response mediated by this brain region. Opioids are not essential at the level of the spinal cord either, as inhibitory interneurons often co-express enkephalin and GABA (Todd et al., 1992) and the activation of 5-HT_3 receptors releases GABA from interneurons in the dorsal horn (Fukushima et al., 2011). Spinal GABAergic inhibition is an important mechanism of nociceptive control that is impaired during chronic painful conditions (Paul et al., 2014). Drugs that positively modulate spinal $GABA_A$ receptors have been shown to reduce hyperalgesia in mouse models of neuropathic pain

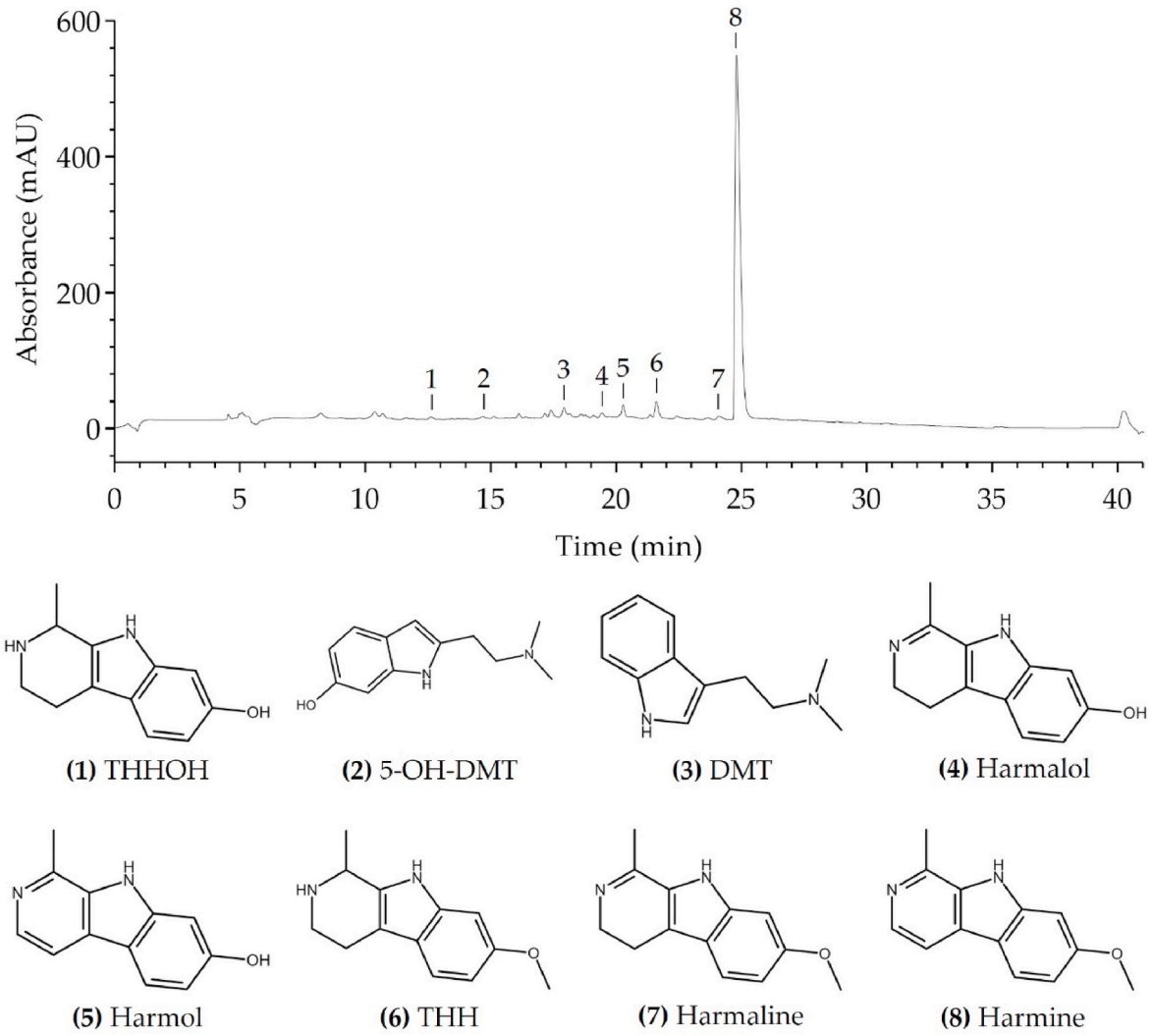

Fig. 8. Chromatogram of ayahuasca with alkaloids identified by HPLC-ESI-MS/MS. Chemical structures of tentatively identified components are preceded by the number of their corresponding peaks. Abbreviations: 7-hydroxy-tetrahydroharmine (THHOH); 5-hydroxy-N,N-dimethyltryptamine (5-OH-DMT); N,N-dimethyltryptamine (DMT); tetrahydroharmine (THH).

(Knabl et al., 2008; Witschi et al., 2011).

Although our results suggest the recruitment of descending pain inhibitory pathways, the kinetics of Fos expression does not match the times of antinociceptive effect of ayahuasca. An important limitation of using Fos as a proxy for neuronal activity is that Fos expression, unlike *in vivo* electrophysiology or imaging methods, does not necessarily correlate with real-time changes in the pattern of brain activity (McReynolds et al., 2018). It has been shown that in murine models of pain, pharmacological treatments can either promote antinociception without altering Fos expression in the brain or induce changes in Fos expression without antinociception (Coggeshall, 2005). Notwithstanding, the antinociceptive effects of ayahuasca more likely result from a multimodal action, which is consistent with its chemical complexity and subjective effects in the mind.

3.8. Single or multiple administrations of ayahuasca do not induce signs of toxicity in mice

Neither the acute oral treatment with ayahuasca (600–3000 μL/kg) in healthy mice nor daily treatments with ayahuasca (600 μL/kg) twice a day for 14 days in neuropathic mice affected their motor coordination and locomotion, as determined by the rotarod (Fig. S2) and the open field (Fig. S3) tests. Similarly, daily oral treatments with ayahuasca (600 μL/kg) twice a day for 14 days did not affect the body weight or the food and water intake (Fig. S4), did not induce any changes in hematological parameters or in plasma biochemical markers of hepatorenal (Table S1), did not induce macroscopic alterations in the stomach, kidneys, and liver (Table S2), and did not cause histopathological changes in these organs (Fig. S5). Overall, our results suggest the safety of daily oral treatments with ayahuasca at the dose of maximum antinociception.

In a recent systematic review, Daldegan-Bueno et al. (2023) analyzed 32 studies that assessed the effects of ayahuasca in experimental animals, 16 of which evaluated parameters of toxicity. In agreement with our results, most studies reported no toxic effects induced by ayahuasca, including those that performed multiple administrations for up to 70 days. Investigations carried out with human subjects also attest the safety of ayahuasca consumed in religious ceremonies by both novice and experienced users (Domínguez-Clavé et al., 2016; Dos Santos et al., 2016; Riba et al., 2001). The most frequent adverse effects are vomiting and diarrhea, although these are not perceived as negative effects in the religious context, but rather as mechanisms of spiritual and bodily purging (Costa et al., 2005). Importantly, patients using serotonergic agents such as monoamine reuptake inhibitors and tricyclic antidepressants should avoid ayahuasca due to the risk of serotonin syndrome (Callaway and Grob, 1998; Volpi-Abadie et al., 2013).

3.9. Chemical composition of ayahuasca

The HPLC-ESI-MS/MS analysis allowed the detection of eight alkaloids in ayahuasca: 7-hydroxy-tetrahydroharmine (THHOH), 5-hydroxy-N,N-dimethyltryptamine (5-OH-DMT), N,N-dimethyltryptamine (DMT), harmalol, harmol, tetrahydroharmine (THH), harmaline, and harmine (Fig. 8). Components were identified by interpreting the fragmentation patterns obtained from mass spectra (MS^2 and MS^3 experiments). Data provided by reference standards (harmine and harmaline) and from the literature (McIlhenny et al., 2009; McIlhenny et al., 2012) were also employed for the comprehensive evaluation of the sample. The retention times and mass spectrum data along with the peak assignments for components identified using positive ionization mode can be found in the supplementary material (Table S3).

Eight different alkaloids were detected in the brew; all of them had been previously described in other ayahuasca samples (McIlhenny et al., 2009; McIlhenny et al., 2012; Rodríguez et al., 2022). Nevertheless, the proportions of these components greatly vary among samples, making each ayahuasca rather unique. Chemical variability can be explained by the different methods employed in the making of ayahuasca. Although the species *Banisteriopsis caapi* and *Psychotria viridis* are frequently mentioned in the literature as the ingredients of ayahuasca, other plant species can be used as sources of DMT and β-carbolines (Nižnanský et al., 2022; Rodríguez et al., 2022). Moreover, each tribe or religious group has its own protocols for brewing ayahuasca, which include variable proportions of each plant, cooking time, dilutions, etc. (Rodríguez et al., 2022).

The phytochemical analysis of ayahuasca also revealed that harmine was the major component present in the sample. The harmine peak in ayahuasca had a retention time of 25.2 min, matching the peak of harmine standard (Fig. 9). The concentration of harmine in ayahuasca was calculated by simple linear regression after building a calibration curve with multiple concentrations of harmine standard (Fig. S6). The average harmine content found in ayahuasca was 5.87 ± 0.16 mg/mL, which is within an expected range compared to other ayahuasca samples (McIlhenny et al., 2009).

3.10. Harmine promotes long-lasting antinociception during experimental neuropathy

To investigate if harmine contributes to the antinociceptive effect of ayahuasca, the component was tested in the partial sciatic nerve ligation model of neuropathic pain (Fig. 10). Based on the results of harmine quantification in ayahuasca, the dose of harmine contained in the maximum effective dose of ayahuasca (600 μL/kg) was estimated to be 3.5 mg/kg. This dose was used as a starting point to build a dose-response curve for harmine (0.035–35 mg/kg) in this model (Fig. 10A; time: $F(14, 588) = 55.49$; $p < 0.0001$; treatment: $F(7, 42) = 228.5$; $p < 0.0001$; interaction time × treatment: $F(98, 588) = 10.85$; $p < 0.0001$). Harmine dose-dependently reduced mechanical allodynia following a single oral administration (Fig. 10A; $p < 0.05$). At the maximum effective dose (3.5 mg/kg), the antinociceptive effect of harmine peaked at 1 h and lasted for up to 10 h post-treatment. Daily oral treatments with harmine (3.5 mg/kg) twice a day for 14 days promoted a plateau of antinociception from the fifth day onwards (Fig. 10B; time: $F(31, 868) = 17.06$; $p < 0.0001$; treatment: $F(4, 28) = 466.8$; $p < 0.0001$; interaction time × treatment: $F(124, 868) = 10.37$; $p < 0.0001$). Harmine (35 mg/kg) did not affect the permanence time of mice on the rotarod apparatus (data not shown).

Harmine is a competitive and reversible inhibitor of the enzyme MAO (McKenna et al., 1984). MAO inhibitors were the first class of antidepressants and are still used in the present, although to a lesser extent (Jha and Mathew, 2023). Previous studies have shown the antidepressant effects of harmine, which could be attributed to MAO inhibition (Farzin and Mansouri, 2006; Liu et al., 2017). The use of antidepressants for treating neuropathic pain is well-established, although tricyclic antidepressants and monoamine reuptake inhibitors are usually the drug classes of choice (Ferreira et al., 2023; Reinert et al., 2023). Nevertheless, studies have suggested that MAO inhibitors can be useful in the management of chronic pain syndromes (Mattia and Coluzzi, 2007; Tort et al., 2012) and the antinociceptive effects of different MAO inhibitors have been experimentally demonstrated in the chronic constriction injury model of neuropathic pain in mice (Villarinho et al., 2013). The antinociceptive effect of harmine during experimental neuropathy is probably mediated by mechanisms that are not common among drugs currently used in the treatment of neuropathic pain, which might represent an alternative in the management of refractory painful conditions of neuropathic origin.

In the only previous study that evaluated the antinociceptive effect of ayahuasca in mice, Pires et al. (2018) reported that tetrahydroharmine was the major component of their ayahuasca sample at 1.67 mg/mL, while harmine was the third most abundant component at 0.32 mg/mL.

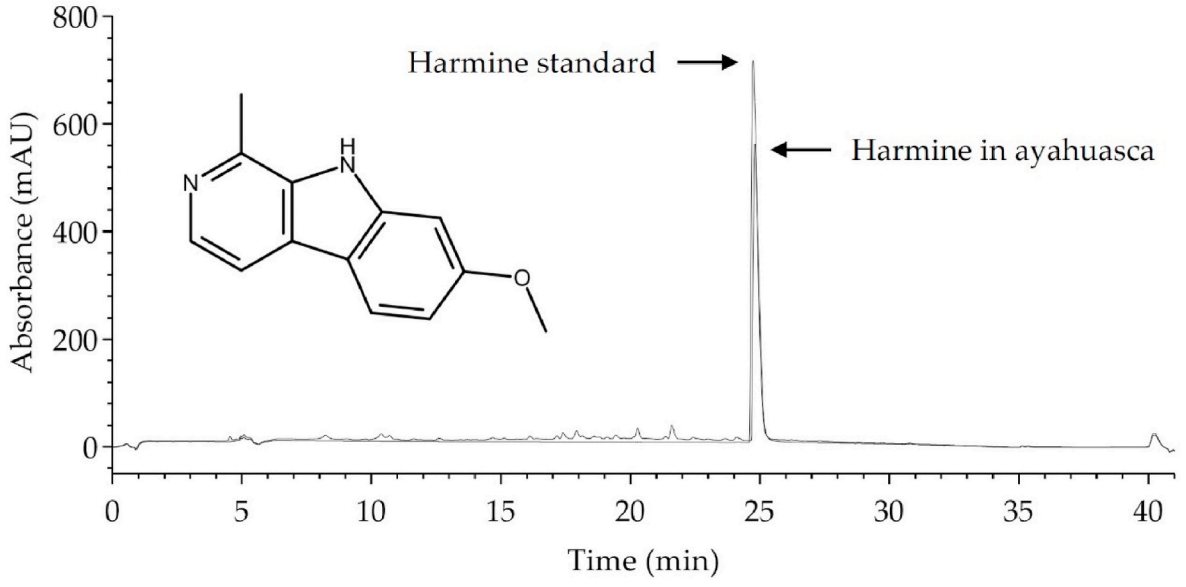

Fig. 9. Superposed chromatograms of ayahuasca and harmine standard. Under the same chromatographic conditions, the retention time of harmine standard aligns with that of the major peak of ayahuasca. Chemical structure of harmine is represented in an inset on the chromatogram.

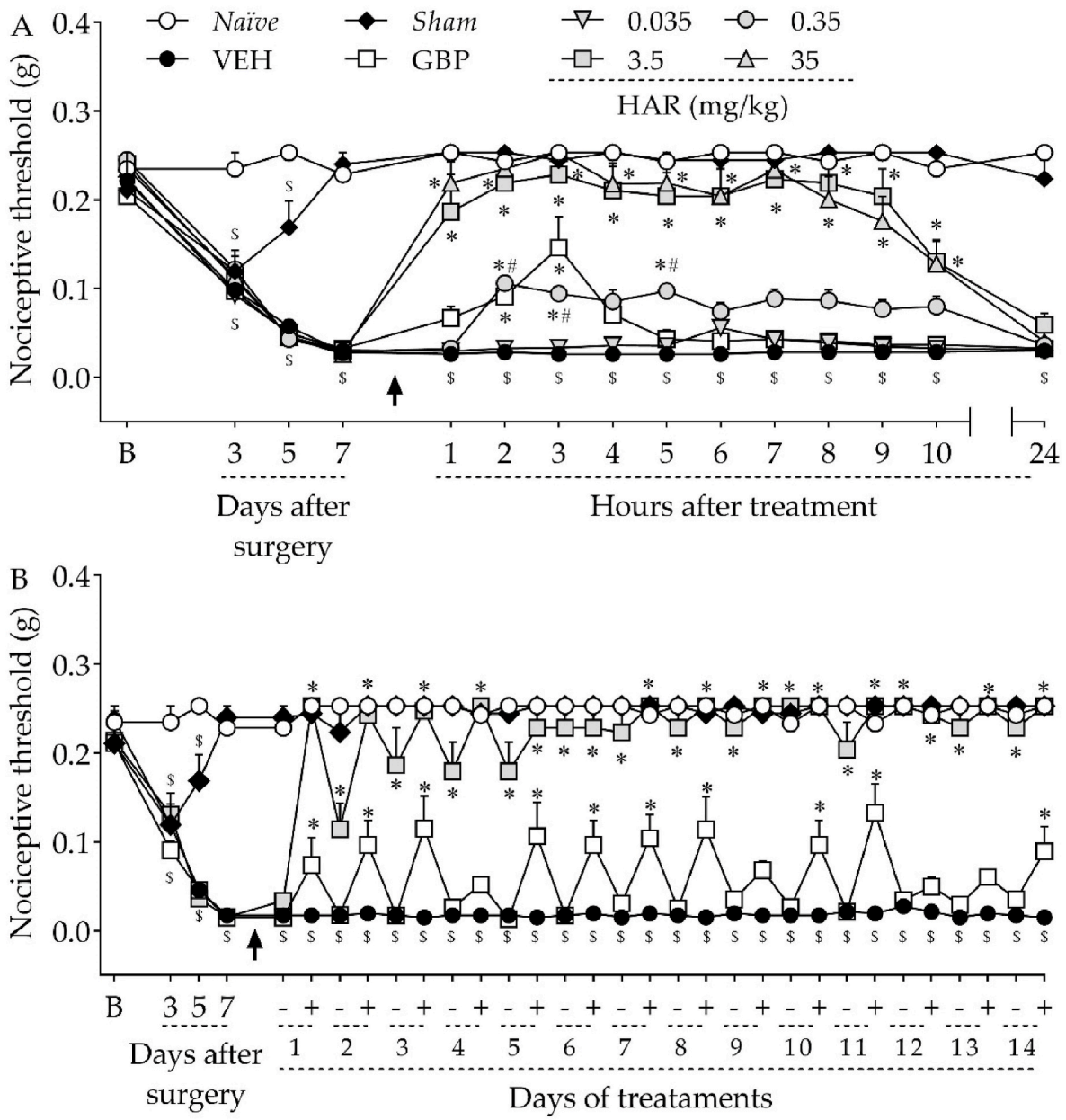

Fig. 10. Antinociceptive effect of single and repeated administrations of harmine during experimental neuropathic pain. Neuropathic mice submitted to partial sciatic nerve ligation were orally treated with harmine (HAR; 0.035–35 mg/kg), water (vehicle; VEH), or gabapentin (GBP; reference drug; 70 mg/kg). *Naïve* mice were not submitted to surgical procedures or treatments. *Sham* mice underwent surgical manipulation without nerve ligation. Mechanical nociceptive thresholds (ordinate axis) represent the filament weight (g) to which mice respond in 50% of trials and were assessed before the experiment (baseline; B), after surgery (days 3–7), and at different times after treatments. **Panel A:** Effect of single treatments. The black arrow indicates when treatments were performed. **Panel B:** Effect of daily treatments twice a day for 14 days. Nociceptive thresholds were assessed during the 14 days of treatments in two moments: before treatments (−) and at the time of maximum effect (+). The black arrow indicates when daily treatments started. For both panels, data are expressed as mean ± standard deviation (n = 6 to 7 mice per group). $^{\$}$ $p < 0.05$ compared to the *naïve* group; $^{*}p < 0.05$ compared to the VEH group; $^{\#}p < 0.05$ compared to the 3.5 mg/kg group, as determined by two-way ANOVA followed by Bonferroni's test.

Comparatively, the concentration of harmine in the ayahuasca used in the present study (5.87 mg/mL) was more than 18 times greater. Despite the clear differences in chemical composition between the two samples, the doses of crude ayahuasca used by Pires et al. (2018) contained 0.032–3.2 mg/kg of harmine, which almost perfectly matches the dose range of harmine used in our work. The fact that, in both studies, the antinociceptive dose ranges of ayahuasca had an equivalent amount of harmine despite important methodological differences corroborates the role of harmine in the antinociception promoted by ayahuasca. Nevertheless, the two ayahuasca samples showed different profiles of antinociceptive effect, suggesting that other components in ayahuasca could also modulate nociceptive transmission resulting in different global effects.

The present results showed that the antinociceptive effect of ayahuasca during experimental neuropathy had a different kinetic profile when compared to harmine alone. The acute oral administration of harmine (3.5 mg/kg) promoted antinociceptive effects during experimental neuropathy that peaked at 1 h and lasted for up to 10 h. This pattern agrees with pharmacokinetic studies in humans showing that, following oral ingestion of ayahuasca, plasma concentrations of harmine peak at 1 h and are still detectable after 10 h (McIlhenny et al., 2012; Nižnanský et al., 2022). On the other hand, the acute oral

administration of ayahuasca (600 μL/kg) containing the equivalent dose of harmine promoted a late antinociceptive effect. This could indicate that other components are delaying the action of harmine by competing for the same binding sites. Another explanation would be the occurrence of pharmacokinetic interactions among the components of ayahuasca, slowing down the absorption of harmine. In fact, the bioavailability of harmine is different in ayahuasca samples with different chemical composition (Callaway et al., 1996; Riba et al., 2003). Interestingly, it has been shown in human subjects that the effects of ayahuasca in the brain follow a biphasic pattern in response to the different plasma levels of DMT and β-carbolines over time (Schenberg et al., 2015). This suggests that the components present in ayahuasca can interact in multiple ways to produce different biological effects. Surely these comparisons are not conclusive, as translatability of pharmacological parameters across species is not perfect. Nevertheless, these data still provide valuable information that could partially explain our observations.

4. Conclusions

The pharmacological treatment of neuropathic pain is still a clinical challenge to be overcome. This study characterized the antinociceptive effects of ayahuasca in different behavioral models of acute and persistent pain in mice. This was the first experimental demonstration of the therapeutic potential of ayahuasca and its major component harmine in the treatment of chronic neuropathic pain. Daily treatments with ayahuasca or harmine completely abolished the nociceptive sensitization that characterizes experimental and clinical neuropathic pain. The antinociceptive effects of ayahuasca seem to be at least partially mediated by descending pain inhibitory pathways and involve serotonergic and $GABA_A$ receptors. Ayahuasca showed a good safety profile when given repeatedly, inducing no detectable signs of systemic toxicity after 14 days of daily oral administrations twice a day. An important limitation of this work is that there is a broad chemical variability among ayahuasca samples, so our results should be interpreted considering the chemical composition shown here. Further studies ought to investigate the analgesic potential of ayahuasca in human subjects and the mechanisms involved.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) [grant number INCT-RENNOFITO 465536/2014–0] and was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) [finance code 001].

CRediT authorship contribution statement

Pedro Santana Sales Lauria: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Juliana de Medeiros Gomes:** Formal analysis, Investigation, Methodology, Writing – original draft. **Lucas Silva Abreu:** Formal analysis, Investigation, Methodology, Writing – original draft. **Rejane Conceição Santana:** Investigation, Methodology. **Victor Luiz Correia Nunes:** Formal analysis, Investigation. **Ricardo David Couto:** Investigation, Methodology. **Paulo Oliveira Colavolpe:** Conceptualization. **Marcelo Sobral da Silva:** Methodology, Resources. **Milena Botelho Pereira Soares:** Resources, Writing – review & editing. **Cristiane Flora Villarreal:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank Sidney Prytherch for the English review of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2024.117710.

Abbreviations

(ANOVA) Analysis of variance
(AYA) ayahuasca
(BIC) bicuculline
(DAB) 3,3′-diaminobenzidine-tetrahydrochloride
(DEXA) dexamethasone
(DMT) N,N-dimethyltryptamine
(DZP) diazepam
(E_{max}) maximum effect
(ESI-MS/MS) electrospray ionization tandem mass spectrometry
(GABA) gamma-aminobutyric acid
(GBP) gabapentin
(HAR) harmine
(HPLC-DAD) high-performance liquid chromatography with diode-array detection
(HRESIMS) high-resolution electrospray ionization mass spectrometry
(i.p.) intraperitoneal
(INDO) indomethacin
(LSD) lysergic acid diethylamide
(MET) methysergide
(MOR) morphine
(NLX) naloxone
(p.o.) orally
(PB) phosphate buffer
(PBS) phosphate buffered saline
(PFA) paraformaldehyde
(PHA) phaclofen
(RIMO) rimonabant
(RMg) nucleus raphe magnus
(RVM) rostral ventromedial medulla
(s.c.) subcutaneous
(VEH) vehicle
(VLPAG) ventrolateral periaqueductal gray

References

Abreu, L.S., Alves, I.M., Espírito Santo, R.F.d., Nascimento, Y.M.d., Dantas, C.A.G., dos Santos, G.G.L., Le Hyaric, M., Guedes, M.L.S., da Silva, M.S., Villarreal, C.F., Velozo, E.d.S., Tavares, J.F., 2019. Antinociceptive compounds and LC-DAD-ESIMSn profile from *Dictyoloma vandellianum* leaves. PLoS One 14 (10), e0224575.

Attal, N., Bouhassira, D., Colvin, L., 2023. Advances and challenges in neuropathic pain: a narrative review and future directions. Br. J. Anaesth.131 1, 79–92.

Barbosa, P.C., Cazorla, I.M., Giglio, J.S., Strassman, R., 2009. A six-month prospective evaluation of personality traits, psychiatric symptoms and quality of life in ayahuasca-naïve subjects. J. Psychoact. Drugs 41 (3), 205–212.

Bates, D., Schultheis, B.C., Hanes, M.C., Jolly, S.M., Chakravarthy, K.V., Deer, T.R., Levy, R.M., Hunter, C.W., 2019. A comprehensive algorithm for management of neuropathic pain. Pain Med. 20 (Suppl. ment_1), S2–S12.

Battaglia, M., Groenewald, C.B., Campbell, F., Scaini, S., De Koninck, Y., Stinson, J., Quinn, P.D., 2023. We need to talk: the urgent conversation on chronic pain, mental health, prescribing patterns and the opioid crisis. J. Psychopharmacol. 37 (5), 437–448.

Beitz, A.J., 1990. Relationship of glutamate and aspartate to the periaqueductal gray-raphe magnus projection: analysis using immunocytochemistry and microdialysis. J. Histochem. Cytochem. 38 (12), 1755–1765.
Bernetti, A., Agostini, F., de Sire, A., Mangone, M., Tognolo, L., Di Cesare, A., Ruiu, P., Paolucci, T., Invernizzi, M., Paoloni, M., 2021. Neuropathic pain and rehabilitation: a systematic review of international guidelines. Diagnostics 11 (1).
Callaway, J.C., Grob, C.S., 1998. Ayahuasca preparations and serotonin reuptake inhibitors: a potential combination for severe adverse interactions. J. Psychoact. Drugs 30 (4), 367–369.
Callaway, J.C., Raymon, L.P., Hearn, W.L., McKenna, D.J., Grob, C.S., Brito, G.S., Mash, D.C., 1996. Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. J. Anal. Toxicol. 20 (6), 492–497.
Carvalho, C.d., Alves, N.C., Monteiro, A.C., Pelógia, N.C.C., 2011. Antinociceptive and anti-inflammatory effect of Agaricus blazei Murill in rats submitted to the modified formalin test. Revista Dor 12 (1), 35–38.
Castro-Neto, E.F.d., da Cunha, R.H., da Silveira, D.X., Yonamine, M., Gouveia, T.L., Cavalheiro, E.A., Amado, D., Naffah-Mazzacoratti Mda, G., 2013. Changes in aminoacidergic and monoaminergic neurotransmission in the hippocampus and amygdala of rats after ayahuasca ingestion. World J. Biol. Chem. 4 (4), 141–147.
Coggeshall, R.E., 2005. Fos, nociception and the dorsal horn. Prog. Neurobiol. 77 (5), 299–352.
Costa, M.C.M., Figueiredo, M.C., Cazenave, S.d.O.S., 2005. Ayahuasca: uma abordagem toxicológica do uso ritualístico. Archives of Clinical Psychiatry (São Paulo) 32 (6), 310–318.
Cui, M., Feng, Y., McAdoo, D.J., Willis, W.D., 1999. Periaqueductal gray stimulation-induced inhibition of nociceptive dorsal horn neurons in rats is associated with the release of norepinephrine, serotonin, and amino acids. J. Pharmacol. Exp. Therapeut. 289 (2), 868–876.
da Silva, M.G., Daros, G.C., de Bitencourt, R.M., 2021. Anti-inflammatory activity of ayahuasca: therapeutical implications in neurological and psychiatric diseases. Behav. Brain Res. 400, 113003.
Daldegan-Bueno, D., Simionato, N.M., Favaro, V.M., Maia, L.O., 2023. The current state of ayahuasca research in animal models: A systematic review. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 125, 110738. https://doi.org/10.1016/j.pnpbp.2023.110738.
Damas, J., Liégeois, J.F., 1999. The inflammatory reaction induced by formalin in the rat paw. N. Schmied. Arch. Pharmacol. 359 (3), 220–227.
de Oliveira Silveira, G., Guimarães dos Santos, R., Rebello Lourenço, F., Novak Rossi, G., Hallak, J.E.C., Yonamine, M., 2020. Stability evaluation of DMT and harmala alkaloids in ayahuasca tea samples. Molecules 25 (9).
Dixon, W.J., 1965. The up-and-down method for small samples. J. Am. Stat. Assoc. 60 (312), 967–978.
Domínguez-Clavé, E., Soler, J., Elices, M., Pascual, J.C., Alvarez, E., de la Fuente Revenga, M., Friedlander, P., Feilding, A., Riba, J., 2016. Ayahuasca: pharmacology, neuroscience and therapeutic potential. Brain Res. Bull. 126 (1), 89–101.
Dos Santos, R.G., Balthazar, F.M., Bouso, J.C., Hallak, J.E., 2016. The current state of research on ayahuasca: a systematic review of human studies assessing psychiatric symptoms, neuropsychological functioning, and neuroimaging. J. Psychopharmacol. 30 (12), 1230–1247.
Dos Santos, R.G., Hallak, J.E.C., 2021. Ayahuasca, an ancient substance with traditional and contemporary use in neuropsychiatry and neuroscience. Epilepsy Behav. : E&B 121 (Pt B), 106300.
Dubuisson, D., Dennis, S.G., 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4, 161–174. Supp C).
Espírito-Santo, R.F., Meira, C.S., Costa, R.D.S., Souza Filho, O.P., Evangelista, A.F., Trossini, G.H.G., Ferreira, G.M., Velozo, E.D.S., Villarreal, C.F., Pereira Soares, M.B., 2017. The anti-inflammatory and immunomodulatory potential of braylin: pharmacological properties and mechanisms by in silico, in vitro and in vivo approaches. PLoS One 12 (6), e0179174.
Estrella-Parra, E.A., Almanza-Perez, J.C., Alarcon-Aguilar, F.J., 2019. Ayahuasca: uses, phytochemical and biological activities. Nat Prod Bioprospect 9 (4), 251–265.
Farzin, D., Mansouri, N., 2006. Antidepressant-like effect of harmane and other beta-carbolines in the mouse forced swim test. Eur. Neuropsychopharmacol : the journal of the European College of Neuropsychopharmacology 16 (5), 324–328.
Ferreira, G.E., Abdel-Shaheed, C., Underwood, M., Finnerup, N.B., Day, R.O., McLachlan, A., Eldabe, S., Zadro, J.R., Maher, C.G., 2023. Efficacy, safety, and tolerability of antidepressants for pain in adults: overview of systematic reviews. Br. Med. J. 380, e072415.
Flanagan, T.W., Nichols, C.D., 2018. Psychedelics as anti-inflammatory agents. Int. Rev. Psychiatr. 30 (4), 363–375.
Fukushima, T., Tsuda, M., Kofuji, T., Hori, Y., 2011. Physiological properties of enkephalin-containing neurons in the spinal dorsal horn visualized by expression of green fluorescent protein in BAC transgenic mice. BMC Neurosci. 12, 36.
Galvão-Coelho, N.L., de Menezes Galvão, A.C., de Almeida, R.N., Palhano-Fontes, F., Campos Braga, I., Lobão Soares, B., Maia-de-Oliveira, J.P., Perkins, D., Sarris, J., de Araujo, D.B., 2020. Changes in inflammatory biomarkers are related to the antidepressant effects of Ayahuasca. J. Psychopharmacol. 34 (10), 1125–1133.
Gama, K.B., Santos, D.S., Evangelista, A.F., Silva, D.N., de Alcântara, A.C., Dos Santos, R. R., Soares, M.B.P., Villarreal, C.F., 2018. Conditioned medium of bone marrow-derived mesenchymal stromal cells as a therapeutic approach to neuropathic pain: a preclinical evaluation. Stem Cell. Int. 2018, 8179013.
Garber, J.C., Barbee, R.W., Bielitzki, J.T., Clayton, L.A., Donovan, J.C., Hendriksen, C.F. M., Kohn, D.F., Lipman, N.S., Locke, P.A., Melcher, J., Quimby, F.W., Turner, P.V., Wood, G.A., Würbel, H., 2011. Guide for the Care and Use of Laboratory Animals, 8 ed. The National Academies Press, Washington, DC.
Giovannini, S., Coraci, D., Brau, F., Galluzzo, V., Loreti, C., Caliandro, P., Padua, L., Maccauro, G., Biscotti, L., Bernabei, R., 2021. Neuropathic pain in the elderly. Diagnostics 11 (4).
Goulart da Silva, M., Daros, G.C., Santos, F.P., Yonamine, M., de Bitencourt, R.M., 2022. Antidepressant and anxiolytic-like effects of ayahuasca in rats subjected to LPS-induced neuroinflammation. Behav. Brain Res. 434, 114007.
Hadar, A., David, J., Shalit, N., Roseman, L., Gross, R., Sessa, B., Lev-Ran, S., 2023. The psychedelic renaissance in clinical research: a bibliometric analysis of three decades of human studies with psychedelics. J. Psychoact. Drugs 55 (1), 1–10.
Harasawa, I., Johansen, J.P., Fields, H.L., Porreca, F., Meng, I.D., 2016. Alterations in the rostral ventromedial medulla after the selective ablation of μ-opioid receptor expressing neurons. Pain 157 (1), 166–173.
Hedau, V.N., Anjankar, A.P., 2022. Psychedelics: their limited understanding and future in the treatment of chronic pain. Cureus 14 (8), e28413.
Henderson-Redmond, A.N., Crawford, L.C., Sepulveda, D.E., Hale, D.E., Lesperance, J.J., Morgan, D.J., 2021. Sex differences in tolerance to delta-9-tetrahydrocannabinol in mice with cisplatin-evoked chronic neuropathic pain. Front. Mol. Biosci. 8, 684115.
Hess, S., Padoani, C., Scorteganha, L.C., Holzmann, I., Malheiros, A., Yunes, R.A., Delle Monache, F., de Souza, M.M., 2010. Assessment of mechanisms involved in antinociception caused by myrsinoic acid B. Biol. Pharmaceut. Bull. 33 (2), 209–215.
Holden, J.E., Jeong, Y., Forrest, J.M., 2005. The endogenous opioid system and clinical pain management. AACN Clin Issues 16 (3), 291–301.
IASP, 2021. Neuropathic Pain. https://www.iasp-pain.org/Advocacy/GYAP.aspx?ItemNumber=5054. (Accessed 14 May 2021).
Irwin, S., Houde, R.W., Bennett, D.R., Hendershot, L.C., Seevers, M.H., 1951. The effects of morphine methadone and meperidine on some reflex responses of spinal animals to nociceptive stimulation. J. Pharmacol. Exp. Therapeut. 101 (2), 132–143.
Jha, M.K., Mathew, S.J., 2023. Pharmacotherapies for treatment-resistant depression: how antipsychotics fit in the rapidly evolving therapeutic landscape. Am. J. Psychiatr. 180 (3), 190–199.
Jiménez, J.H., Bouso, J.C., 2022. Significance of mammalian N, N-dimethyltryptamine (DMT): a 60-year-old debate. J. Psychopharmacol. 36 (8), 905–919.
Jones, C.K., Peters, S.C., Shannon, H.E., 2005. Efficacy of duloxetine, a potent and balanced serotonergic and noradrenergic reuptake inhibitor, in inflammatory and acute pain models in rodents. J. Pharmacol. Exp. Therapeut. 312 (2), 726–732.
Knabl, J., Witschi, R., Hösl, K., Reinold, H., Zeilhofer, U.B., Ahmadi, S., Brockhaus, J., Sergejeva, M., Hess, A., Brune, K., Fritschy, J.M., Rudolph, U., Möhler, H., Zeilhofer, H.U., 2008. Reversal of pathological pain through specific spinal GABAA receptor subtypes. Nature 451 (7176), 330–334.
Kooijman, N.I., Willegers, T., Reuser, A., Mulleners, W.M., Kramers, C., Vissers, K.C.P., van der Wal, S.E.I., 2023. Are psychedelics the answer to chronic pain: a review of current literature. Pain Practice. the official journal of World Institute of Pain.
Labate, B.C., 2012. Ayahuasca religions in Acre: cultural heritage in the Brazilian borderlands. Anthropol. Conscious 23 (1), 87–102.
Labate, B.C., Cavnar, C., 2014. The Therapeutic Use of Ayahuasca, 1 ed. Springer Berlin, Heidelberg.
Lanaro, R., Calemi, D.B.d.A., Togni, L.R., Costa, J.L., Yonamine, M., Cazenave, S.d.O.S., Linardi, A., 2015. Ritualistic use of ayahuasca versus street use of similar substances seized by the police: a key factor involved in the potential for intoxications and overdose? J. Psychoact. Drugs 47 (2), 132–139.
Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animal models of nociception. Pharmacol. Rev. 53 (4), 597–652.
Leite dos Santos, G.G., Casais e Silva, L.L., Pereira Soares, M.B., Villarreal, C.F., 2012. Antinociceptive properties of Micrurus lemniscatus venom. Toxicon 60 (6), 1005–1012.
Liu, F., Wu, J., Gong, Y., Wang, P., Zhu, L., Tong, L., Chen, X., Ling, Y., Huang, C., 2017. Harmine produces antidepressant-like effects via restoration of astrocytic functions. Progress in neuro-psychopharmacology & biological psychiatry 79 (Pt B), 258–267.
Maia-Marques, R., Nascimento, I.M.R., Lauria, P.S.S., Silva, E., Silva, D.F., Casais, E.S.L. L., 2021. Inflammatory mediators in the pronociceptive effects induced by *Bothrops leucurus* snake venom: the role of biogenic amines, nitric oxide, and eicosanoids. Toxicology 448, 152649.
Maia, L.O., Daldegan-Bueno, D., Wießner, I., Araujo, D.B., Tófoli, L.F., 2023. Ayahuasca's therapeutic potential: what we know – and what not. Eur. Neuropsychopharmacol 66, 45–61.
Mattia, C., Coluzzi, F., 2007. Indantadol, a novel NMDA antagonist and nonselective MAO inhibitor for the potential treatment of neuropathic pain. Idrugs : the investigational drugs journal 10 (9), 636–644.
McIlhenny, E.H., Pipkin, K.E., Standish, L.J., Wechkin, H.A., Strassman, R., Barker, S.A., 2009. Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian botanical medicine ayahuasca by liquid chromatography-electrospray ionization-tandem mass spectrometry. J. Chromatogr. A 1216 (51), 8960–8968.
McIlhenny, E.H., Riba, J., Barbanoj, M.J., Strassman, R., Barker, S.A., 2012. Methodology for determining major constituents of ayahuasca and their metabolites in blood. Biomed. Chromatogr. : BMC (Biomed. Chromatogr.) 26 (3), 301–313.
McKenna, D.J., Towers, G.H.N., Abbott, F.S., 1984. Monoamine oxidase inhibitors in South American hallucinogenic plants part 2: constituents of orally-active myristicaceous hallucinogens. J. Ethnopharmacol. 12 (2), 179–211.
McReynolds, J.R., Christianson, J.P., Blacktop, J.M., Mantsch, J.R., 2018. What does the Fos say? Using Fos-based approaches to understand the contribution of stress to substance use disorders. Neurobiology of Stress 9, 271–285.
Millan, M.J., 2002. Descending control of pain. Prog. Neurobiol. 66 (6), 355–474.
Moreira, P., MacRae, E., 2011. Eu venho de longe: Mestre Irineu e seus companheiros. EDUFBA, Salvador, Brazil.

Morgan, M.M., Clayton, C.C., Boyer-Quick, J.S., 2005. Differential susceptibility of the PAG and RVM to tolerance to the antinociceptive effect of morphine in the rat. Pain 113 (1–2), 91–98.
Nascimento, O.A., Espírito-Santo, R.F., Opretzka, L.C., Barbosa-Filho, J.M., Gutierrez, S. J., Villarreal, C.F., Soares, M.B., 2016. Pharmacological properties of riparin IV in models of pain and inflammation. Molecules 21 (12).
Nižnanský, Ľ., Nižnanská, Ž., Kuruc, R., Szórádová, A., Šikuta, J., Zummerová, A., 2022. Ayahuasca as a decoction applied to human. Analytical Methods, Pharmacology and Potential Toxic Effects 11 (4), 1147.
Opretzka, L.C.F., Espírito-Santo, R.F.D., Nascimento, O.A., Abreu, L.S., Alves, I.M., Döring, E., Soares, M.B.P., Velozo, E.D.S., Laufer, S.A., Villarreal, C.F., 2019. Natural chromones as potential anti-inflammatory agents: pharmacological properties and related mechanisms. Int. Immunopharm. 72, 31–39.
Orozco, A.L.C., 2020. Por uma dramaturgia da dor: Tecido Blando – uma peça criada a partir de narrativas de mulheres com experiência em fibromialgia. Universidade Federal da Bahia, Salvador, Brazil, p. 229.
Ossipov, M.H., Morimura, K., Porreca, F., 2014. Descending pain modulation and chronification of pain. Curr. Opin. Support. Palliat. Care 8 (2), 143–151.
Ott, J., 1996. Pharmacotheon: Entheogenic Drugs Their Plant Sources and Histories, 2 ed. Natural Products Co., Kennewick, USA.
Paul, J., Yévenes, G.E., Benke, D., Lio, A.D., Ralvenius, W.T., Witschi, R., Scheurer, L., Cook, J.M., Rudolph, U., Fritschy, J.-M., Zeilhofer, H.U., 2014. Antihyperalgesia by α2-GABAA receptors occurs via a genuine spinal action and does not involve supraspinal sites. Neuropsychopharmacology 39 (2), 477–487.
Paxinos, G., Franklin, K.B.J., 2001. The Mouse Brain in Stereotaxic Coordinates, 2 ed. Academic Press, San Diego, California.
Pic-Taylor, A., da Motta, L.G., de Morais, J.A., Junior, W.M., Santos Ade, F., Campos, L. A., Mortari, M.R., von Zuben, M.V., Caldas, E.D., 2015. Behavioural and neurotoxic effects of ayahuasca infusion (Banisteriopsis caapi and Psychotria viridis) in female Wistar rat. Behav. Process. 118, 102–110.
Pires, J.M., Mendes, F.R., Pires, A.P.S., Yonamine, M., Amaral, J.L.G.d., Carlini, E.A., 2018. Pre-clinical interaction of ayahuasca, a brew used in spiritual movements, with morphine and propofol. Brazilian Journal of Pharmaceutical Sciences 54 (1), e17174.
Reinert, J.P., Veronin, M.A., Medina, C., 2023. Tricyclic antidepressants in nociceptive and neuropathic pain: a review of their analgesic properties in combination with opioids. J. Pharm. Technol. : jPT : official publication of the Association of Pharmacy Technicians 39 (1), 35–40.
Renthal, W., Carle, T.L., Maze, I., Covington 3rd, H.E., Truong, H.T., Alibhai, I., Kumar, A., Montgomery, R.L., Olson, E.N., Nestler, E.J., 2008. Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. J. Neurosci. : the official journal of the Society for Neuroscience 28 (29), 7344–7349.
Riba, J., Rodríguez-Fornells, A., Urbano, G., Morte, A., Antonijoan, R., Montero, M., Callaway, J.C., Barbanoj, M.J., 2001. Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. Psychopharmacology 154 (1), 85–95.
Riba, J., Valle, M., Urbano, G., Yritia, M., Morte, A., Barbanoj, M.J., 2003. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J. Pharmacol. Exp. Therapeut. 306 (1), 73–83.
Rodríguez, L., López, A., Moyna, G., Seoane, G.A., Davyt, D., Vázquez, Á., Hernández, G., Carrera, I., 2022. New insights into the chemical composition of ayahuasca. ACS Omega 7 (14), 12307–12317.
Santos, D.S., Lauria, P.S.S., Evangelista, A.F., Azeredo, F.J., Costa, J.A.V., Soares, M.B.P., Druzian, J.I., Villarreal, C.F., 2020. Beyond inflammation: centrally mediated antinociceptive properties of *Spirulina platensis* LEB-18 biomass via the opioid system. J. Funct.Foods 72.
Schenberg, E.E., Alexandre, J.F., Filev, R., Cravo, A.M., Sato, J.R., Muthukumaraswamy, S.D., Yonamine, M., Waguespack, M., Lomnicka, I., Barker, S. A., da Silveira, D.X., 2015. Acute biphasic effects of ayahuasca. PLoS One 10 (9), e0137202.
Todd, A.J., Spike, R.C., Russell, G., Johnston, H.M., 1992. Immunohistochemical evidence that Met-enkephalin and GABA coexist in some neurones in rat dorsal horn. Brain Res. 584 (1–2), 149–156.
Tort, S., Urrútia, G., Nishishinya, M.B., Walitt, B., 2012. Monoamine oxidase inhibitors (MAOIs) for fibromyalgia syndrome. Cochrane Database Syst. Rev. (4), Cd009807
Urzedo-Rodrigues, L.S., Ferreira, H.S., Santana, R.C., Luz, C.P., Perrone, C.F., Fregoneze, J.B., 2014. Blockade of 5-Ht3 receptors in the septal area increases Fos expression in selected brain areas. Auton. Neurosci. 181, 55–68.
Van Arman, C.G., Begany, A.J., Miller, L.M., Pless, H.H., 1965. Some details of the inflammations caused by yeast and carrageenin (with appendix on kinetics of the reaction). J. Pharmacol. Exp. Therapeut. 150 (2), 328–334.
Vieceli, P.S., Juiz, P.J.L., Lauria, P.S.S., Couto, R.D., Tomassini, T.C.B., Ribeiro, I.M., Soares, M.B.P., Villarreal, C.F., 2021. Physalis angulata reduces the progression of chronic experimental periodontitis by immunomodulatory mechanisms. J. Ethnopharmacol. 273, 113986.
Villarinho, J.G., Pinheiro, K.d.V., Pinheiro, F.d.V., Oliveira, S.M., Machado, P., Martins, M.A.P., Bonacorso, H.G., Zanatta, N., Fachinetto, R., Ferreira, J., 2013. The antinociceptive effect of reversible monoamine oxidase-A inhibitors in a mouse neuropathic pain model. Prog. Neuro Psychopharmacol. Biol. Psychiatr. 44, 136–142.
Volpi-Abadie, J., Kaye, A.M., Kaye, A.D., 2013. Serotonin syndrome. Ochsner J. 13 (4), 533–540.
Witschi, R., Punnakkal, P., Paul, J., Walczak, J.S., Cervero, F., Fritschy, J.M., Kuner, R., Keist, R., Rudolph, U., Zeilhofer, H.U., 2011. Presynaptic alpha2-GABAA receptors in primary afferent depolarization and spinal pain control. J. Neurosci. : the official journal of the Society for Neuroscience 31 (22), 8134–8142.