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Ibogaine induces juvenile-like plasticity and modulates functional and structural regulators of plasticity in the adult mouse visual cortex

Alejo Acuña¹, Federico Billeri², Valentino Totaro^{2*}, Ignacio Carrera³, Juan M. Mesa³, Tommaso Pizzorusso^{2,4} and Francesco M. Rossi^{1*}

Abstract

Background Psychedelics have emerged as powerful modulators of neural plasticity, yet whether the atypical psychedelic ibogaine can enhance plasticity remains poorly understood. Here, we investigated whether a single administration of ibogaine can reinstate juvenile-like experience-dependent plasticity in the adult mouse visual cortex, a canonical model for studying neuroplasticity.

Results Adult mice were treated with ibogaine (40 mg/kg, i.p.) or vehicle, and 24 h later subjected to 4 days of monocular deprivation (MD). Behavioral visual acuity was quantified using the optomotor response test, and structural plasticity was assessed through dendritic spine density analysis following Golgi staining. Ibogaine alone did not alter visual acuity or dendritic spine density in non-deprived adults. However, when coupled with MD, ibogaine restored youthful levels of plasticity: MD significantly reduced visual acuity in the deprived eye and decreased dendritic spine density in the binocular visual cortex of ibogaine-treated, but not vehicle-treated, adult mice. To examine mechanistic correlates of these findings, we quantified perineuronal nets (PNNs), parvalbumin-positive interneurons (PVs), and inhibitory synaptic puncta labeled with the vesicular GABA transporter (vGAT). We found that ibogaine reduced PNN and PV staining intensity and density, decreased the proportion of PVs enwrapped by PNNs, and lowered vGAT-positive puncta density.

Conclusions These results show that ibogaine re-establishes experience-dependent plasticity in the adult visual cortex and that this effect is accompanied by reductions in structural and inhibitory “brakes” on plasticity. Our findings suggest that ibogaine’s long-lasting therapeutic actions can arise, at least partially, from its ability to re-open windows of heightened cortical adaptability.

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Keywords Psychedelics, Ibogaine, Visual cortex, Experience-dependent plasticity, Monocular deprivation, Behavioral visual acuity, Dendritic spine density, Perineuronal nets, Parvalbuminergic neurons, Vesicular GABA transporter

Background

Psychedelics are a large family of substances capable of profoundly altering perception, cognition and mood [1]. Research on these substances has markedly accelerated over the past fifteen years, greatly due to accumulating clinical evidence showing their potential for the treatment of a wide range of mental disorders [1]. For example, clinical trials have shown that psychedelics such as psilocybin and ketamine are beneficial in the treatment of Major Depressive Disorder (MDD), while 3,4-methylenedioxymethamphetamine (MDMA) is effective in the treatment of Post-Traumatic Stress Disorder (PTSD) [2–5].

Ibogaine is the main indole alkaloid derived from the root bark of the shrub *Tabernanthe iboga* and related plants of the Apocynaceae family [6, 7]. Traditionally, it is used in West-African religious, spiritual and healing ceremonies due to its psychedelic effects [6, 7]. In western culture, it initially gained attention after anecdotal reports claiming that its consumption had led people with substance use disorders (SUDs) to suddenly stop abusing substances [6, 7]. Following these reports a great deal of pre-clinical studies investigated into ibogaine's putative anti-addictive properties, finding consistent effects on rodent models of substance abuse [8]. Currently, a few observational studies and open label trials have found promising results in ibogaine for treating substance abuse [9–12] see [13] for a review). Furthermore, other studies have found it can reduce chronic disability related to Traumatic Brain Injury (TBI), as well as associated symptoms of post-traumatic stress disorder (PTSD), depression, anxiety and altered cognitive functioning [14–16]. Nevertheless, ibogaine administration may induce cardiac arrhythmias due to blockade of the cardiac hERG potassium channel [17].

While most well-known psychedelics, such as lysergic acid diethylamide (LSD) and psilocin, are pharmacologically characterized as potent partial agonists of the 5HT_{2A} serotonin receptor [18], ibogaine's affinity to this receptor is low or negligible [19, 20], affecting other molecular targets which has led to its classification as a non-classical (atypical) psychedelic [19, 20]. The subjective experience after ibogaine ingestion has also been reported to differ from that of classic (or serotonergic) psychedelics [21, 22]. Users often describe it as a “waking dream”-like state; accordingly, a recent study on rats shows that ibogaine can induce a wakeful state with electrophysiological features resembling those of REM sleep [23].

The pharmacology of ibogaine, and its active metabolite noribogaine is complex, modulating with low potency interactions several types of molecular targets, including ion channels (blockade of NMDA receptors) [24], monoamine transporters (mainly inhibition of SERT and VMAT2) [25], G-protein coupled receptors (κ and μ opioid receptors among others) [26] and kinase signaling pathway potentiation [27]. The term “matrix pharmacology” has recently been introduced to describe ibogaine's ability to modulate multiple molecular targets and signaling pathways through modest-to-weak interactions [25]. This contrasts with “polypharmacology,” which typically denotes potent agonism or antagonism at multiple targets [25].

Despite these differences, both ibogaine and classic psychedelics have been linked to enhanced neuroplasticity, i.e. the brain's capacity to modify neural circuits. Ibogaine has been shown to increase the expression of brain derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) in both cortical and subcortical regions [28, 29], reinstate social reward learning in adulthood [30], and promote the expression of plasticity related genes [31]. Furthermore, both ibogaine and its main metabolite, noribogaine, can increase dendritic spine density in cortical neurons in vitro [32, 33].

Here, we investigated the effect of ibogaine on the mouse visual cortex, a model that has been used for decades to study the mechanisms regulating experience-dependent plasticity, and has allowed the discovery of several treatments that can enhance cortical plasticity in adult animals. Interestingly, several of these treatments are also known to have neurotherapeutic effects [34–38]. Furthermore, critical period plasticity of the visual cortex has been recently proposed as a useful framework for studying the effects of psychedelics on neural plasticity [39].

Specifically, we focused on studying in adults two well reported plastic phenomena that are present only in juvenile mice during a period of heightened plasticity in the visual cortex (critical period), but that are absent during adulthood: (i) a reduction of dendritic spine density in the binocular visual cortex after monocular deprivation (MD) of the contralateral eye [40–43]; but see also [44, 45]; (ii) a reduction in visual acuity of the deprived eye following MD [43, 46]. We found that, in both cases, adult mice treated with ibogaine show changes in response to MD that are characteristic of juvenile mice.

We then sought to investigate mechanistic correlates that might be enabling the plastic changes observed in

ibogaine-treated adult mice. Global inhibition levels of a brain region, measured through different physiological and molecular techniques, are low during critical periods, and then increase during adulthood [47]. Pharmacologically increasing inhibition during the critical period, results in a premature ending [48], while diminishing inhibition during adulthood, reopens juvenile-like plasticity [49]. Thus, considering the role of inhibition as a brake for ocular dominance plasticity appearing on the end of critical periods, we studied perineuronal nets (PNNs) and parvalbuminergic neurons (PVs), which are largely colocalized [50], as well as the vesicular GABA transporter (vGAT) as an inhibitory synaptic marker [51]. Importantly, it has been shown that PNNs finish maturing at the end of the critical period, and that their pharmacological removal in adulthood causes juvenile-like plasticity to be restored in the visual cortex [52], as well as in other brain regions [53]. PV inhibitory neurons have been implicated in the regulation of critical periods and are considered of central importance in the regulation of brain plasticity [54–56].

Our results show that ibogaine administration reduces PNN and PV presence in the adult mouse visual cortex, with pronounced effects in layers IV and V, and also reduces vGAT levels.

Methods

Animals

All experiments were approved by the Uruguayan Research Ethics Committees (protocol approval #1621, ExpFCien 240012-000026-22). Uruguayan legislation governing animal experimentation is in compliance with Directive 2010/63/EU of the European Union. A total of 42 C57BL6/J mice (both sexes) at postnatal (P) day 60 (P60) provided by the Unidad de Reactivos y Biomodelos de Experimentación, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay, were used. Mice were bred under specific pathogen-free conditions, housed (6 per cage) in ventilated cages with positive pressure (20 ± 1 °C, relative humidity 40–60%, 14/10 h light-dark cycle), fed with standard mouse diet *ad libitum*, and had free access to water.

Ibogaine treatment

Ibogaine-HCl (IBO) was prepared in the Laboratorio de Síntesis Orgánica (Departamento de Química Orgánica, Facultad de Química) by decarboxylation of voacangine isolated from *Voacanga africana* root bark according to previously published method [57] (see Suppl. material and Suppl. Table 1 for details). For administration to animals, ibogaine-HCl was dissolved at 4 mg/ml in warm saline/EtOH (90:10), and administered intraperitoneally (i.p.) at a dose of 40 mg/kg. Control animals were administered with vehicle (VEH; EtOH 10%). This dose was

selected based on previous studies demonstrating that it increases the expression of neurotrophic factors, reduces depression-like behavior, and exhibits anti-addictive effects, whereas lower doses produce lower or no significant effects [28, 29, 58].

Monocular deprivation

Monocular deprivation was performed 24 h after ibogaine administration, at P61, under ketamine-xylazine-anesthetized, 100–10–3 mg/Kg anesthesia. This time point was chosen based on previous reports on drugs inducing experience-dependent plasticity in the adult visual cortex [34] and to study its effects after clearing of ibogaine and its metabolite off the body [58]. The eyelids of the right eye were sutured close as previously described [43], and kept this way for four days. The integrity of the suture was checked daily and mice were used only if the eyelids remained closed throughout the duration of the deprivation period. The eyelids were reopened the day before performing the optomotor test. The eye was examined under a stereomicroscope, and mice with scarring of the cornea, opacity of the eye or eye abnormalities were discarded from the study. Pupil dilation was tested with a light flash.

Optomotor response test

Behavioral visual acuity was assessed using the Optomotor Response (OMR) test [43, 59, 60]. Mice were put in an arena that consisted of four monitors arranged in a quadrangle (square, 35 cm side length), and two mirrors that were placed on top (roof) and on the bottom (floor) of the arena. The roof mirror had a central 5 cm diameter hole, where a camera was positioned to record the animal's behavior. This camera could be easily moved or removed to optimize positioning before the start of each recording session. A circular platform (height 13 cm, diameter 5 cm, distance from monitors 17.5 cm) was secured on the center of the bottom mirror, where the mouse was placed unrestrained. Stimuli were presented on the four monitors using the PsychoPy® software (PsychoPy v3.0) [61]. The stimulus consisted of vertical sine-wave gratings drifting either clockwise or counterclockwise, with spatial frequency varying from trial to trial. Each trial presented gratings of a specific frequency moving clockwise for 30 s, followed by 30 s of counterclockwise motion, at a speed of 12°/s and 100% contrast. To maintain the animal's attention and facilitate faster testing, brief, salient luminance changes and light taps on the monitors were occasionally introduced during testing to prompt the animal to look toward the monitors. The spatial frequencies tested were: 0.1, 0.2, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, and 0.8 cycles/degree (c/d). The 0.1 c/d frequency was always presented first, as it reliably evokes a response and therefore can be used as a control to confirm that

the mouse is properly positioned and able to respond to the stimulus [43, 59, 60]. The remaining frequencies were presented in random order to avoid potential confounding effects of time or fatigue on responses. Experiments were conducted under scarce light conditions (~ 12 cd/m²). All animals were habituated prior to testing through gentle handling and by being placed on the arena platform for 30 s to 1 min before stimulus presentation began. Videos were analyzed by quantifying head movements and comparing them to the direction of the stimulus. A head movement was counted as a response only if it matched both the direction and speed of the grating. Very short head movements and repositioning of the whole body were not counted as responses. Visual acuity was determined as the highest spatial frequency of the stimulus at which the mouse gave a response. After all mice had performed the optomotor test, they were sacrificed (by cervical dislocation) and their brains collected for Golgi staining.

Golgi staining

After removal, brains were rinsed in physiological solution and then Golgi-stained using the *élite* Golgi Kit (#006690, Bioenno, Tech, LLC), as previously described [43]. Briefly, each brain was immersed in 5 ml of an impregnation solution (provided with the kit), and stored at room temperature (RT) in darkness. The day after, the impregnation solution was renewed and the brains were kept inside it for 5 extra days at RT and darkness. After this, brains were cut with a vibratome (LeicaGeo-Systems VT1000S) in 150 μ m thick coronal slices, and the left visual cortex was collected in phosphate buffer (PB) 0.1 M. Sections were incubated free-floating in the staining and clarity solutions (also provided with the kit), rinsed in phosphate buffer saline 0.01 M containing 0.3% Triton X-100 (PBST), directly mounted on gelatin-coated slides, and left to dry overnight. The following day slices were dehydrated in 95% and 100% EtOH subsequently, and then cleaned with xylene. Finally, slides were covered with coverslips using mounting media (Entellan, Sigma-Aldrich).

Spine density analysis

Photographs of the images were obtained using a D70S Digital Camera (Nikon) attached to a Microphot-FXA microscope (Research Microscope, Nikon) at 40x. In order to be used for quantification, slices had to have strongly stained neurons. Spine density quantification was performed on apical dendrites of pyramidal neurons in layers II-III and V of the binocular visual cortex (bVC). bVC position was determined by comparing sections at 4x, with images from the Paxinos and Franklin (2007) [62] mouse brain atlas (AP: between -4.36 and -3.08 ; ML: between $+3.25$ and $+2.5$). Nissl-stained alternating

sections were used to identify cortical layers. Only neurons showing typical pyramidal neuron morphology were analyzed [63, 64]. In order to be quantified, dendrites had to meet the following criteria: (i) to be viewed clearly, without significant breaks or interrupted staining; (ii) not to be interrupted by other stained neurons; (iii) the soma had to be clearly identifiable and completely stained. Once dendrites were selected, the number of protrusions along the entire dendrite was counted. On average, 5 neurons/animal were analyzed (from an average of 3 sections/animal). Image visualization and analysis was carried out with the free software Fiji (Fiji Is Just Image), NIH).

Immunofluorescence staining

The immunofluorescence staining was performed on non-deprived adult mice treated with ibogaine or vehicle. Mice were deeply anesthetized with ketamine-xylazine-acedan, 100-10-3 mg/Kg anesthesia and perfused via intracardiac infusion with cold phosphate buffer saline (PBS) and then 4% paraformaldehyde (PFA, w/vol, in PBS). Brains were extracted and post-fixed overnight in PFA 4% in PBS at 4 °C, then transferred to 30% (w/vol) sucrose in PBS for 48 h at 4 °C. For each brain, 50 μ m coronal sections spanning the whole visual cortex were collected free-floating in PBS, using a freezing microtome (Leica).

WFA and PV immunostaining

Sections were blocked at RT for 2 h in a solution containing 3% BSA (Sigma-Aldrich, A7906) in PBS, and then incubated o/n at 4 °C with a solution containing biotinylated *Wisteria Floribunda* Lectin (WFA; Vector Laboratories, B-1355-2) 1:200 in blocking solution. Following PBS washes, sections were incubated in blocking solution containing 1:400 Streptavidin, Alexa Fluor™ 555 conjugate (Invitrogen, S21381), at RT for 2 h, and rinsed in PBS. Then slices were blocked in 10% BSA, 0.3% Triton X-100, PBS, for 1 h, washed in PBS and incubated o/n at 4 °C with 1:400 mouse anti-parvalbumin antibody (Sigma Aldrich, P3088), 1% BSA, 0.1% Triton X-100, in PBS. Then, sections were rinsed in PBS, incubated with 1:500 Goat anti-Mouse Alexa Fluor™ 488 (Invitrogen, A32723), 1% BSA, Triton X-100 0.1%, PBS, at RT for 2 h, and washed again in PBS. Finally, sections were mounted on microscopy slides with VECTASHIELD® antifade mounting medium (Vector Laboratories, H-100), and stored at 4 °C.

vGAT immunostaining

Slices were blocked at RT for 1 h in a solution containing 5% BSA, 0.3% Triton X-100, in PBS. Then, slices were incubated o/n at 4 °C with a solution containing rabbit polyclonal anti-vesicular GABA transporter (vGAT;

Synaptic Systems, 131003), 1:1000 in blocking solution. On the following day, sections were rinsed in PBS at RT and incubated with 1:400 Goat anti-Mouse Alexa Fluor™ 488 (Invitrogen, A32723) in blocking solution. Finally, sections were mounted on microscopy slides with VECTASHIELD® antifade mounting medium (Vector Laboratories, H-100), and stored at 4 °C.

Image acquisition and quantification

Images were acquired using a Zeiss ApoTome.2 microscope and digitized by an AxioCam MR R3 12-bit camera. The acquisition software ZEN blue (RRID: SCR_013672) was used to control image acquisition.

WFA and PV

Sections stained with WFA and the anti-PV antibodies were imaged with a 10x objective. For the WFA channel, excitation light passed through a 538–562 nm bandpass filter and a 570 nm dichroic mirror, while emitted light was filtered with a 570–640 nm bandpass filter. For the PV channel, excitation light passed through a 450–490 nm bandpass filter and a 495 nm dichroic mirror, while emitted light passed through 500–550 nm bandpass. Exposure time was 225 ms for the WFA channel, and 1600 ms for the PV channel, while excitation light intensity was the same for all images. Each brain slice was obtained as a multi-image experiment on a single z-plane selected at the depth of maximal staining intensity. A tilted z-plane was acquired in order to reduce artifacts in image intensity that may arise if the tissue section is not sitting exactly perpendicular to the optical path. The angle of the z-plane was determined by linearly interpolating between 2 and 4 manually selected focus points. For all sections, 3 apotome images were acquired for optical sectioning. After the acquisition, multi-image tiles were stitched in ZEN and exported as 8-bit TIFF files for further processing. On average, 8 sections/animal were analyzed. X and Y coordinates of WFA staining and PV-immunolabeling were detected using a convolutional neural network (CNN) developed by [50], that was trained to detect PNNs and PV cells. After this, a binary mask delineating the visual cortex was manually drawn using the free software Fiji (Fiji Is Just ImageJ, NIH), and the number of PNNs and PVs falling within this mask was counted.

To analyze intensity, small image crops (50×50 pixels for PNNs, 25×25 for PVs) centered on the coordinates where the CNN detected PNN or PVs were obtained. Pixels belonging to the PNN or PV of each crop were detected using Otsu's binarization method. Intensity was defined as the average of the intensity values of all the pixels belonging to a PNN or PV.

Finally, “energy”, a metric that integrates both density and staining intensity of cells, was also estimated for both

structures (50). Energy was calculated by summing the intensity of each PNN or PV in a cortical layer, and then dividing by the area of the layer. In this way energy is a metric that weighs the contribution of each structure to density, based on intensity.

We also assessed the colocalization of PNNs and PVs based on their (X, Y) positions in the original image. A PNN and a PV were considered co-localized if the distance between them was less than or equal to 15 pixels (9.675 µm). If multiple PVs or PNNs met this criterion for a given reference object, the closest one was selected as the co-localized counterpart. If no PV or PNN was within this distance, the reference object was classified as uncolocalized. Finally, we calculated the percentage of PV cells that were surrounded by a PNN (PNN+/PV cells, calculated as the ratio between colocalized PVs and total PVs), and the percentage of PNNs surrounding a PV cell (PV+/PNNs, calculated as the ratio between colocalized PNNs and total PNNs).

PV+ cells were classified into different intensity staining classes. This analysis was exploratory, and based on previous studies showing that PV neurons can be grouped in four classes based on their PV staining intensity (low PV, intermediate low PV, intermediate high PV, and high PV) [50, 54]. These classes, particularly the high PV and low PV, likely reflect distinct timing of neurogenesis and connectivity, and have been associated with varying levels of plasticity. To classify PV cells into discrete intensity groups, we used an approach based on Gaussian Mixture Modeling (GMM), as implemented in the mclust package in R. Specifically, the distribution of PV staining intensities from the vehicle-treated group was modeled as a mixture of four Gaussian components. Cutoff points between intensity classes were defined as the intersection points between adjacent components. The resulting cutoff values were then used to assign PV+ cells from both vehicle- and ibogaine-treated groups into its corresponding intensity class. Finally, for each mouse, the percentage of cells in each class relative to the total amount of cells was calculated.

vGAT

A 63x oil immersion objective was used to image sections stained with the anti-vGAT antibody. Filters were the same as for PV imaging. For each section, serial optical sections at 0.33 µm intervals were imaged for a total of at least 15 optical Sect. (5 µm). Maximum intensity projections (MIPs) were generated from the group of 5 consecutive sections yielding the higher mean pixel intensity. Four separate pictures were taken from each slice (2 in superficial layers and 2 in deep layers). After the acquisition, images were exported as 8-bit TIFF files for further processing. On average, 6 sections/animal were analyzed. To identify vGAT positive puncta, images were

first pre-processed. A 5-pixel mean filter was applied to each image, and the resulting output was multiplied by the original image to reduce background signal. To further enhance signal-to-noise ratio, a Gaussian filter with a sigma of 1.2 was applied to the new images, and the resulting blurred image was subtracted to it. Segmentation was then performed using the Otsu automatic thresholding method and puncta were detected and quantified using the Puncta Analyzer plug-in in the free software Fiji (Fiji Is Just ImageJ, NIH).

Statistical analysis

The researcher was blind to both drug and MD treatment during all image acquisition steps, and also during quantification. All data were analyzed using linear models, implemented in the lme4 package of RStudio [65, 66]. For visual acuity analysis, a Linear Mixed Model (LMM) was used, in which visual acuity was entered as the dependent variable. Drug treatment (ibogaine or vehicle), monocular deprivation (absence or presence), and eye (left or right) were introduced as fixed effects. Subject identity was included as a random intercept.

WFA and PV measures (energy, intensity and density), PNN/PV colocalization density, and vGAT-positive puncta density, were also modeled with LMM. For each model, object density (or intensity or energy) was introduced as the dependent variable, and drug treatment and layers as a fixed effect. In the case of PNN/PV colocalization density, layer was not included as a fixed effect, and instead object type (un-colocalized PNN, un-colocalized PV, colocalized structures) was included. Subject identity was included as a random intercept in all models.

Dendritic spine counts were modeled with a Generalized Linear Mixed Model (GLMM), of the Poisson family, with a logarithmic link function. Object count was introduced as the dependent variable, drug treatment and monocular deprivation as fixed effects, and neuron identity nested within subject identity was used as a random effect. The logarithm of dendritic segment length was included as an offset variable, to account for differences in counts due to varying segment lengths.

Finally, between groups differences in the percentage of PNN+/PV cells and PV+/PNNs were analyzed separately, using a binary GLMM. The dependent variable was a binary variable, indicating if the structure (PNN or PV) was colocalized with its counterpart (PV or PNN, respectively) or not. Treatment and layer, as well as their interaction, were entered as fixed effect. Subject identity was entered as a random effect.

Wald Chi square tests were used to evaluate the significance of main effects and interactions in all models (implemented in the car package of RStudio [67]). When post hoc tests were computed, Tukey's correction for

multiple comparisons was applied (using the emmeans package in RStudio).

The percentage of PV+ cells across intensity classes was modeled using a GLMM with a beta-binomial error distribution, implemented in the glmmTMB package of RStudio. Number of cells belonging to each intensity class relative to the total number of cells per subject was entered as the dependent variable, while drug treatment and intensity class, as well as their interaction, were included as fixed effects. Subject identity was included as a random intercept. We selected a beta-binomial distribution to account for over dispersion in the proportion data.

Results

In order to study whether ibogaine induces experience-dependent plasticity in the visual cortex of adult mice, we analyzed the effect of monocular deprivation on visual acuity, as a parameter of functional plasticity, and on dendritic spine density, as a parameter of structural plasticity.

Ibogaine restores functional plasticity in adult mice

First, we studied whether ibogaine by itself affects behavioral visual acuity (Fig. 1). Adult (P60) non-deprived mice were treated with vehicle or ibogaine (40 mg/Kg) and 6 days later visual acuity was measured for both eyes separately with the optomotor response test (OMR). Our results show that ibogaine did not affect visual acuity of either eye, when comparing against the vehicle-treated group ($\chi^2_1=0.004$, $p=0.948$). Additionally, visual acuity of the left (L) and right (R) eye was similar in animals treated with vehicle, as well as in animals treated with ibogaine (VEH L: 0.308 ± 0.015 (c/d) vs. VEH R: 0.325 ± 0.011 (c/d), $n=6$, $t_{26}=-0.190$, $p=0.851$; IBO L: 0.320 ± 0.015 (c/d) vs. IBO R: 0.316 ± 0.012 (c/d), $n=6$, $t_{26}=-0.379$, $p=0.707$).

Then, in order to study if ibogaine has an effect on experience-dependent plasticity, adult mice were treated with ibogaine or vehicle, and monocularly deprived for 4 days (right eye). Behavioral visual acuity was measured with the OMR test after reopening of the deprived eye (Fig. 2). Our results show that in ibogaine-treated mice, visual acuity of the right (R) deprived eye was reduced in comparison to the left (L) non-deprived eye (IBO L: 0.393 ± 0.016 (c/d) vs. IBO R: 0.256 ± 0.022 (c/d), $n=8$, $t_{26}=7.221$, $p<0.001$). On the contrary, visual acuity of R deprived and L non-deprived eye was similar in vehicle-treated mice (VEH L: 0.327 ± 0.012 (c/d) vs. VEH R: 0.340 ± 0.015 , $n=10$, $t_{26}=-0.734$, $p=0.469$).

Ibogaine restores structural plasticity in adult mice

To study if ibogaine facilitates structural plasticity, we analyzed dendritic spine density on apical dendrites from pyramidal neurons of layer II-III and V of the left

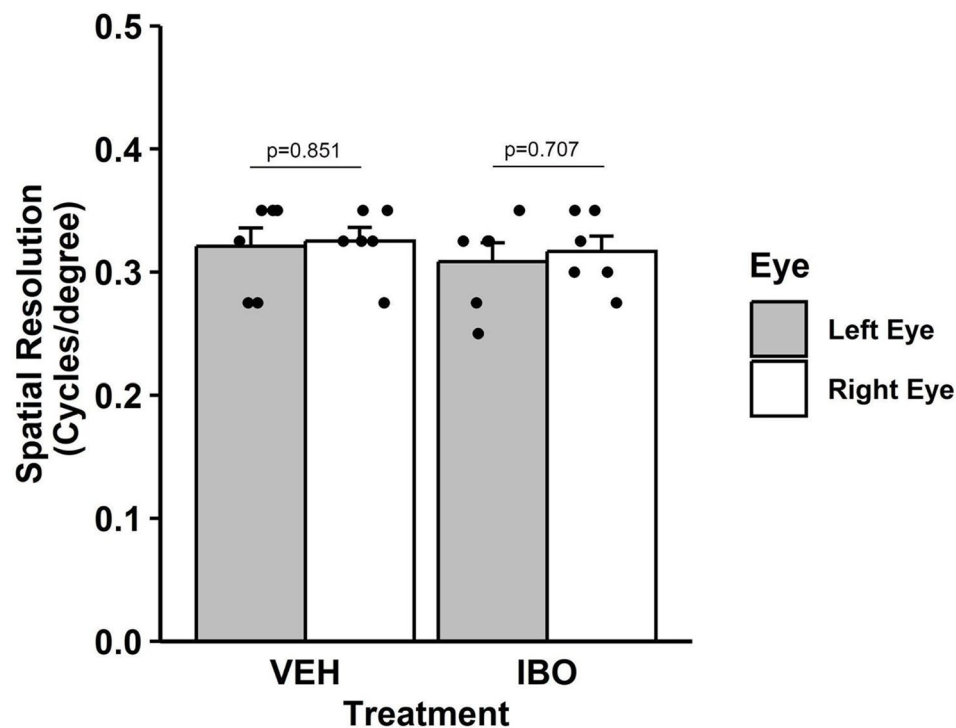


Fig. 1 Visual acuity is not affected by ibogaine treatment in adult mice. The graph shows the maximal spatial frequency (c/d) detected through the left (L, grey bars) and right (R, white bars) eye of vehicle- (VEH) and ibogaine- (IBO) treated adult mice. Data are expressed as mean spatial frequency ± SEM, and each black dot represents one animal. No difference was detected between treatments ($\chi^2_1=0.004$, $p=0.948$) nor between the left and right eye of vehicle-treated and of ibogaine-treated mice (VEH L: 0.308 ± 0.015 vs. VEH R: 0.325 ± 0.011 , $n=6$, $t_{26}=-0.190$, $p=0.851$; IBO L: 0.320 ± 0.015 vs. IBO R: 0.316 ± 0.012 , $n=6$, $t_{26}=-0.379$, $p=0.707$)

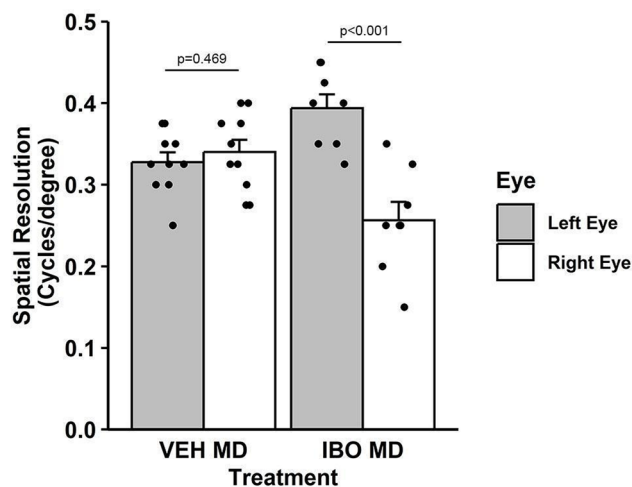


Fig. 2 Ibogaine induces functional plasticity in adult mice. The graph shows the maximal spatial frequency (c/d) detected through the left (L, grey bars) and right deprived (R, white bars) eye of vehicle- (VEH) and ibogaine- (IBO) treated adult mice. Data are expressed as mean spatial frequency ± SEM, and each black dot represents one animal. No difference was detected between left and right deprived eye in vehicle-treated adult mice (VEH L: 0.327 ± 0.012 vs. VEH R: 0.340 ± 0.015 , $n=10$, $t_{26}=-0.734$, $p=0.469$). On the contrary, in ibogaine-treated mice, visual acuity of the right MD eye was reduced in comparison to the left non-deprived eye (IBO L: 0.393 ± 0.016 vs. IBO R: 0.256 ± 0.022 , $n=8$, $t_{26}=7.221$, $p<0.001$)

(contralateral to the deprived eye in MD mice) binocular visual cortex (Fig. 3). Following the optomotor test, adult mice of all groups were sacrificed and tissues processed with the Golgi staining method. Our results show that dendritic spine density was similar between vehicle- and ibogaine-treated non-deprived adult animals (IBO: 0.825 ± 0.049 (spines/ μm), $n=19$ dendrites, $n=3$ mice, vs. VEH: 0.815 ± 0.023 (spines/ μm), $n=13$ dendrites, $n=3$ mice, $z=0.331$, $p=0.987$). However, following monocular deprivation, dendritic spine density was reduced in ibogaine-treated mice in comparison to vehicle-treated ones (IBO MD: 0.681 ± 0.061 (spines/ μm), $n=14$ dendrites, $n=4$ mice, vs. VEH MD: 0.827 ± 0.040 , $n=32$ dendrites, $n=6$ mice, $z=-2.743$, $p=0.006$).

Ibogaine reduces PNN/PV staining

Following treatment with vehicle or ibogaine, visual cortex sections of non-deprived adult mice were processed by double-immunohistochemistry with the *Wisteria Floribunda* Lectin, as a marker of PNNs, and with the anti-parvalbumin antibody as a marker of parvalbumin-positive neurons (PVs). We then calculated the “energy” of both structures, a single metric that integrates both density and staining intensity of cells. This metric, analogous to the one used by the Allen Institute gene

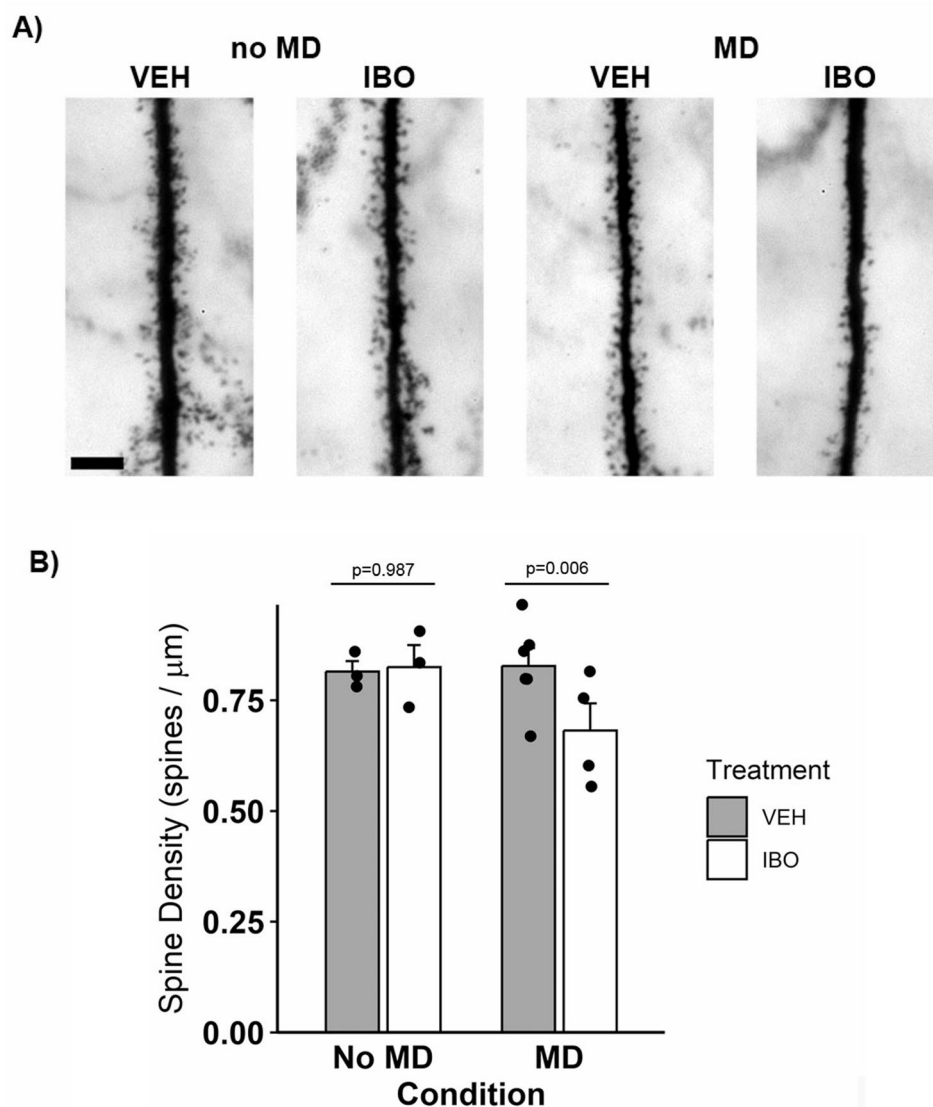


Fig. 3 Ibogaine induces structural plasticity in adult mice. **A)** Representative images of apical dendrites of the left (contralateral) visual cortex of non-deprived (no MD) and deprived (MD) adult mice treated with vehicle (VEH) or ibogaine (IBO). Bar: 5 μm . **B)** The graph shows the dendritic spine density (spines/ μm) of the left (contralateral) visual cortex of non-deprived (no MD) and deprived (MD) adult mice treated with vehicle (VEH) or ibogaine (IBO). Data are expressed as mean dendritic spine density $\pm$ SEM, and each black dot represents one animal. No difference was detected between vehicle- and ibogaine-treated non-deprived mice (IBO: 0.825 ± 0.049 , $n = 13$ dendrites, $n = 3$, vs. VEH: 0.815 ± 0.023 , $n = 19$ dendrites, $n = 3$, vs. $z = 0.331$, $p = 0.987$). On the contrary, MD reduced dendritic spine density in ibogaine-treated vs. vehicle-treated adult mice (IBO MD: 0.681 ± 0.061 , $n = 14$ dendrites, $n = 4$, vs. VEH MD: 0.827 ± 0.040 , $n = 32$ dendrites, $n = 6$, $z = -2.743$, $p = 0.006$).

expression dataset, has been used previously to analyze PNN and PV distribution [50]. Our results show that PNN energy was diminished in the visual cortex of adult mice treated with ibogaine (Fig. 4), compared to mice treated with vehicle, although between group differences were marginally significant (IBO: 3220.410 ± 3.902 (a.u.), $n = 6$, vs. VEH: 5119.520 ± 960.337 (a.u.), $n = 6$, $\chi^2_1 = -3.599$, $p = 0.057$).

In order to analyze whether the decrease in PNN staining observed in ibogaine-treated adult mice was selective of specific cortical layers, a layer analysis was done

(Table 1). Results showed that PNN energy was mainly reduced in layer IV in ibogaine- vs. vehicle-treated mice (treatment by layer interaction, $\chi^2_4 = -45.828$). Since energy metrics are calculated taking into consideration density and intensity, we also analyzed these parameters separately in the whole visual cortex and through the different cortical layers. Results obtained with this analysis paralleled those obtained analyzing the energy parameter (Suppl. Figure 4, Suppl. Tables 2 and 3).

Similarly, we found that parvalbumin energy (Fig. 5) was reduced in the visual cortex of ibogaine-treated

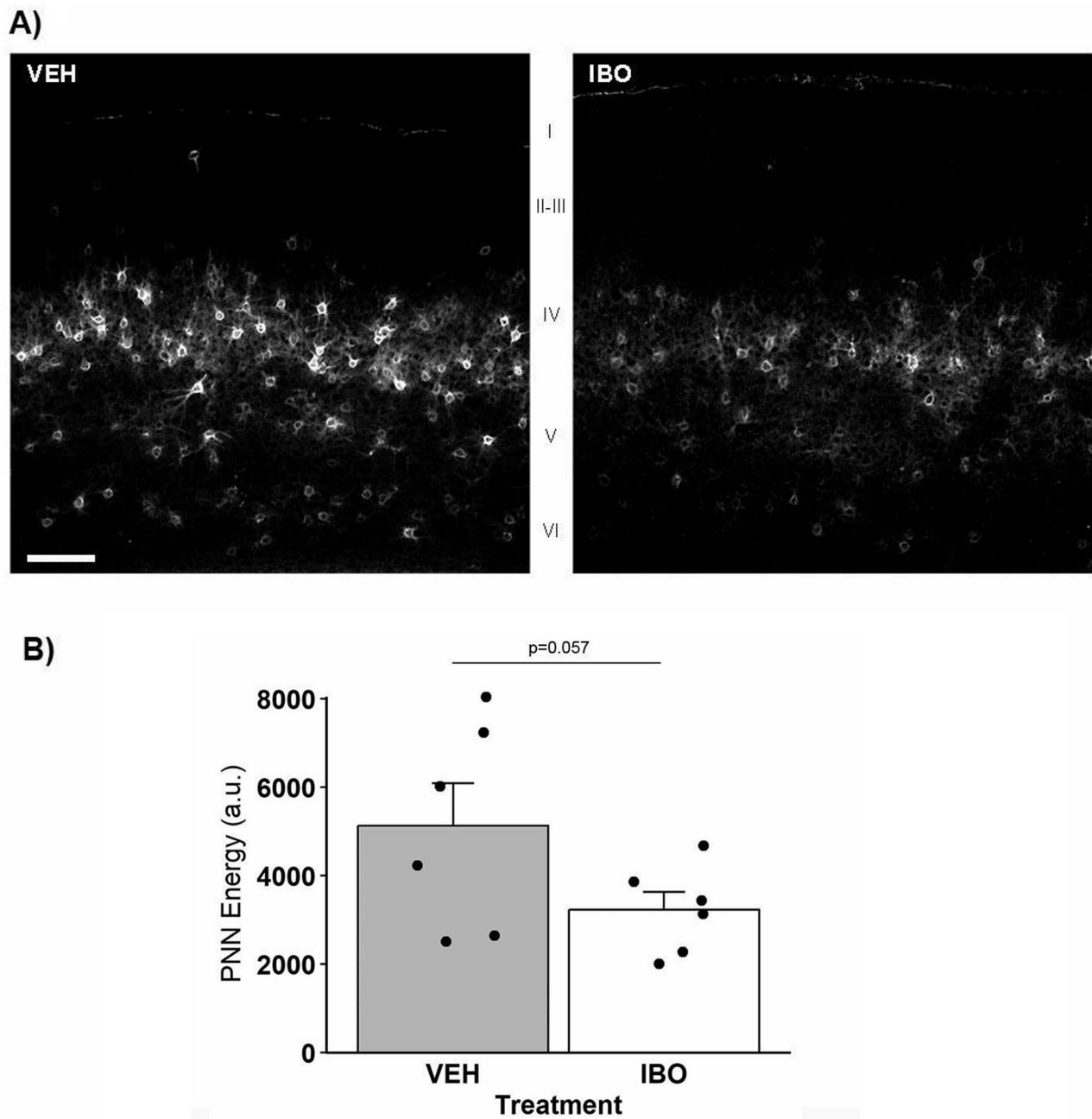


Fig. 4 Ibogaine induces a reduction of PNN energy in the visual cortex of adult mice. **A)** Representative images of WFA-stained 50 μ m sections of the visual cortex of adult mice treated with vehicle (VEH) or ibogaine (IBO). Bar = 150 μ m. Cortical layers are indicated. **B)** The graph shows the PNN energy (a.u.) of the visual cortex of VEH (grey bar) and IBO (white bar) adult mice. Data are expressed as mean PNN energy $\pm$ SEM, and each black dot represents one animal. Ibogaine induces a decrease of PNN energy in ibogaine-treated vs. vehicle-treated adult mice (IBO: 3220.410 $\pm$ 3.902 (a.u.), $n=6$, vs. VEH: 5119.520 $\pm$ 960.337 (a.u.), $n=6$, $\chi^2_1=3.599$, $p=0.057$)

adult mice as compared to vehicle-treated mice (VEH: 11437.911 $\pm$ 1882.036 (a.u.), $n=6$, vs. IBO: 5222.456 $\pm$ 915.487 (a.u.), $n=6$, $\chi^2_1=9.087$, $p=0.002$).

A layer specific analysis of PV energy indicated a treatment by layer interaction effect ($\chi^2_4=38.231$, $p<0.001$). Between group comparisons showed PV staining was significantly reduced in ibogaine-treated mice in L2/3, L4

and L5 and (Table 2). Similarly to what we observed in PNNs, density and intensity results for PVs were similar to those obtained for PV energy (Suppl. Figure 5, Suppl. Tables 4 and 5).

Previous studies have suggested that PV+ neurons can be classified into four distinct subclasses based on their PV staining levels (low PV, intermediate low PV,

Table 1 Comparison of PNN energy across layers in the visual cortex of ibogaine- and vehicle-treated adult mice

L	VEH	IBO	P
I	310.057 ± 73.691	193.761 ± 28.598	0.205
II-III	1793.918 ± 388.655	1065.854 ± 163.678	0.495
IV	14073.726 ± 2272.501	9243.356 ± 1066.732	0.001
V	5459.219 ± 1057.806	3587.312 ± 467.124	0.105
VI	1655.734 ± 295.329	1036.886 ± 115.457	0.587

Table 1. PNN energy is reduced in layer IV of ibogaine-treated mice. The table reports the values corresponding to PNN energy (mean ± SEM) in the different layers (L) of the visual cortex of ibogaine- (IBO) and vehicle- (VEH) treated adult mice. P indicates the p values obtained comparing PNN energy of IBO and VEH mice between the same cortical layer

Table 2 Comparison of PV energy across layers in the visual cortex of ibogaine- and vehicle-treated adult mice

L	VEH	IBO	P
I	995.501 ± 221.511	671.052 ± 148.051	0.727
II-III	9295.963 ± 1810.578	7952.504 ± 1533.515	0.042
IV	19074.523 ± 3314.563	9295.963 ± 1810.578	0.001
V	15366.794 ± 2688.356	7506.193 ± 1235.996	0.004
VI	5749.268 ± 936.381	2690.310 ± 485.116	0.146

Table 2. PV energy is reduced in cortical layers of ibogaine-treated mice. The table reports the values corresponding to PV energy (mean ± SEM) in the different layers (L) of the visual cortex of ibogaine- (IBO) and vehicle- (VEH) treated adult mice. P indicates the p values obtained comparing PV energy of IBO and VEH mice between the same cortical layer

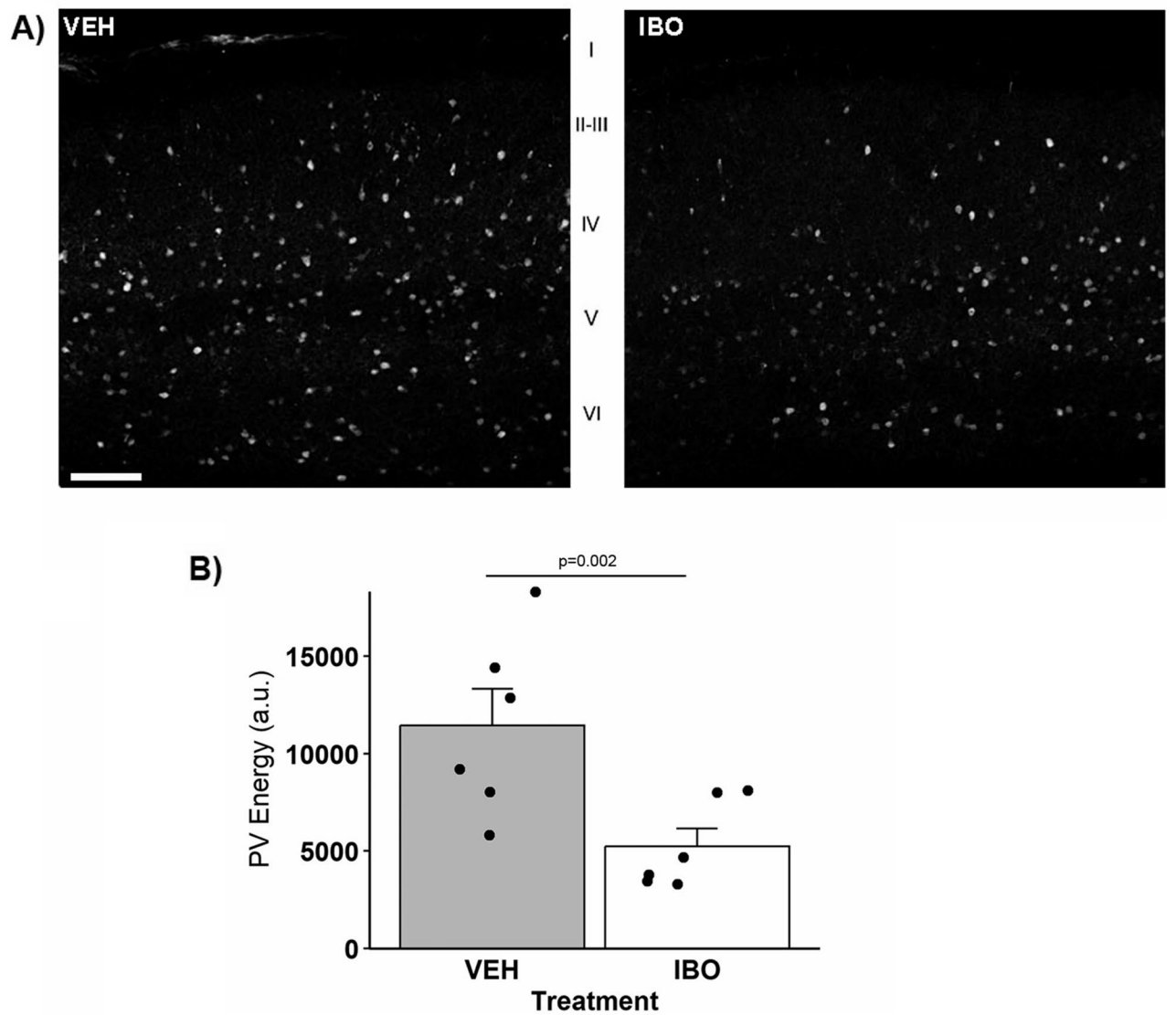


Fig. 5 Ibogaine induces a reduction of PV energy in the visual cortex of adult mice. **A)** Representative images of PV-stained 50 µm sections of the visual cortex of adult mice treated with vehicle (VEH) or ibogaine (IBO). Bar = 150 µm. Cortical layers are indicated. **B)** The graph shows the PV energy (a.u.) of the visual cortex of VEH (grey bar) and IBO (white bar) adult mice. Data are expressed as mean PV energy ± SEM, and each black dot represents one animal. PV energy decreases in ibogaine-treated vs. vehicle-treated adult mice (VEH: 11437.911 ± 1882.036 (a.u.), n=6, vs. IBO: 5222.456 ± 915.487 (a.u.), n=6, $\chi^2_1=9.087, p=0.002$)

intermediate high PV, and high PV) [50, 54]. Importantly, the low PV class is associated with a network more permissive towards plasticity, while the high PV class is associated with more limited plasticity. As an exploratory analysis, we decided to study if there were between group differences on the percentage of cells belonging to each class. Therefore, we classified our PV+ cells into four different classes using Gaussian Mixture Modeling (GMM, see Methods section). Cutoff points between intensity classes were defined as the intersection points between adjacent components, resulting in the following classification: low PV class: intensity between 17.4 and 38.5; intermediate low PV class: intensity between 38.5 and 68.1; intermediate high PV class: intensity between 68.1 and 109; high PV class: intensity between 109 and 241. After classifying cells we calculated the percentage of cells in each class (relative to the total amount of classified cells) in both vehicle-treated and ibogaine-treated mice (Suppl. Figure 6). Our results show that the vehicle-treated group had a lower percentage of the low PV+ cell class compared to the ibogaine-treated group (low PV cell class: VEH: $14.209 \pm 6.149\%$, $n=6$, vs. IBO: $41.342 \pm 7.970\%$, $n=6$, $t = -4.687$, $p < 0.001$), as well as a higher percentage of the intermediate high class (intermediate high PV cell class: VEH: $44.001 \pm 2.604\%$, $n=6$, vs. IBO: $35.750 \pm 3.109\%$, $n=6$, $t = 2.079$, $p = 0.037$). There were no between group differences on the percentage of intermediate low PV cells (intermediate low PV cell class: VEH: $28.500 \pm 3.930\%$, $n=6$, vs. IBO: $14.703 \pm 3.121\%$, $n=6$, $t = 1.011$, $p = 0.312$), nor on the percentage of high PV cells (intermediate high PV cell class: VEH: $13.272 \pm 3.863\%$, $n=6$, vs. IBO: $6.252 \pm 3.125\%$, $n=6$, $t = 1.840$, $p = 0.065$).

To explore if ibogaine affected the relationship between PNNs and PV cells in the visual cortex, we calculated the percentage of PV cells that were surrounded by PNNs (PNN+/PV cells) and the percentage of PNNs surrounding PV cells (PV+/PNNs) (Fig. 6). We found that the percentage of both PNN+/PV cells and PV+/PNNs were reduced in ibogaine-treated mice (PNN+/PV cells: VEH: $31.178 \pm 2.198\%$, $n=6$, vs. IBO: $37.431 \pm 2.992\%$, $n=6$, $\chi^2_1 = 4.739$, $p = 0.029$; PV+/PNNs: VEH: $66.326 \pm 4.907\%$, $n=6$, vs. IBO: $47.665 \pm 4.723\%$, $n=6$, $\chi^2_1 = 7.146$, $p = 0.007$).

To further understand this result we looked at between-group differences in the density of colocalized and un-colocalized structures (Suppl. Figure 7). We found that ibogaine reduced both the density of colocalized structures and PV+ cells that were not colocalized with PNNs, but the density of un-colocalized PNNs remained the same (un-colocalized PNN density: VEH: 29.977 ± 6.260 (PNNs/mm²), $n=6$, vs. IBO: 35.538 ± 5.800 (PNNs/mm²), $n=6$, $t = 0.274$, $p = 0.786$; un-colocalized PV density: VEH: 97.568 ± 12.44 (PVs/mm²), $n=6$, vs. IBO: 68.661 ± 5.635 (PVs/mm²), $n=6$, $t = 3.286$, $p = 0.003$;

colocalized PNNs/PVs: VEH: 58.668 ± 5.201 (PNNs/PVs/mm²), $n=6$, vs. IBO: 32.972 ± 3.107 (PNNs/PVs/mm²), $n=6$, $t = 3.885$, $p < 0.001$). Overall, these results show that ibogaine by itself could set the ground for enhanced plasticity by reducing PNN and PV energy, as well as their colocalization.

Ibogaine reduces vGAT staining

Finally, we were interested in investigating if ibogaine affects the expression of the vesicular GABA transporter, vGAT (Fig. 7). We observed that the density of vGAT positive puncta was reduced in the visual cortex of non-deprived adult mice treated with ibogaine, compared to vehicle-treated animals (IBO: 0.552 ± 0.020 (puncta/ μm^2), $n=6$, vs. VEH: 0.657 ± 0.039 (puncta/ μm^2), $n=6$, $\chi^2_1 = 5.644$, $p = 0.017$).

Analysis of the mean size particle showed no difference between vehicle- and ibogaine-treated mice (VEH: 0.0624 ± 0.0010 μm^2 , $n=6$, vs. IBO: 0.0642 ± 0.0005 μm^2 , $n=6$, $\chi^2_1 = 0.432$, $p = 0.510$). Thus ibogaine also affects inhibitory neurons at the synaptic level, by reducing vGAT puncta density.

Discussion

Our results show that ibogaine can enhance plasticity in the adult mouse visual cortex. Specifically, we showed that a protocol of monocular deprivation, that usually does not induce changes in adult mice, reduces visual acuity of the deprived eye, as well as dendritic spine density in the binocular visual cortex contralateral to the deprived eye, only when coupled with ibogaine treatment. Furthermore, we found that ibogaine administration correlates with a reduction in perineuronal nets, parvalbumin-positive interneurons, and vesicular GABA transporter (vGAT), all factors that are known brakes of cortical plasticity.

To estimate visual acuity, we used the optomotor response (OMR) test [43, 59, 60]. It has been previously shown that the OMR is a useful tool to detect experience-dependent plastic changes in visual acuity: MD induces a decrease of visual acuity of the deprived eye of young mice during the critical period for ocular dominance plasticity, but not during adulthood [43, 46]. Here we showed that ibogaine treatment in adult mice restores sensitivity to MD, detected as a decrease in visual acuity of the deprived eye, indicating that ibogaine can restore juvenile-like plasticity levels.

Then, to study plasticity at a morphological level we analyzed dendritic spine density by using Golgi-like staining and counting all protrusions along dendrites of LII-III and V pyramidal neurons. Other works have shown that MD during the critical period reduces dendritic spine density in the contralateral visual cortex of the deprived eye (40–43; but see also 44,45). Similar to

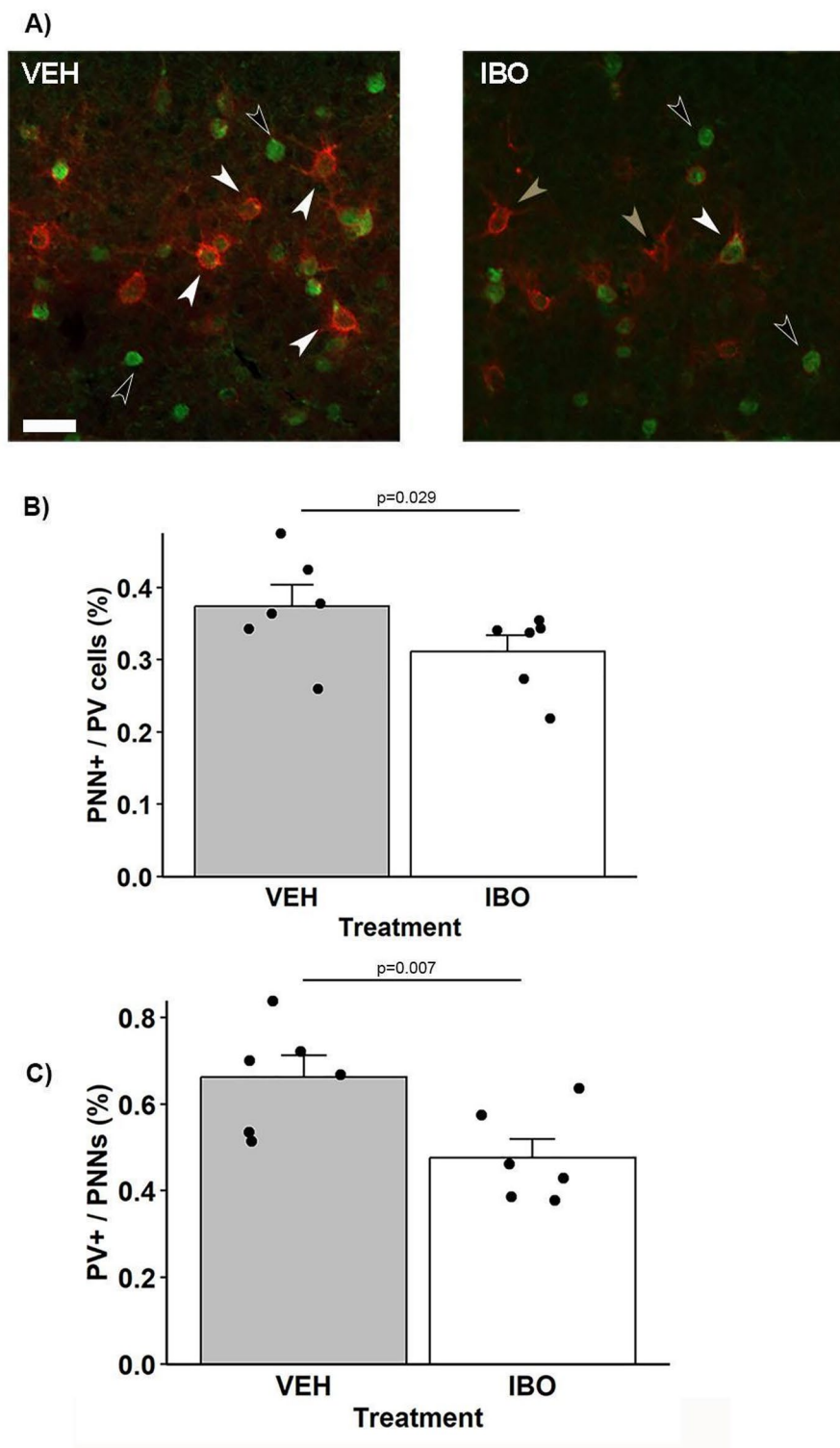


Fig. 6 Ibogaine reduces the percentage of PNNs/PVs colocalized structures in the visual cortex of adult mice. **A)** Representative images of PNN (red) / PV (green)-stained 50 μ m sections of the visual cortex of adult mice treated with vehicle (VEH) or ibogaine (IBO). Bar = 50 μ m. Arrows indicate PV+ only cells (black), PNN+ only structures (grey), and PV+/PNN+ structures (white). **B)** The graphs show the percentage of PVs surrounded by PNNs (PNN+/PVs cells, left) and the percentage of PNNs surrounding PV cells (PV+/PNNs, right) in the visual cortex of VEH (grey bar) and IBO (white bar) adult mice. Data are expressed as mean % of colocalization $\pm$ SEM, and each black dot represents one animal. PNN+/PV cells: VEH: 31.178 $\pm$ 2.198%, n = 6, vs. IBO: 37.431 $\pm$ 2.992%, n = 6, χ^2_1 = 4.739, p = 0.029; PV+/PNNs: VEH: 66.326 $\pm$ 4.907%, n = 6, vs. IBO: 47.665 $\pm$ 4.723%, n = 6, χ^2_1 = 7.146, p = 0.007

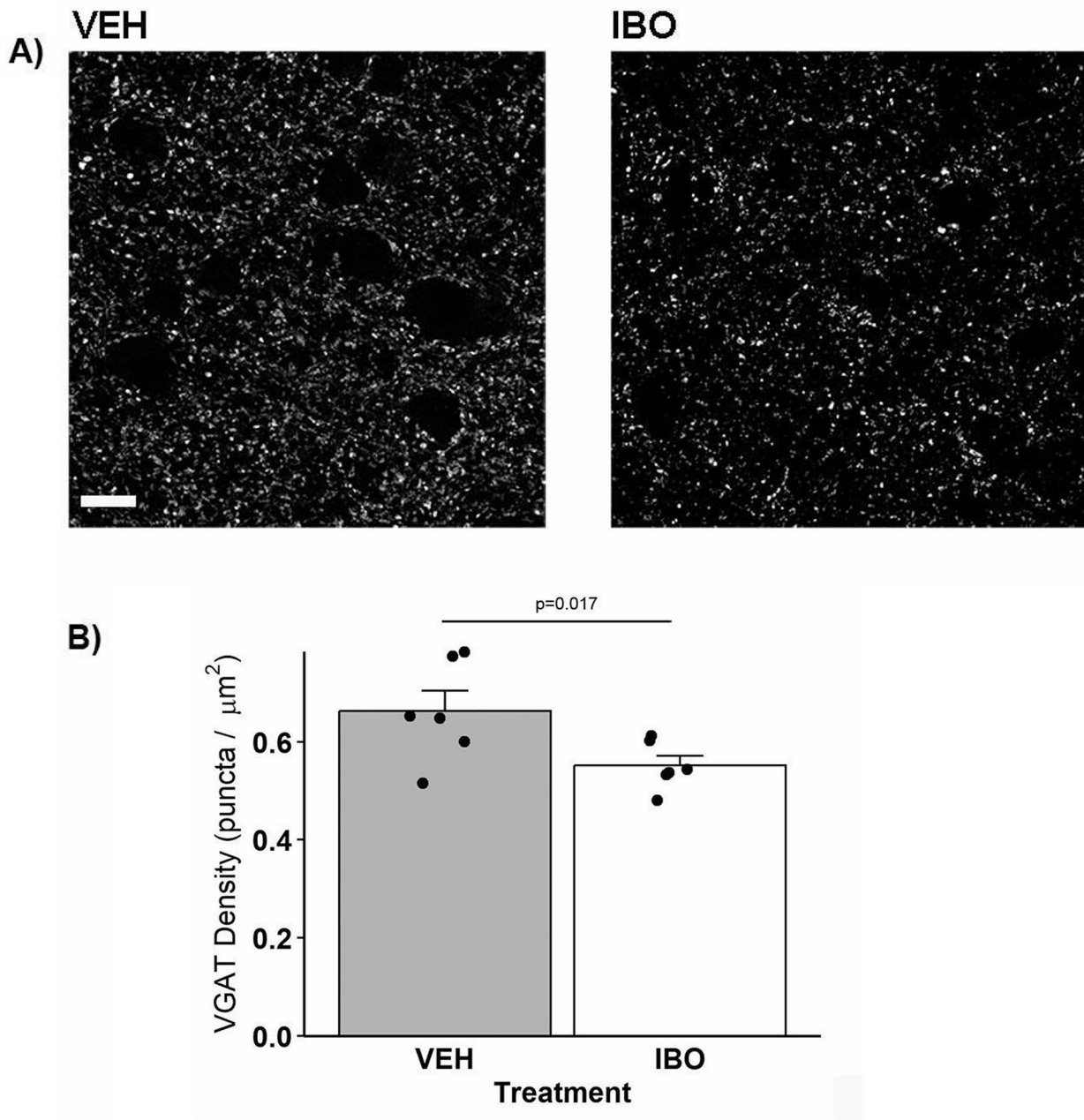


Fig. 7 Ibogaine reduces vGAT staining in the visual cortex of adult mice. **A)** Representative images of vGAT-stained 50 μm sections of the visual cortex of adult mice treated with vehicle (VEH) or ibogaine (IBO). Bar = 10 μm . **B)** The graph shows the density of vGAT-positive puncta (puncta/ μm^2) of the visual cortex of VEH (grey bar) and IBO (white bar) adult mice. Data are expressed as mean vGAT density $\pm$ SEM, and each black dot represents one animal. VEH: 0.657 ± 0.039 , $n=6$, vs. IBO: 0.552 ± 0.020 , $n=6$, $\chi^2_1=5.644$, $p=0.017$

visual acuity, we found that changes in ibogaine-treated MD mice mimic those found in critical period MD mice, reasserting that ibogaine restores plasticity in the visual cortex.

Interestingly, ibogaine per se did not induce effects on visual acuity nor on dendritic spine density but only when coupled to MD, reasserting that it potentiates experience-dependent plasticity. Other studies have

found that ibogaine, or its main metabolite noribogaine, increases dendritic spine density in cortical neurons [32, 33]. Importantly, the in vitro nature of those experiments limits the extent to which they can be directly compared with our in vivo results.

An important feature of our study is that visual experience manipulation by MD was performed 24 h after ibogaine administration, when both the drug and its main

metabolite have been cleared off the rodent's body [58]. This indicates that the potential for plastic changes outlasts pharmacokinetic elimination of the drug and its metabolite. Long-lasting effects of ibogaine administration on behavioral and molecular markers of plasticity have also been observed in other experimental models [29, 30].

As for the mechanisms underlying the plasticity-inducing effects of ibogaine, we focused on the analysis of markers of the two main factors controlling the opening, duration and closure of the critical period and plasticity levels: the inhibitory tone and the maturation of structural brakes [47, 53, 68]. Our results show that ibogaine treatment in adult mice induces a decrease in PV, PNN, and the colocalized PV/PNN structures, as well as vGAT, indicating an effect on both structural and inhibitory limiting factors. The observation that there are more PV neurons lacking PNNs following ibogaine treatment agrees with the plasticity-inducing effect of ibogaine. It is considered that when PNN surrounding PVs are reduced or absent, PV neuron physiology changes and this can increase the plasticity of cortical circuits [69, 70]. A reduction in vGAT staining has been observed also following other strategies that, like ibogaine, potentiate plasticity in the visual cortex of adult mice, such as fluoxetine treatment and enriched environment [71, 72]. It has been previously reported that PV-positive cells of the low intensity class are more permissive toward plasticity, while PV-positive cells of the high intensity class limit plasticity [50, 54]. We found that the pattern of PV intensity staining in ibogaine-treated mice is more resembling of a configuration of PV cells that has been associated with higher plasticity.

Finally, it is worth noting that our results show that, even though ibogaine has a complex pharmacology that differs with respect to other psychedelics, it shares the ability to re-open a period of juvenile-like plasticity. For example, it has been shown that both ketamine [35, 73–75] and LSD [76] also increase experience-dependent plasticity in the visual cortex. A complementary finding is that both of these drugs, along with ibogaine, psilocybin and MDMA reinstate social reward learning in adulthood [30]. Furthermore, changes in parvalbumin positive cells have been found after the administration of ketamine, 2,5-dimethoxy-4-iodoamphetamine (DOI) and psilocybin [75, 77–79], while changes in PNNs or extracellular matrix gene transcription have been found after the administration of psilocybin, ketamine, LSD and MDMA [30, 75, 77]. Therefore, as suggested previously [30, 80], it seems that despite diverse mechanisms of action, psychedelics, as a class, share the property of increasing plasticity while altering factors that include both structural and functional changes related to the remodeling of PNNs and PVs respectively.

A limitation of the present study is that it does not delve into the pharmacological mechanisms behind the plasticity-inducing effect of ibogaine. Given ibogaine's complex pharmacology, this is a difficult task, with several possibilities. It has been suggested that up-regulation of a signaling pathway involving AMPAR-BDNF-TrkB-mTOR might be a common mechanism of psychedelic's effect on brain plasticity [80]. In the case of ketamine, activation of this pathway seems to be related to altered glutamatergic transmission resulting from its antagonism towards the NMDA receptor [80]. The modulation of the AMPAR-BDNF-TrkB-mTOR pathway has been less characterized following ibogaine administration. However, the fact that ibogaine too is an NMDA antagonist and can alter neuronal excitability in the cortex [23, 24, 81, 82], suggests this pathway as a plausible mechanism of action also for ibogaine.

Another line of evidence suggests that psychedelics induce a heightened sensitivity to BDNF through allosteric modulation of the TrkB receptor [76]. While no evidence exists to support this mechanism for ibogaine, atomistic molecular dynamics simulations might be useful to study it [73, 76].

Another limitation of our study is that, when delivered i.p., ibogaine gets rapidly converted to noribogaine through first-pass liver metabolism by the CYP2D6 enzyme [6]. Therefore, in the brain, both molecules can be found simultaneously, making it difficult to identify if the observed effects are due to ibogaine, noribogaine, or both acting at the same time [58]. Future studies administering only noribogaine i.p. or using the i.v. route (which avoids first-pass liver metabolism) might help in disambiguating these effects.

Conclusions

In conclusion, we found that a single administration of ibogaine to adult mice renders their visual cortex susceptible to plastic changes that are only found during the critical period of ocular dominance plasticity. This increase in plasticity correlates with a reduction of PNNs surrounding PV neurons, as well as a decrease of the inhibitory synaptic marker vGAT. This shows that increased cortical plasticity can be a mechanism behind ibogaine's long lasting therapeutic effects.

Abbreviations

BDNF	Brain Derived Neurotrophic Factor
BSA	Bovine Serum Albumine
bVC	Binocular Visual Cortex
CNN	Convolutional neural network
GABA	Gamma-aminobutyric acid
GDNF	Glial cell line-Derived Neurotrophic Factor
GLMM	Generalized Linear Mixed Model
IBO	Ibogaine
L	Left
LSD	Lysergic acid diethylamide
MD	Monocular Deprivation

MDD	Major Depressive Disorder
MDMA	3,4-methylenedioxymethamphetamine
NMDA	N-methyl-D-aspartate
OMR	Optomotor Response Test
PB	Phosphate Buffer
PBS	Phosphate Buffer Saline
PBST	Phosphate Buffer Saline Triton X-100
PFA	Paraformaldehyde
PNN	Perineuronal Net
PTSD	Post-Traumatic Stress Disorder
PV	Parvalbumin
Px	Postnatal day x
R	Right
REM	Rapid Eye Movement
RT	Room Temperature
SEM	Standard Error of the Mean
SERT	Serotonin Transporter
SUD	Substance Use Disorder
TBI	Traumatic Brain Injury
VEH	Vehicle
vGAT	Vesicular GABA transporter
VMAT2	Vesicular Monoamine Transporter 2
WFA	Wisteria Floribunda Lectin

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12868-026-01005-6>.

Supplementary Material 1

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Author contributions

A.A. and F.M.R. have contributed to the conception and design of the work, the acquisition, analysis and interpretation of data, and have drafted the work and substantively revised it. F.B. and V.T. have contributed to the acquisition and analysis of data. I.C. has contributed to the conception of the work and interpretation of data, and has drafted the work and substantively revised it. J.M.M. has contributed to the acquisition of data; T.P. has contributed to the conception of the work, to the interpretation of data, and has drafted the work and substantively revised it. All authors have approved the submitted version, and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files. Raw data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All experiments were performed in accordance with the EC Directive 86/609/EEC for animal experiments (directive from the European Commission of the European Economic Community) and the Uruguayan Research Ethic Committees (protocol approval #1621, ExpFCien 240012-000026-22).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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