




Environmental enrichment alleviates cognitive and psychomotor alterations and increases adult hippocampal neurogenesis in cocaine withdrawn mice

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Abstract

Cocaine is a widely used psychostimulant drug whose repeated exposure induces persistent cognitive/emotional dysregulation, which could be a predictor of relapse in users. However, there is scarce evidence on effective treatments to alleviate these symptoms. Environmental enrichment (EE) has been shown to be associated with improved synaptic function and cellular plasticity changes related to adult hippocampal neurogenesis (AHN), resulting in cognitive enhancement. Therefore, EE could mitigate the negative impact of chronic administration of cocaine in mice and reduce the emotional and cognitive symptoms present during cocaine abstinence. In this study, mice were chronically administered with cocaine for 14 days, and control mice received saline. After the last cocaine or saline dose, mice were submitted to control or EE housing conditions, and they stayed undisturbed for 28 days. Subsequently, mice were evaluated with a battery of behavioural tests for exploratory activity, emotional behaviour, and cognitive performance. EE attenuated hyperlocomotion, induced anxiolytic-like behaviour and alleviated cognitive impairment in spatial memory in the cocaine-abstinent mice. The EE protocol notably upregulated AHN in both control and cocaine-treated mice, though cocaine slightly reduced the number of immature neurons. Altogether, these results demonstrate that EE could enhance hippocampal neuroplasticity ameliorating the behavioural and cognitive consequences of repeated administration of cocaine. Therefore, environmental stimulation may be a useful strategy in the treatment cocaine addiction.

KEYWORDS

adult hippocampal neurogenesis, cocaine use, cognitive impairment, environmental enrichment

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1 | INTRODUCTION

Cocaine use is a global problem that entails serious health, economic, legal and social consequences. In 2019, roughly 20 million people worldwide or 0.4% of the adult population aged 15–64 had used cocaine at some point in their life.¹ In fact, it is estimated that 20% of cocaine users become dependent on cocaine at some point in their lives^{2,3} losing the control over drug intake. Moreover, according to data from the European Drug Report (2020), among cocaine users who start treatment, approximately 52% have already been treated previously, suggesting a high relapse rate in cocaine addiction. However, regardless of the establishment of addiction and compulsive drug seeking, cocaine use and withdrawal are associated with cognitive, emotional, and motivational impairment.⁴ These behavioural symptoms are attributed to maladaptive neuroadaptations, which are, at least in part, induced by the repeated use of the drug.⁴ Indeed, some clinical studies have shown that neurocognitive impairment—including attention deficits, working and reference memory deficits and behavioural inhibition or cognitive flexibility deficits—correlates directly with the time spent using cocaine or with the amount of cocaine consumed; and these alterations can be reversed after a sufficiently long abstinence from the drug.^{4–7}

Cocaine-induced neurocognitive and behavioural impairments are also revealed by animal research, which allows to control the administration of the drug. According with other studies (reviewed in Mañas-Padilla et al.⁸), we have previously shown that young male mice exposed to 12–14 days of repeated cocaine administration display behavioural alterations that persist after protracted cocaine abstinence.^{9–11} Mainly, mice abstinent from chronic cocaine exposure are impaired in hippocampal-dependent memory, evidenced by object or place recognition and spatial navigation tasks,^{9–11} and they may demonstrate exacerbated exploratory activity and motor activation when they are exposed to challenging situations or novel environments.¹⁰ In this regard, the hippocampus is a brain region widely communicated with main addiction-related brain areas that has a key role for declarative memory, emotional behaviour and response to novel stimuli; and it modulates the hyperlocomotive effects of cocaine—through excitatory connections with the accumbens.^{12,13} Therefore, the hippocampus is a strong candidate to modulate the cocaine-induced behavioural symptoms, and so, strategies that stimulate hippocampal plasticity may be valuable in the therapies destined to treat the cocaine abuse.

Environmental enrichment (EE) refers to housing conditions that consist of the exposure to a combination of sensory stimulation that promotes exploration (i.e., novel objects), social interaction and physical activity. This multisensory stimulation has been shown to have widespread effects on the brain and behaviour such as in exploration, emotional regulation,^{14,15} learning and memory, synaptogenesis and angiogenesis both in healthy rodents and in pathological conditions such as stress and neurodegenerative disorders (reviewed in previous works^{16–19}). Importantly, it is well known that EE upregulates proliferation, maturation and functional integration of new neurons in the adult hippocampus—adult hippocampal neurogenesis (AHN)—and

these new neurons are required for at least some of the cognitive and emotional effects related to EE.^{20,21}

The relationship between EE and drug-related behaviours has received increasing attention in recent years, suggesting that EE has both preventive and therapeutic actions against drug effects.^{22,23} There is some evidence suggesting that EE can attenuate cocaine induced conditioned place preference in rodents^{24,25} and reduces cocaine self-administration.^{26,27} The investigation with humans also suggests that ‘enrichment-related’ therapies may provide a valuable adjuvant intervention in drug addiction. For example, physical exercise, meditation, as well as behavioural interventions, have showed positive results on addicted patients (reviewed in Sampedro-Piquero et al.²⁸). Nevertheless, although there is a substantial amount of data describing the influences of housing conditions on addiction-related responses such as processing of drug-associated memories or the self-administration of drugs,²⁹ it has not been investigated whether exposure to EE during abstinence from chronic cocaine could reduce its persistent cognitive and behavioural symptoms. Interestingly, cocaine-abstinent mice show normal AHN levels in basal conditions; but they show a dysregulation—either an abnormal increase or decrease—of their number of hippocampal immature neurons when they are exposed to behavioural tasks.^{9–11} Therefore, they could be benefited of a pro-AHN intervention such as EE. The present study aims to investigate whether EE improves the emotional and cognitive symptoms caused by chronic cocaine administration and, whether EE modulates the AHN in cocaine-abstinent mice.

2 | MATERIALS AND METHODS

2.1 | Animals

Forty young-adult male C57BL/6J mice (Janvier Labs, Le Genest-Saint-Isle, France) were used in this study. All of them were maintained in standard conditions (temperature: 22 ± 2°C; 12-h light/dark cycle; lights on at 8:00 AM) with ad libitum access to water and food. Procedures followed the European (Directive 2010/63/UE) and Spanish regulations (Royal Decrees 53/2013 and 1386/2018, and Law 32/2007) for animal research. The experimental protocols were approved by the research ethics committee of the University of Málaga (code: CEUMA 81-2016-A) and Junta de Andalucía (code: 30/03/2017/055).

2.2 | Cocaine treatment

At 13 weeks of age mice were divided into two groups with similar average body weight and assigned either to a cocaine or saline treatment. The cocaine group (‘COC’, $n = 20$) received a chronic cocaine treatment consisting of a daily intraperitoneal (i.p.) 20-mg/kg dose of cocaine (Alcaliber S.A., Madrid, Spain; diluted in 10-ml/kg volume of

saline -0.9% NaCl-) for 14 consecutive days in their home cage, while the vehicle group ('VEH' $n = 20$) received an equivalent i.p. volume of saline solution. This dosage regimen was chosen because it has been shown to induce persistent cognitive and exploratory alterations.⁹⁻¹¹

2.3 | Environmental enrichment procedure

After the last cocaine or saline dose, mice were randomly assigned to the CTRL or EE conditions yielding a total of four treatment groups: cocaine-control (COC-CTRL, $n = 10$), cocaine-enrichment (COC-EE, $n = 10$), vehicle-control (VEH-CTRL, $n = 10$) and vehicle-enrichment (VEH-EE, $n = 10$). The CTRL environment consisted of individually housed mice in a standard 15 cm × 33 cm × 13 cm transparent polypropylene cage containing nesting material. Enriched mice were housed in group of five to stimulate social interaction, in a larger cage (44 cm × 27 cm × 28 cm; Dayang Pet Products, Foshan City, China) containing nesting material, two floors communicated by a

large slide-like plastic tube and two running wheels equipped with a magnetic counter that allow to assess wheel revolutions. Cognitive stimulation was provided by an assortment of objects that may include two rodent dwellings, a NOVOMAZE (ViewPoint, Lyon, France) and four to six miscellaneous toys. Cages were cleaned twice per week, with new enrichment objects in different configurations introduced at each cleaning to maintain novelty. As a measure of the quantity of voluntary exercise, the distance ran on the wheel (number of revolutions multiplied by the wheel perimeter) was monitored daily. Both groups remained in these conditions from the throughout the duration of the experiment (Figure 1A for a representative image of the cages).

2.4 | Bromodeoxyuridine administration

Bromodeoxyuridine (BrdU, Sigma-Aldrich, Madrid, Spain) was administered during the cocaine withdrawal period on days 18, 25, 32 and

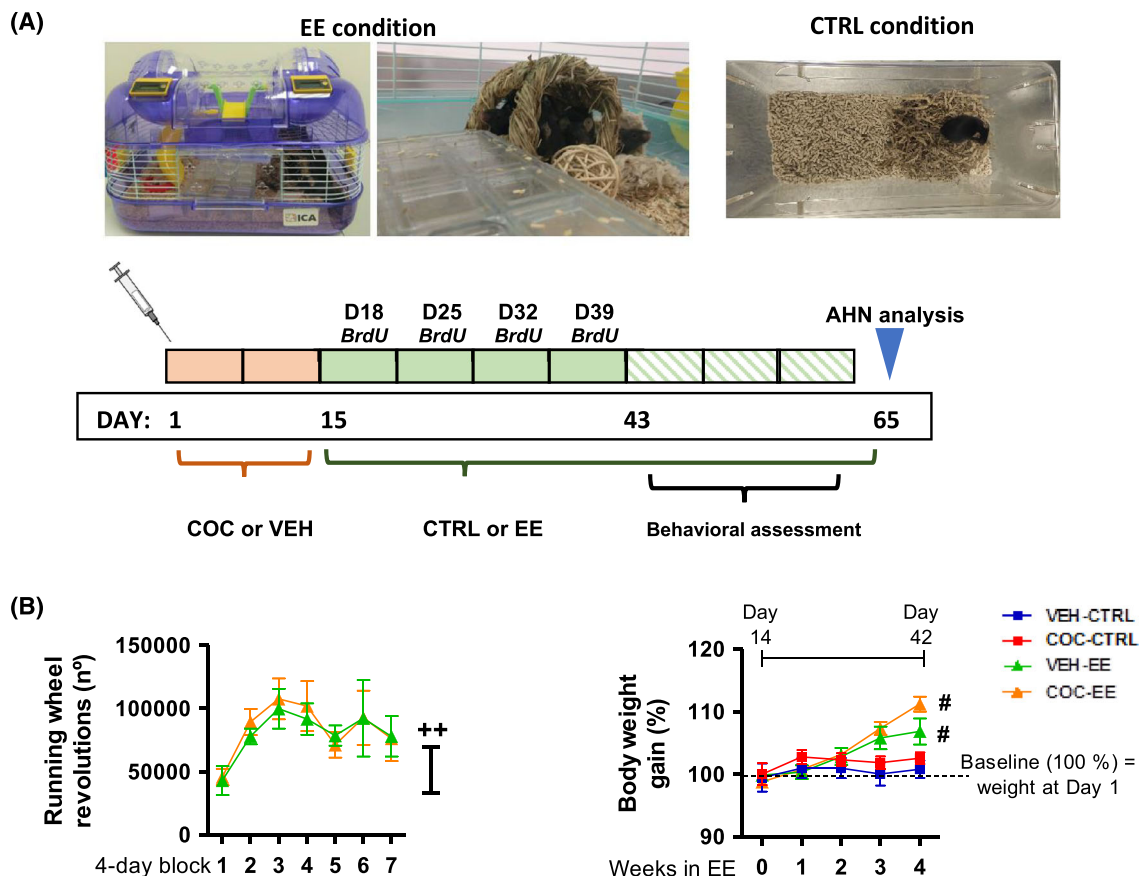


FIGURE 1 Experimental protocol (A). Mice were treated with cocaine or vehicle for 14 consecutive days (days 1–14). Cocaine-abstinent mice or vehicle-treated mice were then housed in standard or environmentally enriched conditions from day 15 and then submitted to a behavioural testing battery (days 43–60) to evaluate the long-term consequences of cocaine. Mice were sacrificed 5 days after completing the behavioural experiments (day 65) for histological analysis. Photographs show the enriched housing (environmental enrichment, EE) and the standard–control–housing (CTRL) conditions. According to the presence of cocaine and/or EE, four experimental conditions were studied: VEH-CTRL ($n = 9$), COC-CTRL ($n = 10$), VEH-EE ($n = 10$) and COC-EE ($n = 10$). Mice in the EE condition progressively increased wheel running (B) and body weight gain (C) assessed during the four first weeks of EE. analysis of variance (ANOVA) effect for ‘day’: $++p < 0.001$; post hoc LSD: Difference of the EE groups versus their CTRL group: $\#p < 0.05$. Data are expressed as mean ± SEM.

39 (Figure 1A) to label the newly generated cells. Mice received two daily 75-mg/kg intraperitoneal BrdU administrations, separated by 4 h.³⁰ With this BrdU administration protocol the aim is to know whether the condition of 'enriched environment' can modulate hippocampal neuroplasticity and adult neurogenesis, compared to the control condition, without specifying the exact moment in which this effect occurs.

2.5 | Behavioural assessment

Behavioural testing started 28 days after the last cocaine or saline dose (Figure 1A). Mice were carried to a noise-isolated room at 9:00 AM, and they were habituated for at least 20 min before starting the behavioural assessment. A battery of behavioural tests for exploratory activity, emotional behaviour and cognitive performance was performed based on our previously published protocols.^{9–11}

The behavioural paradigms included were the elevated plus maze (EPM) and the open field to assess anxiety-like behaviour and locomotor activity (days 43 and 45), the Y maze test for spontaneous alternation behaviour (day 44), the novel object recognition (day 46) and the novel place recognition test (day 47), the forced swimming test for despair-like behaviour (day 47) and the water maze for spatial learning (days 50–62) (Figure 1A). Specifically, the water maze included different tasks: habituation to the maze (1 days), visible platform training (2 days), spatial reference memory training with a hidden platform (4 days), probe trials for long term memory retention at 24- and 72-h intervals, platform reversal trials for cognitive flexibility (1 day) and delayed matching-to-place spatial working memory training (2 days). All behavioural protocols were performed as detailed in the supporting information and in previous publications.^{9–11}

2.6 | Immunohistochemistry and quantification

Our histological procedures are extensively described in the supporting information. On day 65 (Figure 1A), mice from all experimental conditions were intracardially perfused and histological studies were performed as described in the supporting information. Coronal sections (40 μ m) were obtained on a Thermo Scientific 650 V vibratome. AHN was quantified in the dorsal hippocampus. AHN-related markers were determined by free-floating immunofluorescence and confocal microscopy. The different stages of differentiation were studied by the expression and colocalization of doublecortin (DCX, expressed in immature neurons up to 3–4 weeks of age³²) and calretinin (CR, expressed in early postmitotic stage of granule cells up to 4–5 weeks of age, coinciding in a period of time with the expression of DCX³¹). Also, BrdU was used to detect the labelled cells aged 41 to 43 days old that survived until the end of the experiment. To confirm their neuronal phenotype, colocalization of BrdU with the mature neuron marker neuronal nuclei (NeuN; that is expressed by neurons from their approximately third week of age onwards³²) was analysed by immunofluorescence and confocal microscopy.

The AHN-related primary antibodies used were goat anti-doublecortin (1:500, Santa Cruz, sc-8066); rabbit anti-calretinin (1:3000, Swant, 76994); rat anti-BrdU (1:500, Abcam, 6326) and mouse anti-NeuN de mouse (1:500, Abcam, 104224).

One random series was chosen for each immunohistochemistry that was comprised of one of every six hippocampal sections. Another randomly chosen series was used for DAPI Staining to determine the area of the subgranular cell zone.

Stereology was used for cell quantification in a confocal microscopy. BrdU labelled cells were counted by the optical fractionator method. The physical-dissector method, adapted to confocal microscopy as previously described,³³ was used to estimate the density of DCX+, CR+ and NeuN cells. Next, the Cavalieri method was used to obtain both the total area of the dentate SGZ and the total volume of the dentate GCL, in order to estimate total cell numbers, by multiplying the cell density by the total area or volume of the dentate region under study, as Llorens-Martín et al.³³ described previously.

2.7 | Statistical analysis

Groups were compared by Student's *t* tests or by analysis of variance (repeated-measures analysis of variance [ANOVA] or factorial ANOVA) followed by the post hoc Fisher's least significant difference tests. Only significant results ($p \leq 0.05$) are shown.

3 | RESULTS

3.1 | Mice housed in EE increased wheel running and body weight gain irrespectively of their cocaine treatment

Wheel running and body weight gain were evaluated across the four first weeks of EE. Mice housed under EE progressively increased wheel running behaviour until they reached a plateau (repeated measures ANOVA 'cocaine \times day': effect of 'day': $F(6, 36) = 5.628$, $p = 0.000$; Figure 1B), and they gained more weight than mice housed in the control condition (repeated measures ANOVA 'cocaine \times EE \times day': effect of 'day': $F(4, 140) = 37.371$, $p = 0.000$; 'EE \times day': $F(4, 140) = 26.158$, $p = 0.000$; post hoc is shown in Figure 1C). Neither wheel running nor body weight gain were influenced by the previous cocaine treatment (Figure 1B,C). Furthermore, it is worth to mention that objects and materials appeared used (i.e., shredded and chewed) in all EE cages, suggesting that both the VEH-EE and the COC-EE mice interacted with these elements.

3.2 | EE attenuated hyperlocomotion in the cocaine-abstinent mice and induced anxiolytic-like behaviour

In the EPM task, mice housed in EE cages spent more time in the unprotected open arms of the apparatus (factorial ANOVA

'cocaine × EE': effect of 'EE': $F(1, 35) = 12.042, p = 0.001$ (Figure 2A) and therefore less in the close arms (factorial ANOVA 'cocaine × EE': effect of 'EE': $F(1, 35) = 17.743, p = 0.00017$) (data not shown); and reduced their latency to first enter an open arm (factorial ANOVA 'cocaine × EE': effect of 'EE': $F(1, 35) = 11.040, p = 0.002$) while no differences were found in locomotor activity

(Figure 2A). This task shows an anxiolytic effect of EE, independently of the cocaine or vehicle treatment (factorial ANOVA 'cocaine × EE': effect of 'EE': $F(1, 35) = 14.481, p = 0.001$) (Figure 2A).

Exploration of a squared open field evidenced locomotor hyperactivity in the cocaine-abstinent mice, which was counteracted by EE. In the habituation session (5 min) in the novel environment, the

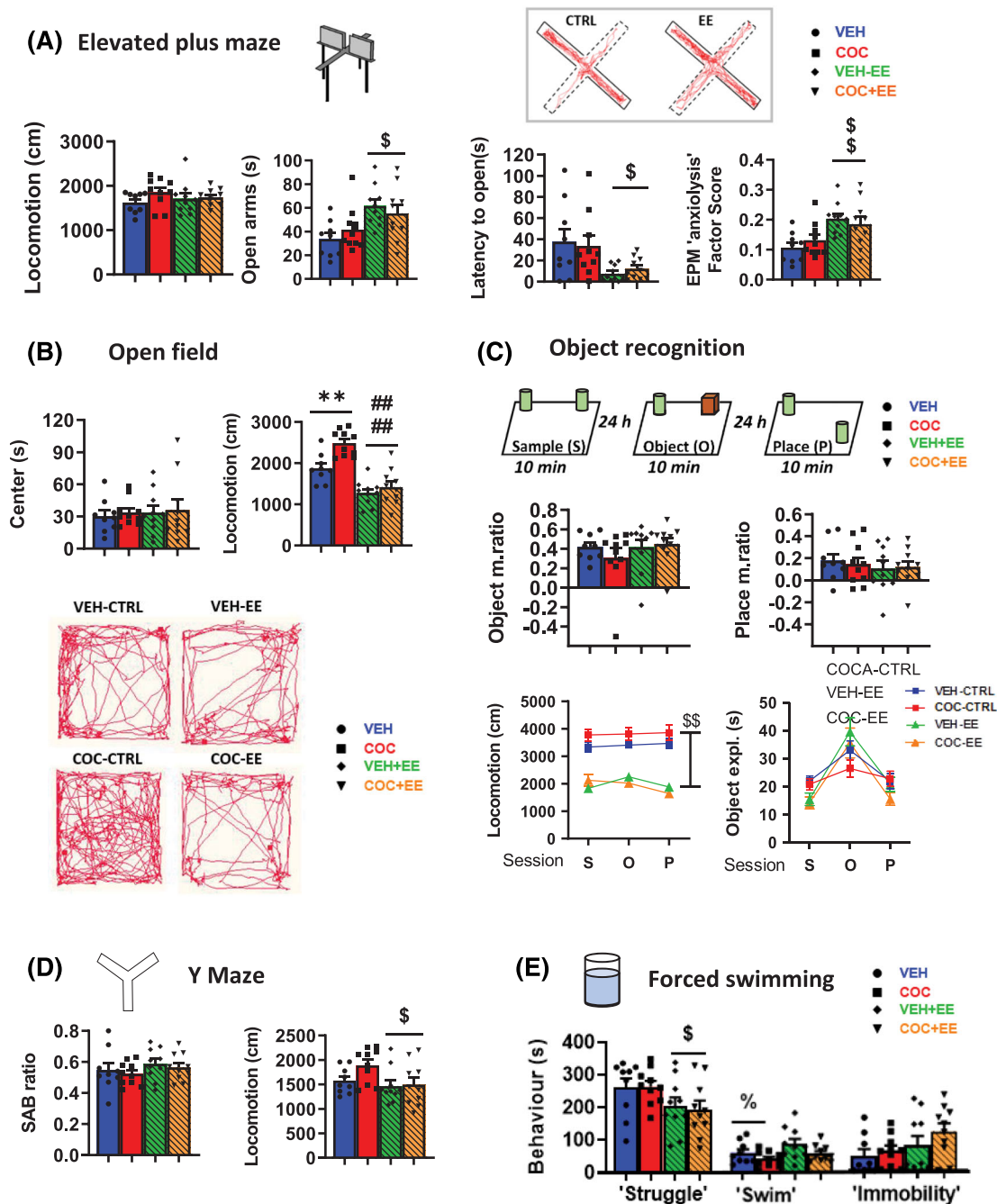


FIGURE 2 Effect of cocaine abstinence and environmental enrichment (EE) on exploratory, anxiety and cognitive tasks. (A) The EE-housed mice showed anxiolysis in the elevated plus maze (EPM) irrespective of their cocaine treatment. (B,C) EE-induced hypolocomotion across the open-field (OF) testing (habituation, sample and test sessions) (D) and in the Y maze task. Furthermore, EE attenuated locomotor hyperactivity induced by cocaine abstinence in the open-field habituation session (E). Both cocaine and EE modulated mice behaviour in the forced swimming test. Analysis of variance (ANOVA) effect for 'cocaine': $\%p < 0.05$; ANOVA effect for 'EE': $\$p < 0.05$; $\$\$p < 0.001$; post hoc LSD: difference of the VEH-CTRL group versus the COC-CTRL group: $**p < 0.001$; difference of the EE groups versus their CTRL group: $\#p < 0.05$; $\#\#\#p < 0.001$. Data are expressed as mean \pm SEM.

COC-CTRL mice were the most active, but both VEH-EE and COC-EE mice reduced locomotion compared to their non-enriched counterparts (factorial ANOVA 'cocaine × EE': effect of 'cocaine': $F(1, 35) = 11.127, p = 0.002$; 'EE': $F(1, 35) = 53.845, p = 0.000$; 'cocaine × EE': $F(1, 35) = 4.194, p = 0.048$; post hoc is shown in Figure 2B). In subsequent exposures to the open field (i.e., 10 min 'sample', 'object' and 'place' sessions of the object recognition test), when the environment was familiar and objects were included to explore, the COC-CTRL mice no longer showed an abnormally increased motor activation. Nevertheless, locomotion was still notably reduced in both VEH-EE and COC-EE groups (repeated measures ANOVA 'cocaine × session × EE': effect of 'EE': $F(1, 35) = 137.710, p = 0.000$; Figure 2C). Regarding the total time of object exploration, a repeated measures ANOVA ('cocaine × EE × session) revealed significant effects of both 'session' ($F(2, 70) = 16.546, p = 0.000$) and 'EE × session' ($F(2, 70) = 3.503, p = 0.036$), which was attributed to a slight tendency of the EE mice to explore the objects during less time in the sample session. Nevertheless, in the object and place memory tests all experimental groups showed a similar preference for the novel or the displaced object (Figure 2C).

In the Y maze test, there were no differences among groups in spontaneous alternation (SAB ([number of spontaneous alternations]/[total the number of arm entries – 2]) (Figure 2D) nor in the total number of arm entries (data not shown). However, locomotion supported results found in the open field test, since EE reduced locomotor activation (factorial ANOVA 'cocaine × EE': effect of EE: $F(1, 35) = 4.738, p = 0.036$; Figure 2D). Though no effect of 'cocaine' was found in the ANOVA analysis, a student's *t* test comparing the VEH-CTRL and the COC-CTRL groups only would confirm increased locomotor activity in the latter ($t(17) = -2.146; p = 0.047$) (Figure 2D).

Finally, the forced swimming test revealed that cocaine did not influence the total time mice spent immobile but affected the total time mice spent 'swimming' (factorial ANOVA 'cocaine × EE': effect of 'cocaine': $F(1, 35) = 5.084, p = 0.031$). On the other hand, EE reduced the amount of time mice 'struggled'—that is, were highly active trying to escape from the water—(factorial ANOVA 'cocaine × EE': effect of 'EE': $F(1, 35) = 6.223, p = 0.017$; Figure 2E) and showed a tendency to increase immobility (factorial ANOVA 'cocaine × EE': effect of 'EE': $F(1, 35) = 3.982, p = 0.054$ —unsignificant) and to reduce the 'swim' (effect of 'EE': $F(1, 35) = 4.010, p = 0.053$ —unsignificant) (Figure 2E).

3.3 | EE alleviated cocaine-induced impairment in spatial memory

In the water maze, mice from all experimental conditions were able to find a visible platform (visual training; data not shown). However, when the platform was hidden in a fixed location (spatial reference memory training), the COC-CTRL mice were notably slower than the VEH-CTRL mice in learning the platform position across the acquisition days, which was also evident when the platform was displaced to

a new location (in the platform reversal day). Importantly, EE prevented this acquisition deficit, so the COC-EE mice learned to find the hidden platform similarly as drug-naïve controls (repeated measures ANOVA 'cocaine × EE × session' on platform latency measures: effect of 'cocaine × EE': $F(1, 34) = 4.782, p = 0.036$; 'session': $F(9, 306) = 21.253, p = 0.000$; post hoc is shown in Figure 3A). In the probe trials, the COC-CTRL mice evidenced impaired long-term spatial memory retention when tested at a 72-h interval, a deficit that was alleviated by EE (factorial ANOVA 'cocaine × EE': time in the target quadrant: effect of 'cocaine × EE': $F(1, 34) = 5.511, p = 0.025$; distance to platform: 'cocaine × EE': $F(1, 34) = 9.416, p = 0.004$; platform crossings: 'cocaine': $F(1, 34) = 11.088, p = 0.002$; post hoc analyses are shown in Figure 3B). Finally, mice were tested for spatial working memory. While there were no differences in the sample trials, analysis of the test trials revealed a defective performance in the COC-CTRL mice that was ameliorated by EE (repeated measures ANOVA 'cocaine × EE × day': effect of 'EE' $F(1, 34) = 4.648, p = 0.038$; 'cocaine × EE': $F(1, 34) = 4.680, p = 0.038$; 'cocaine × day': $F(1, 34) = 8.095, p = 0.007$; post hoc is shown in Figure 3C). The beneficial impact of EE on spatial memory was limited to the cocaine-abstinent mice, because EE did not affect spatial memory measures in drug naïve animals.

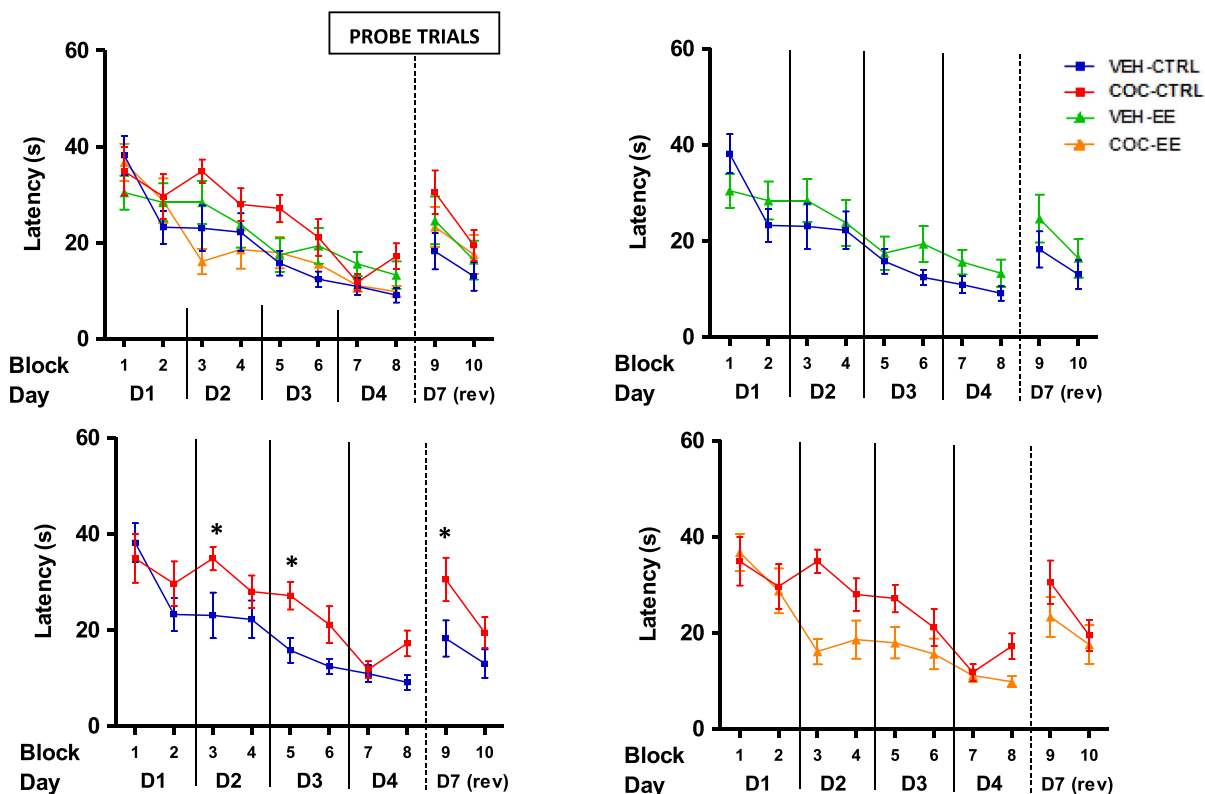
3.4 | Hippocampal plasticity (AHN) was potentiated by EE in the cocaine-abstinent mice

After completion of the experiment, AHN-related markers were analysed in the hippocampal dentate gyrus (Figure 4). We measured AHN by immunohistochemistry of BrdU staining, double staining of BrdU/NeuN and double staining of DCX/CR.

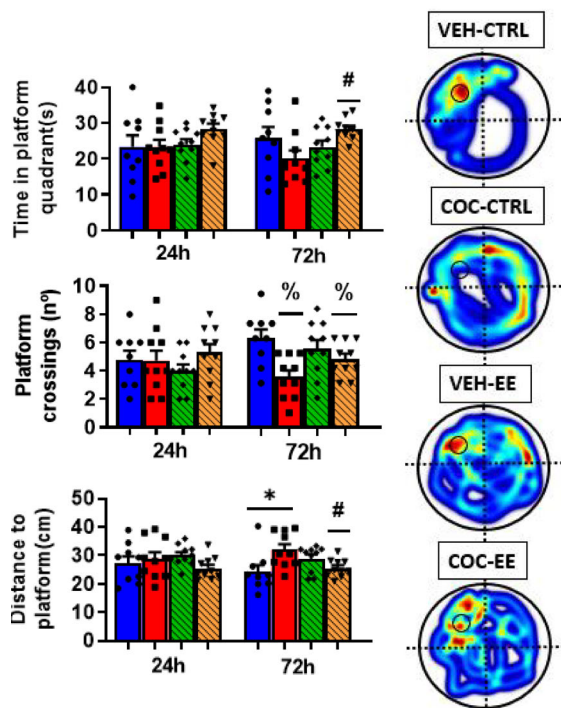
Interestingly, the cocaine treatment did not affect the number of BrdU+ cells that were generated during the 4 weeks of cocaine withdrawal and survived until the end of the experiment (Figure 4B). However, EE notably increased the number of BrdU+ cells in the DG (factorial ANOVA 'cocaine × EE': effect of 'EE': $F(1, 35) = 64.786, p = 0.000$) irrespectively of previous drug exposure. Double staining of BrdU/NeuN confirmed that practically all the newly born cells were differentiated into mature neurons in all four treatments (Figure 4B; data not shown).

The total number of immature granule neurons was determined by evaluating the number of DCX and CR-positive cells. Both the total number of DCX+ and DCX+/CR+ immature neurons were significantly increased in EE animals compared to controls but, importantly, previous cocaine treatment slightly reduced their number (for DCX+/CR+ cells: factorial ANOVA 'cocaine × EE': effect of 'cocaine': $F(1, 34) = 4.548, p = 0.040$; 'EE': $F(1, 34) = 30.501, p = 0.001$; for the total of DCX+ cells: factorial ANOVA 'cocaine × EE': effect of 'cocaine': $F(1, 34) = 4.610, p = 0.039$; 'EE': $F(1, 34) = 26.657, p = 0.001$) (Figure 4C). No significant differences were found in the total number of DCX+/CR– cells, which only represented an ~5%–10% of the total DCX+ population (data not shown).

(A) Reference memory acquisition



(B)



(C)

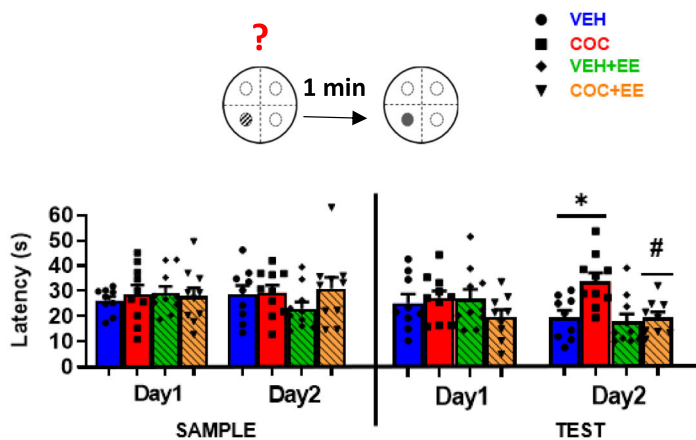


FIGURE 3 Environmental enrichment (EE) improved both reference and working spatial memory during cocaine abstinence. Cocaine impaired both spatial reference memory acquisition (A) and its long-term maintenance (B), a deficit that was not present in the COC-EE animals. EE also ameliorated spatial working memory deficits induced by cocaine (C). Analysis of variance (ANOVA) effect for 'cocaine': % $p < 0.05$; post hoc LSD: Difference of the VEH-CTRL group versus the COC-CTRL group: * $p < 0.05$; ** $p < 0.001$; difference of the EE groups versus their CTRL group: # $p < 0.05$; ## $p < 0.001$. Data are expressed as mean \pm SEM. In (A), each block of sessions included four sessions each. rev: reversal

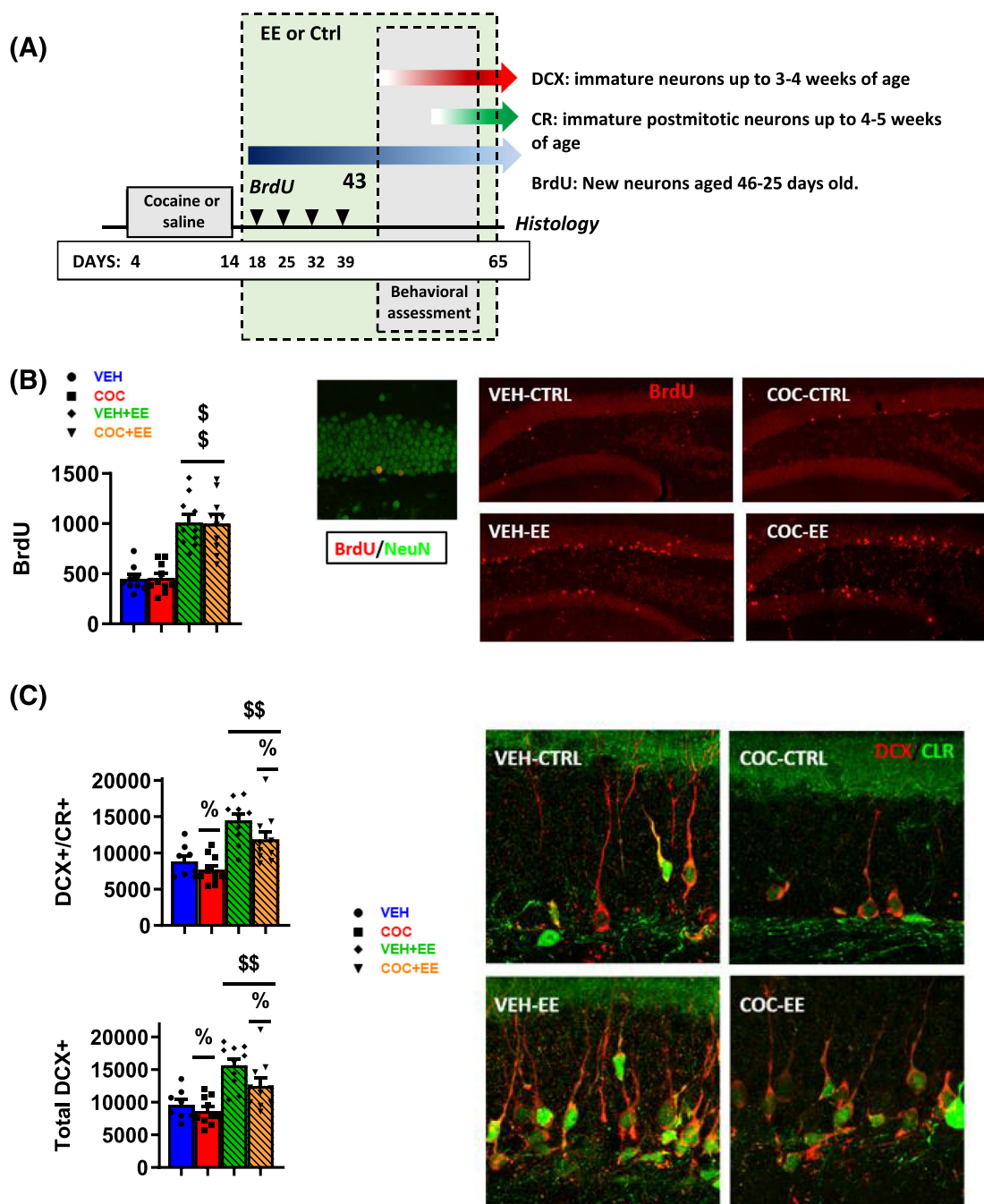


FIGURE 4 Hippocampal plasticity (AHN) potentiated by environmental enrichment (EE) in the cocaine abstinent mice. (A) Schedule of the experiment (as shown in Figure 1A) displaying the timing of expression of each AHN marker analysed. EE notably increased the number of BrdU+ cells in the DG. Double staining of BrdU/NeuN confirmed that the newly born cells were differentiated into mature neurons in all four treatments (Figure 4B). (C) Total number of DCX+ and DCX+/CR+ immature neurons were significantly increased in EE animals compared controls and the previous cocaine treatment slightly reduced their number. Panels (B) and (C) show representative confocal images. Analysis of variance (ANOVA) effect for 'EE': \$ $p < 0.05$; \$\$ $p < 0.001$; ANOVA effect for 'cocaine': % $p < 0.05$; data are expressed as mean \pm SEM.

4 | DISCUSSION

In recent years, great progress has been made in investigating the influence of environmental conditions on the development of drug abuse and addiction.²⁹ Although recent data show that an enriched environment recovers cognitive deficits and improves synaptic

plasticity in mice abstinent from ethanol,³⁴ little is known about the effect of EE on cognitive impairment due to cocaine. The present study provided environmental stimulation after discontinuation of chronic cocaine administration. It has been previously shown that mice exposed to repeated cocaine administration display behavioural alterations that persist after protracted cocaine abstinence (reviewed

in Mañas-Padilla et al.⁸); thus, the present study suggests that environmental conditions could reduce the behavioural and cognitive consequences of repeated administration of this drug.

Regarding exploratory activity, cocaine groups showed hyperlocomotion when exploring novel environments such as the OF and the Y maze tasks, as reported in our previous work.¹⁰ Such exacerbated motor activation was counteracted by EE. In fact, EE-housed animals, irrespectively of their previous cocaine treatment, showed reduced locomotor activity in these tasks compared to controls. These results could be related by a lack of novelty preference in enrichment animals (due to the new enrichment objects repeatedly introduced in their cage) or to a faster habituation to novelty in EE animals, in line with previous research (reviewed in Bellés et al.³⁵). The EE-housed mice also showed an increase in body weight. While it is normally assumed that physical activity attenuates, or even prevents, increases in body mass, this assumption is not consistent. A few studies showed that running wheel activity may not affect body weight gain,^{36–38} and it may be accompanied by increased caloric intake.^{36,38} Furthermore, we must bear in mind that in the present study, in addition to physical exercise, the animals were exposed to multisensory stimulation. There are different works that suggest that mice in enriched housing conditions consume significantly more food than mice in standard housing, which may be consistent with the higher body weight.^{39–41} Therefore, the amount of food consumed is a factor that was not evaluated in this study but could explain why the weight of our animals under the EE condition was higher than those housed under standard conditions.

Regarding emotional behaviours, increased anxiety may be notorious at early stages of cocaine abstinence (i.e., first few days⁴²), but exacerbated anxiety responses are rarely found in mice abstinent from cocaine for 3 or 4 weeks.^{9,10} EE reduced anxiety-like behaviour in mice (reviewed in Fox et al.⁴³) independently of their cocaine or vehicle treatment. This was evidenced on all the parameters measured with EPM test—that is, the time in the unprotected open arms, latency to enter in open arm and anxiolysis factor score—though no significant differences were noted on typical anxiety-like measures—that is, the time in the centre zone—in the OF test between those enriched mice and their standard condition. This is perhaps not surprising since several studies have explored the discrepancies that are commonly found between these tasks, despite both being tests of anxiety-like behaviour.^{44,45} Interestingly, in the forced swimming test, EE-housed animals struggled less time in the water. Struggling is a highly active behaviour that has been interpreted both as a correlate of increased motor activity⁴⁶ and as a panic-like anxious response.⁴⁷ Therefore, a reduced struggling behaviour seems in accordance with the hypolocomotive and anxiolytic phenotype shown by the EE-treated mice in the previous tasks.

Results from the Morris Water Maze, a paradigm for measuring spatial memory, showed that EE ameliorated both reference and working spatial memory in the cocaine-abstinent mice. Like our previous study¹¹ the cocaine-abstinent mice were notably slower than the control mice in learning the platform position across the reference memory acquisition days, which was also evident when the platform

was displaced to a new location (i.e., reversal trial). However, EE prevented this acquisition deficit, so the mice that had received cocaine but were exposed to enrichment learned to find the hidden platform similarly as drug-naïve controls and improved in measures of long-term memory consolidation (72 h). Similar results were found in the working memory task, considering that the deficits of cocaine-abstinent mice were ameliorated by EE in the second day of the test, while the cocaine-abstinent mice did not learn the task on either of the 2 days of the test, unlike their control group. However, the beneficial impact of EE on spatial memory was limited to the cocaine-abstinent mice, since mice in the control group that had access to enrichment had not a better performance in the water maze than their control group. This result may seem surprising given that previous reports described an improved spatial memory task performance in healthy mice that have been exposed to enrichment protocols.^{20,48–50} The divergent results may be attributed to a number of factors that may differ among studies, including (1) the strain or the age of mice used and the control group used, (2) the duration of enrichment, (3) the number of objects and social companions present in the home cage, or their frequency of change, (4) the use of exercise in the protocol of EE or (5) differences in the water maze protocol. For example, the beneficial effects of certain EE protocols may affect older mice or mice in conditions in which deficits exist, but not healthy young mice.^{51–54} This could also explain that the increased AHN in the control group may not be accompanied by an improvement in performance in memory tests due to a possible ‘ceiling effect’, as control mice showed a good performance in the spatial memory tasks.

Unlike previous studies—including several from our own laboratory (reviewed in Mañas-Padilla et al.⁸)—in this occasion we have not found a deleterious effect of cocaine in the object and place recognition memories nor in SAB behaviour.¹¹ The discrepancy among findings might be explained by certain differences in protocols used or by other unknown experimental variables. Indeed, the notable impact of both intra- and inter-laboratory variability on mouse research has been stressed recently,^{55,56} attributed to uncontrollable variables such as unexpected fluctuations of the phenotypes of control mice between batches. Also, there are studies that show how different results are obtained depending on the test used to assess domains such as memory.⁵⁷ This may be due to factors such as the complexity of the task or the memory load required in each of them.

Finally, regarding AHN, the cocaine treatment did not affect the number of BrdU+ cells nor their differentiation into mature neurons (BrdU+/NeuN+), which were normal compared with vehicle-treated mice. This supports the assumption that extended cocaine withdrawal is not associated with persistent alterations of basal AHN (reviewed in Castilla-Ortega and Santín⁵⁸). Moreover, both groups of the enrichment condition showed an increased number of BrdU+ cells, so it can be concluded that EE upregulates the survival of the adult-born neurons generated during the EE protocol.^{48,59} It is important to add that an additional potentiation of hippocampal proliferative activity by EE could not be completely ruled out, since markers of cell proliferation have not been evaluated in this work. Regarding the population of the young immature neurons, they were slightly downregulated by

cocaine treatment in despite of being augmented by EE. Considering that the immature neurons (up to ~3 weeks of age) are highly sensitive to modulation by the environmental demands,⁶⁰ this outcome is likely to reflect an abnormal response of AHN to the behavioural protocol. This is in concordance with our previous results showing that cocaine-treated mice abnormally regulated their immature DCX+ neurons in response to learning experiences, but displayed normal numbers of these cells in conditions of no stimulation.^{9–11} In conclusion, cocaine decreased this population of young immature neurons, but the enriched environment counteracted this effect and increased their numbers.

Increased neurogenesis is a well-known effect of EE in adult rodents.⁶¹ Previous work⁶² has shown additive effects of exercise and novelty exposure on neurogenesis, as exercise may be more involved in promoting cell proliferation while other aspects of enrichment promote cell survival and differentiation. However, despite variable results when using EE without a running wheel,⁶³ several studies have shown that cognitive or social stimulation applied alone can induce neurogenesis.^{17,64–67} The current study employed an enrichment protocol that included social enrichment, novelty, and exercise. Therefore, a limitation is that we cannot attribute the enrichment effects to any particular aspect of our protocol. Future studies would be needed to determine which aspects of our enrichment protocol are necessary and sufficient to counteract the effects of cocaine on cognition, behaviour and AHN in cocaine abstinent mice. Along with this, it would be interesting to know how much exercise each mouse performs individually to establish different correlations between physical exercise and AHN. Indeed, in previous studies we have verified that the amount of exercise performed is important to explain the increase in AHN.⁶⁸ Nevertheless, it is important to stress that the amount of running in this study was similar for both VEH and COC-treated mice, so this variable could not explain differences in hippocampal plasticity or behaviour among treatments in this study.

A second limitation to the current study is that we conducted this work in males only. It might be interesting to study if the effect of the EE on the cognitive decline caused by cocaine is the same in females than in males. Numerous works have shown how that EE has a positive influence in female rodents for relevant aspects related with the consumption of drugs, like drug-seeking,⁶⁹ self-administration⁷⁰ or in anxiety and in depressive-like behaviour.⁷¹ Moreover, we cannot assure that the benefits found with enrichment are specifically caused by AHN. For example, the increase in AHN by EE can be induced by growth or brain-derived neurotrophic factor (BDNF), which are not specific regulators of AHN as they could also facilitate other neuroplastic processes—such as axonal branching, neurotransmission, and successful synapse formation— in the ‘old’ neurons.^{72,73}

Despite these limitations, this study supports that, in addition to pharmacological and cognitive therapies, positive and stimulating environmental conditions reduce the drug-induced cognitive decline and hence, could be key factors in the long-term treatment of addiction facilitating abstinence from drug use.^{74,75}

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

LJS and ECO conceived and designed the study; MCMP, LV and LSS conducted the behavioural experiments; MCMP, PT, EC, SGR and JLT conducted the histological study; MCMP and ECO analysed the data; MCMP and ECO wrote the manuscript; MCMP, JLT, LJS and ECO critically reviewed the manuscript; all authors approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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