Author manuscript published in final edited form as: Addiction Biology, 2019. 24(3):458-470. doi: 10.1111/adb.12612

Lysophosphatidic acid-induced increase in adult hippocampal neurogenesis facilitates the forgetting of cocaine-contextual memory

David Ladrón de Guevara-Miranda[1], Román Darío Moreno-Fernández[1], Sara Gil-Rodríguez[1], Cristina Rosell-Valle[1,2], Guillermo Estivill-Torrús[3], Antonia Serrano[4], Francisco J. Pavón[4], Fernando Rodríguez de Fonseca[4], Luis J. Santín[1]*, Estela Castilla-Ortega[4]

[1]Departamento de Psicobiología y Metodología de las Ciencias del Comportamiento, Instituto de Investigación Biomédica de Málaga (IBIMA), Facultad de Psicología, Universidad de Málaga, Spain.

[2]Unidad de Producción de Reprogramación Celular, GMP-Iniciativa Andaluza en Terapia Avanzadas, Junta de Andalucía, Spain.

[3]Unidad de Gestión Clínica de Neurociencias, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Regional Universitario de Málaga, Spain.

[4]Unidad de Gestión Clínica de Salud Mental, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Regional Universitario de Málaga, Spain.

*Correspondence to:

Luis J. Santín Núñez. Departamento de Psicobiología y Metodología de las CC, Facultad de Psicología, Universidad de Málaga, Campus de Teatinos S/N, 29071 Málaga, Spain. Fax: +34 952132621. E-mail: luis@uma.es

Abstract

Erasing memories of cocaine-stimuli associations might have important clinical implications for addiction therapy. Stimulating hippocampal plasticity by enhancing adult hippocampal neurogenesis (AHN) is a promising strategy because the addition of new neurons may not only facilitate new learning but also modify previous connections and weaken retrograde memories. To investigate whether increasing AHN prompted the forgetting of previous contextual cocaine associations, mice trained in a cocaine-induced conditioned place preference (CPP) paradigm were administered chronic intracerebroventricular infusions of lysophosphatidic acid (LPA, an endogenous lysophospholipid with pro-neurogenic actions), ki16425 (a $LPA_{1/3}$ receptor antagonist), or a vehicle solution, and they were

tested 23 days later for CPP retention and extinction. The results of immunohistochemical experiments showed that the LPA-treated mice exhibited reduced long-term CPP retention and an ~two-fold increase in the number of adult-born hippocampal cells that differentiated into mature neurons. Importantly, mediation analyses confirmed a causal role of AHN in reducing CPP maintenance. In contrast, the ki16425-treated mice displayed aberrant responses, with initially decreased CPP retention that progressively increased across the extinction sessions, leading to no effect on AHN. The pharmacological treatments did not affect locomotion or general exploratory or anxiety-like responses. In a second experiment, normal and LPA_1 receptor-deficient mice were acutely infused with LPA, which revealed that LPA_1-mediated signaling was required for LPA-induced proliferative actions. These results suggest that the LPA/LPA_1-pathway acts as a potent *in vivo* modulator of AHN and highlight the potential usefulness of pro-AHN strategies to treat aberrant cognition in those addicted to cocaine.

Keywords: Antagonist ki16425, anxiety, causal mediation analysis, cell proliferation, conditioned place preference CPP, LPA_1 receptor

Author manuscript published in final edited form as: Addiction Biology, 2019. 24(3):458-470. doi: 10.1111/adb.12612

INTRODUCTION

Cocaine use, which is a global burden that entails serious health, economic, legal, and social consequences (United Nations Office on Drugs and Crime, 2016), is associated with a significant risk for the development of a life-long addictive disorder (López-Quintero *et al.* 2011). Once addiction is established, cocaine use becomes a habit that is no longer driven by the desire to experience its gratifying effects. Instead, the stimuli repeatedly associated with the reinforcing properties of cocaine, which are the contextual stimuli that are concomitant to drug intake, can elicit an intense desire for the drug (*craving*), which in turn precipitates uncontrollable drug seeking and relapse and maintains the disorder over time (Everitt 2014). Thus, cocaine addiction can be considered a disorder of aberrant cognition in which recurrent drug-related memories that yield maladaptive behaviors usually coexist with a cognitive decline that impedes new memory acquisition (Vonmoos *et al.* 2014; Castilla-Ortega *et al.* 2017).

The harmful nature of the memories of cocaine-stimuli associations is partially explained by their powerful resistance to extinction and forgetting (Kutlu & Gould 2016). At this point, the hippocampus plays a critical role because it is deeply rooted in the cocaine addiction brain circuitry and is a key region for processing declarative and associative memories (Castilla-Ortega *et al.* 2016b). Clinical research has revealed that those addicted to cocaine exhibit increased hippocampal activity in response to cocaine-associated cues that correlates with the intensity of the *craving* they experienced (Fotros *et al.* 2013; Castilla-Ortega *et al.* 2016b). In preclinical experiments, the processing of drug-context associations has been widely studied using the conditioned place preference (CPP) paradigm, which has revealed that the hippocampus is patently involved in the acquisition, extinction, long-term retention and reinstatement of contextual cocaine memories (Hernández-Rabaza *et al.* 2008; Otis *et al.* 2014; Burgdorf *et al.* 2017).

Recently, the role of adult hippocampal neurogenesis (AHN) in both establishing and maintaining hippocampal-dependent memories has been proposed. AHN is a neuroplastic phenomenon that occurs within the dentate gyrus (DG) region. New neurons, which are continuously generated in the DG subgranular zone, can be recruited to participate in hippocampal-dependent learning, especially

when they show enhanced plasticity at 1-3 weeks of age (Tashiro *et al.* 2007; Deng *et al.* 2009). Interestingly, AHN has been recently revealed to have a dual role in memory. In addition to its well-known ability to facilitate hippocampal learning (Castilla-Ortega *et al.* 2011), the DG remodeling induced by the generation and functional integration of new neurons may promote the forgetting and clearance of previously stored memories (Frankland *et al.* 2013; Akers *et al.* 2014). This newly reported ability of AHN, which has been shown in retrograde contextual associative memories as well as spatial memories (Akers *et al.* 2014; Epp *et al.* 2016), could have implications for the cognitive events related to cocaine addiction. Thus, generating a pool of new hippocampal neurons could potentially facilitate the forgetting of harmful retrograde contextual cocaine memories while providing a useful neurobiological resource for overcoming new learning, because the long-lasting cognitive deficits induced by cocaine are concomitant to impaired neuroplasticity in the DG (Ladrón de Guevara-Miranda *et al.* 2017). Although addiction research has focused more on examining how cocaine modulates AHN rather than how AHN manipulations affect cocaine-related behaviors (Castilla-Ortega *et al.* 2016b, 2017), the available evidence suggests an inverse relationship between retrograde cocaine memory retrieval and the generation of newly born hippocampal neurons. In our previous experiment, a post-training reduction in AHN by a DNA-alkylating agent (temozolomide) potentiated long-term retrieval for cocaine-context associative memories (CPP retention) (Castilla-Ortega *et al.* 2016a). Conversely, the post-training stimulation of AHN by environmental enrichment or physical exercise weakens cocaine-CPP maintenance (Mustroph *et al.* 2011, 2016). However, the newly born hippocampal neurons do not seem to be required for this effect (Mustroph *et al.* 2015). When AHN is manipulated via nonspecific strategies that may trigger additional neurobiological effects, statistical approaches may help to elucidate the specific contribution of AHN to the observed behavior. In this regard, causal mediation analysis models are useful in AHN research (Lazic 2012; Lazic *et al.* 2014). Mediation analysis determine the extent of the contribution of a given measure (a *mediator* –i.e., AHN-) on the relationship between other variables (a causal variable or *predictor* –i.e., the experimental treatment- and an outcome variable or *criterion* –i.e., behavior-; Baron & Kenny 1986).

In accordance with the aforementioned rationale, strategies based on the modulation of AHN may be clinically relevant for treating cocaine addiction. Lysophosphatidic acid (1-acyl-2-sn-glycerol-3-phosphate, LPA) is an endogenous phospholipid with multiple biological functions that acts through

specific G-protein-coupled receptors (LPA_{1-6}) that are ubiquitously distributed throughout the body and nervous system (Noguchi *et al.* 2009; Choi & Chun 2013). Specifically, the LPA_1 receptor is the receptor that has been investigated the most because it is required for LPA-mediated neurotrophic actions, including cell proliferation, differentiation, survival, and myelinization (Estivill-Torrús *et al.* 2008; Noguchi *et al.* 2009; Choi & Chun 2013; García-Díaz *et al.* 2015). The LPA_1 receptor is expressed at high levels in the developing brain, where it is necessary for normal neurodevelopment (Estivill-Torrús *et al.* 2008; Noguchi *et al.* 2009; García-Díaz *et al.* 2015), and, importantly, it also modulates AHN. The LPA_1 receptor is expressed by neural precursor cells of the adult mouse DG, where it acts as a functional marker of AHN (Walker *et al.* 2016), while null mice that constitutively lack the LPA_1 receptor show a notable reduction in AHN (Matas-Rico *et al.* 2008). Although the *in vivo* neurogenic effects of exogenous LPA administration have rarely been investigated, 28 days of intracerebroventricular (i.c.v.) LPA infusion have been shown to increase AHN in mice (Walker *et al.* 2016). Furthermore, LPA signaling is relevant to behavior. Acute central LPA infusion potentiates hippocampal-dependent memory (Dash *et al.* 2004) while increasing anxiety-like responses (Castilla-Ortega *et al.* 2014; Yamada *et al.* 2015). However, studies on LPA_1–null mice that constitutively lack this receptor have revealed that this genotype suffers from altered neurodevelopment that results in exploratory, emotional, and cognitive deficits in adulthood (Santin *et al.* 2009; Castilla-Ortega *et al.* 2010; Pedraza *et al.* 2014). When LPA_1–null mice are challenged with cocaine, they show normal locomotor sensitization, but cocaine-induced conditioned locomotion is absent, possibly because of their cognitive impairments (Blanco *et al.* 2012).

This study aimed to elucidate whether the enhancement of AHN by the repeated central administration of LPA affects retrograde memories for cocaine-context associations. Mice previously trained in a cocaine-induced CPP task received chronic i.c.v. treatments with LPA or ki16425, which is a selective antagonist of the $LPA_{1/3}$ receptors that is widely used in preclinical research (Noguchi *et al.* 2009). The general exploratory and anxiety-like behaviors of the mice were assessed before they were tested for long-term CPP retention and extinction. Importantly, the causal role of AHN in CPP retention was evaluated using mediation analyses (Lazic 2012; Lazic *et al.* 2014). In a second experiment, wild-type and LPA_1-null mice were administered acute LPA or ki16425 infusions to determine the involvement of the LPA_1 receptor in the *in vivo* pro-neurogenic actions of LPA.

MATERIALS AND METHODS

Animals

Twenty-eight male C57BL/6J mice (Janvier Labs, Le Genest-Saint-Isle, France) were used in the first study (Experiment I). The second study (Experiment II) involved 14 male mice from the *Málaga* variant of the LPA_1-null mouse that constitutively lacks the LPA_1 receptor [maLPA_1-null mice, characterized in (Estivill-Torrús *et al.* 2008; Matas-Rico *et al.* 2008)] and 14 male wild-type (WT) mice, which were all from a hybrid C57BL/6J × 129X1/SvJ background. The mice were approximately 12 weeks old when the experiments started, and they were individually housed with nesting material and *ad libitum* access to water and food (temperature: 22 ± 2 °C; 12-h light/dark cycle; lights on at 8:00 a.m.). The procedures, were performed in accordance with the European (Directive 2010/63/UE) and Spanish (Real Decreto 53/2013, Ley 32/2007 and 9/2003, Real Decreto 178/2004, Decreto 320/2010) regulations on animal research and approved by the research ethics committee of the University of Málaga (CEUMA Nº8 2014-A).

Drugs

LPA 18:1 (1-oleoyl-LPA; Tocris Bioscience, Bristol, UK) or ki16425 (ApexBio Technology, Houston, TX, USA) was dissolved in vehicle (fatty acid-free bovine serum albumin; Sigma-Aldrich, Madrid, Spain) at a concentration of 3% in saline (0.9% NaCl). The drugs were microinjected in the left lateral cerebral ventricle at a dose of 20 nM for LPA 18:1 or 400 nM for ki16425 (**supporting information**).

Experiment I

Acquisition of cocaine-induced CPP

Two weeks before the start of the behavioral procedures, C57BL/6J mice were implanted with a guide cannula in their left cerebral ventricle (**supporting information**) and underwent at least 10 days of

postoperative recovery (Fig. 1A). The CPP procedure was conducted in a CPP apparatus that consisted of two similar but distinguishable compartments that were connected by a clear corridor (Panlab SL, Barcelona, Spain). The conditioning protocol has been described previously (Castilla-Ortega *et al.* 2016a; Ladrón de Guevara-Miranda *et al.* 2016, 2017); **supporting information**. Briefly, on day 12, the animals were allowed to freely explore the apparatus for 20 min in a habituation session. Then, the mice were randomly assigned to a COC or SAL group and underwent a conditioning phase over five days (days 15 to 19). The COC mice (n = 20) were administered a daily cocaine injection (20 mg/kg, intraperitoneal, i.p.; Sigma-Aldrich) before being confined in one compartment of the apparatus, and they were administered a daily i.p. injection of saline when they were confined in the opposite compartment. The SAL mice (n = 8) underwent a similar protocol but received saline injections in both daily sessions. Lastly, the acquisition of CPP was evaluated in a test session (day 20), during which the mice had free access to both compartments, as in the habituation session (Fig. 1A).

Chronic intracerebroventricular microinjections

Once the CPP was acquired, the mice were subjected to a withdrawal period of 23 days (Castilla-Ortega *et al.* 2016a) (days 20-43), during which i.c.v. infusions were conducted once a day for 17 days (days 22-26, days 28-33, and days 35-40; **supporting information**; Fig. 1A). The mice from the COC group were then randomly assigned to one of three pharmacological treatments such that they were injected with either LPA 18:1 (COC-LPA; n = 8), ki16425 (COC-ki16425; n = 6), or vehicle (COC-VEH; n = 6), while the SAL mice were given microinjections of vehicle (SAL-VEH; n = 8).

Bromodeoxyuridine administration

Bromodeoxyuridine (BrdU) was administered i.p. once a week during CPP withdrawal (days 25, 32, and 39; Fig. 1A) to label proliferating cells. The mice received two daily doses (75 mg/kg, i.p.) dissolved in saline that were separated by 4 h [adapted from (Castilla-Ortega *et al.* 2016a)].

General behavioral monitoring

During CPP withdrawal, the mice were assessed with the following battery of tests in order to examine whether the pharmacological treatments affected their general behavior: the elevated plus maze (EPM; day 27) and open field (OF; day 34), which assessed anxiety-like behaviors and exploration, and the Y-maze test (day 41), which assessed spatial working memory and continuous spontaneous alternations (Fig. 1A) (**supporting information**). The general behavioral monitoring tests were separated in time in order to assess the effect of the pharmacological treatments at different points throughout the chronic administration. Please note that no drugs were administered on the behavioral testing days to avoid potential interferences. Principal component analyses were conducted to reduce the variables assessed in the EPM and OF tests to a few behavioral dimensions (**supporting information**).

Cocaine-CPP retention and extinction

The mice underwent a long-term CPP retention test on day 43 followed by 12 extinction sessions (days 44-59, excluding weekends; Fig. 1A), which were performed in an identical manner as the habituation and test sessions. The mice were intracardially perfused 24 h after the last extinction session for AHN assessment.

Experiment II

Naïve WT and maLPA$_1$-null mice were briefly anesthetized and randomly administered an acute i.c.v. injection of LPA 18:1 (WT-LPA, *n* = 5; maLPA$_1$-null-LPA, *n* = 5), ki16425 (WT-ki16425, *n* = 5; maLPA$_1$-null-ki16425, *n* = 5), or vehicle (WT-VEH, *n* = 4; maLPA$_1$-null-VEH, *n* = 4) (**supporting information**). The animals remained in their home cages and were not disturbed until they were perfused 24 h later for hippocampal cell proliferation assessments (Fig. 4A).

Histological procedures

Author manuscript published in final edited form as: Addiction Biology, 2019. 24(3):458-470. doi: 10.1111/adb.12612

The intracardiac perfusion solution consisted of 0.1 M phosphate-buffered saline (pH 7.4) and 4% paraformaldehyde. After a 48-h postfixation period at 4 °C, the brains were dissected through the midline and cut into 50-μm coronal sections with a vibratome. The left hemisphere was used to confirm the correct placement of the i.c.v. injections, while the right hippocampus (bregma −1.06 mm to −3.08 mm) was processed by free-floating immunohistochemistry using the biotin-avidin method with the chromogen diaminobenzidine (Matas-Rico *et al.* 2008; Castilla-Ortega *et al.* 2016a). For Experiment I, mouse anti-BrdU (1:500; Developmental Studies Hybridoma Bank, Iowa City, IA, USA) was employed to visualize the cells that had incorporated BrdU and then survived until the end of the experiment. Likewise, we performed double fluorescence immunochemistry of BrdU and rabbit anti-neuronal nuclei (NeuN; 1:500; EMD Millipore Corporation, Billerica, MA, USA) to assess the differentiation of the BrdU-labeled cells into mature neurons. In Experiment II, mouse anti-proliferating cell nuclear antigen (PCNA; 1:1.000; Sigma-Aldrich) was used to evaluate cell proliferation. All antibodies were diluted in phosphate-buffered saline, 0.5% Triton X-100, and 2.5% donkey serum. Cell quantification was conducted in the suprapyramidal DG (SupraDG) and infrapyramidal DG (InfraDG) granular cell layers (**supporting information**). The data are expressed as the number of cells per mm^2.

Statistical analysis

General statistical analysis

Between- and intra-group comparisons were performed using analyses of variance (ANOVAs) followed by the post hoc Fisher's least significant difference tests. The relationships between variables were tested using Pearson's correlations. Only significant results ($P \leq 0.05$) are shown.

Processing of CPP data

For the habituation, test, retention, and extinction sessions, a CPP-Score was calculated [(seconds spent in the cocaine-paired compartment − seconds spent in the saline-paired compartment)/seconds spent in both compartments × 100]. Preference for the cocaine-paired compartment was indicated by

a CPP-Score greater than zero (Poltyrev & Yaka 2013; Castilla-Ortega *et al.* 2016a). The compartments were chosen arbitrarily to calculate the CPP-Score in the SAL mice. The CPP-Scores of the extinction sessions were averaged into blocks of three sessions each.

The change in CPP-Score (∆CPP-Score) was calculated to measure how the CPP varied from the habituation session to the test and retention sessions (∆CPP-Score in the test session = CPP-Score in the test session minus CPP-Score in the habituation session; ∆CPP-Score in the retention session = CPP-Score in the retention session minus CPP-Score in the habituation session). Moreover, the ∆CPP-Score(ext) was calculated to examine how the CPP changed from the retention session through extinction [∆CPP-Score(ext) = CPP-Score in each extinction block minus the CPP-Score in the retention session].

Mediation analysis of the role of AHN in cocaine-CPP retention

To examine whether AHN influenced cocaine-CPP retention after chronic LPA or ki16425 administration, a mediation analysis was conducted using IBM SPSS 20 (IBM Corporation, Armonk, NY, USA) and the PROCESS macro (Hayes, 2013). For either the LPA or ki16425 treatments, we implemented a simple mediation model that included each pharmacological treatment (i.e., LPA -*versus* vehicle-; ki16425 -*versus* vehicle-) as a predictor, the long-term CPP retention (i.e., ∆CPP-Score in the retention session) as a criterion, and AHN (i.e., the number of BrdU+ cells/mm^2 in the DG) as a mediator. The measures were standardized (mean = 0 ± standard deviation = 1), and the analysis was conducted according to the causal steps approach (Baron & Kenny 1986). As is recommended for studies with small sample sizes, the significance of the effects was tested using a bootstrapping method with bias-corrected confidence intervals (CIs; Shrout & Bolger 2002) based on 5,000 bootstrap samples. *P* values ≤ 0.05 were considered statistically significant, and 95% CIs excluding the 0 value were used for the bootstrapping.

RESULTS

Author manuscript published in final edited form as: Addiction Biology, 2019. 24(3):458-470. doi: 10.1111/adb.12612

Experiment I: Pharmacologically induced increase in AHN reduces the long-term retention of cocaine-induced CPP

Both chronic LPA and ki16425 treatments reduced long-term CPP retention without affecting the general behavior of the mice

The test session revealed significant conditioning in the COC-treated mice (Fig. 1B). Subsequent chronic treatment with either LPA or ki16425 reduced long-term CPP maintenance such that only the COC-VEH mice differed from the SAL mice, and they showed a significant preference for the cocaine-paired compartment during the 23-day retention session [repeated-measures ANOVA for CPP-Score: 'session': $F(2,48) = 30.957$, $P < 0.001$; 'treatment × session': $F(6,48) = 4.591$, $P = 0.001$; for the ∆CPP-Score: 'session': $F(1,17) = 5.432$, $P = 0.032$; 'treatment × session': $F(2,17) = 3.871$, $P = 0.041$. The post hoc analyses are shown in Fig. 1B,C).

Although the COC-LPA mice continued to show no preference for the cocaine-paired compartment across the extinction sessions, the preference of the COC-ki16425 mice for the cocaine-paired compartment progressively increased despite their initial CPP-retention attenuation [repeated-measures ANOVA for the CPP-Score: 'session': $F(4,96) = 3.841$, $P = 0.001$; 'treatment × session': $F(12,96) = 2.611$, $P = 0.001$; for the ∆CPP-Score(ext): 'treatment': $F(3,17) = 6.814$, $P = 0.006$; post hoc analyses are shown in Fig. 1E,F).

No differences between groups were found in locomotion across the CPP task (Fig. 1D,G), in the general behavioral tests performed during the pharmacological administration period (**supporting information**, Fig. 1H-J) or in body weight (data not shown).

LPA treatment increased AHN

Chronic LPA administration (COC-LPA mice) notably increased the number of BrdU+ cells in the DG (Fig. 2A,B; one-way ANOVA for the total DG: 'treatment': $F(1,3) = 3.585$; $P = 0.029$; repeated measures ANOVA for the DG blades: 'treatment': $F(1,3) = 3.369$, $P = 0.035$; post hoc analyses shown

in Fig. 2B) and enhanced their differentiation into mature neurons (%BrdU-NeuN colocalization; Fig. 2C,D; one-way ANOVA for the total DG: 'treatment': $F(1,3) = 16.870$; $P < 0.001$; repeated-measures ANOVA for the DG blades: 'treatment': $F(3,22) = 16.770$, $P < 0.001$; post hoc analyses shown in Fig. 2C). However, the levels of the AHN-related markers in both the COC-VEH and COC-ki16425 groups were similar to that of the control SAL-VEH animals (Fig. 2B,C).

Reduction of CPP retention was mediated by AHN in LPA-treated mice

As indicated above, although both LPA and ki16425 administration reduced cocaine-CPP retention, they had different effects on AHN. When the data for the vehicle-treated mice (COC-VEH) were grouped with the data for the LPA-treated mice (COC-LPA), the levels of AHN in the mice were strongly and inversely correlated with CPP retention ($r = -0.706$, $P = 0.005$; Fig. 3A), which suggested that a higher number of neurons generated after CPP acquisition predicted a greater reduction in long-term CPP maintenance. However, AHN did not correlate with CPP retention after ki16425 treatment, and ki16425 treatment did not increase the number of adult-born neurons (Fig. 3D). This result suggested that the enhanced AHN accounted for the attenuated CPP retention in the LPA-treated group, while ki16425 influenced CPP retention through other (AHN-independent) mechanisms. To verify this assumption, causal mediation analyses were conducted.

The first mediation model confirmed a total effect of LPA on CPP retention (total effect, path *c*; Fig. 3B). When AHN was included as a mediator of this effect, a relationship was found between LPA treatment and the number of adult-born neurons in the DG (path *a*), which in turn was associated with CPP retention (path *b*) (Fig. 3B). However, the direct effect of LPA on CPP behavior became nonsignificant (path *c'*; Fig. 3B) when controlling for AHN. The bootstrapping analysis confirmed the significance of the AHN-mediated effect ($a \times b = -0.674$, 95% CI [-1.673 to -0.007]), while the AHN-independent effect (path c') was nonsignificant (Fig. 3C). Overall, these results showed a complete mediation effect of AHN in the reduction of LPA-induced long-term CPP retention.

However, although chronic ki16425 treatment showed a total effect in reducing CPP retention (path *c*), no correlations were found between ki16425 treatment and AHN (path *a*) or between AHN and CPP

Author manuscript published in final edited form as: Addiction Biology, 2019. 24(3):458-470. doi: 10.1111/adb.12612

retention (path *b*), thus eliminating a mediation effect due to hippocampal neurogenesis (Fig. 3E). Accordingly, the ki16425 treatment effect still explained the reduction in CPP retention when controlling for AHN-dependent effects (*c'* = −1.450, 95% CI [-2.459 to -0.441]; Fig. 3E,F).

Experiment II: Acute LPA infusion increased DG cell proliferation only in mice expressing the LPA_1 receptor

Twenty-four hours after a single i.c.v. administration, WT-LPA mice showed a significant increase in proliferating PCNA+ cells in the DG. Interestingly, this was a genotype-dependent effect because it was not reproduced when LPA was administered to maLPA$_1$-null mice [Fig. 4B,C; factorial ANOVA (treatment × genotype) for the total DG: 'treatment': $F(2,21) = 4.003$, $P = 0.034$; 'genotype': $F(1,21) = 38.514$, $P < 0.001$; 'treatment × genotype': $F(2,21) = 5.743$, $P = 0.010$; repeated-measures ANOVA for the DG blades (treatment × genotype × DG blade): 'treatment': $F(2,21) = 3.898$, $P = 0.036$; 'genotype': $F(1,21) = 35.963$, $P < 0.001$; 'treatment × genotype': $F(2,21) = 5.743$, $P = 0.010$; DGBlade: $F(1,21) = 25.934$, $P < 0.001$; the post hoc analyses are shown in Fig. 4C]. The maLPA$_1$-null genotype showed less DG cell proliferation, as previously reported (Matas-Rico *et al.* 2008), while acute ki16425 treatment did not alter cell proliferation in any genotype (Fig. 4B,C).

DISCUSSION

This study aimed to test the hypothesis that modulating AHN after establishing cocaine-context associations would influence the maintenance of these associations. The main finding was that chronic central LPA administration notably increased AHN and weakened the long-term retention (23 days) of a previously acquired cocaine-CPP memory. This result was consistent with the reported ability of pro-neurogenic strategies, such as environmental enrichment and voluntary exercise, to reduce the retrieval or reinstatement of cocaine-induced CPP provided that they are administered subsequent to learning (Solinas *et al.* 2008; Mustroph *et al.* 2011, 2016). Nevertheless, one report has specifically demonstrated that a running-induced increase in AHN is not a mechanism necessary for exercise to abolish previously established cocaine-CPP memories (Mustroph *et al.* 2015). Rather than ruling out a potential role of AHN, this outcome emphasizes that nonspecific strategies such as

exercise generate numerous off-target effects that may be sufficient to modulate behavior (Lazic 2012; Mustroph *et al.* 2015). Statistical approaches such as causal models (mediation analyses) are advantageous as they allow for the examination of the specific contribution of AHN to a behavioral effect, even when nonspecific AHN manipulations are used (Lazic 2012; Lazic *et al.* 2014). In this regard, the LPA treatment likely affected brain regions other than the hippocampus. However, when AHN was statistically controlled (i.e., by suppressing the difference between groups in the number of newborn hippocampal cells), the effect of LPA on CPP retention was no longer significant, thus showing that there were no AHN-independent mechanisms that could account for the reduction in CPP retention exhibited by the COC-LPA group. Therefore, the mediation analysis indicated that the attenuation of cocaine-CPP memories after LPA treatment was mediated by post-learning improvements in AHN.

The results of this study support the idea that the functional role of AHN in hippocampal dependent memory, including cocaine contextual memories, is critically defined by the timing of the generation of new neurons (Akers *et al.* 2014; Castilla-Ortega *et al.* 2017). When AHN is increased *before* cocaine-CPP memory formation, the new highly plastic neurons could be recruited to potentiate the formation of the memory of the cocaine experience, thus enhancing CPP learning and/or maintenance (Smith *et al.* 2009; Mustroph *et al.* 2011; Castilla-Ortega *et al.* 2017). Nevertheless, cocaine-induced CPP can also be acquired in AHN-reduced conditions because animals with low AHN may increase the reinforcing value of cocaine [as elucidated by self-administration studies (Noonan *et al.* 2010)], and they may engage alternate brain circuits to learn the cocaine-context associations, which subsequently become harder to extinguish (Castilla-Ortega *et al.* 2016a). The aforementioned evidence agrees overall with the well-known role of adult-born neurons in facilitating the learning of hippocampal contextual conditioning tasks (Castilla-Ortega *et al.* 2011). This study, however, focused on the involvement of hippocampal neurons that are generated *after* drug-context associations are established. We previously demonstrated that the post-learning reduction of AHN exacerbated long-term CPP retention (Castilla-Ortega *et al.* 2016a). Thus, enhancing AHN within this time period conversely contributed to forgetting (i.e., reduced long-term retention) of the cocaine-CPP memory. At the time of the retention test, new neurons that were affected by the LPA treatment were between 3-21 days old, which is a critical time period of enhanced plasticity, which allows immature neurons to

Author manuscript published in final edited form as: Addiction Biology, 2019. 24(3):458-470. doi: 10.1111/adb.12612

modulate hippocampal circuitry in an experience-specific manner (Tashiro *et al.* 2007; Castilla-Ortega *et al.* 2016b). Especially when they are at an immature stage, new neurons form synapses within the DG and with other hippocampal regions, which are more easily recruited by environmental demands than the older, previously formed synapses (Ramirez-Amaya *et al.* 2006; Deng *et al.* 2009). Considering that new neurons compete for inputs with older neurons to achieve their stable functional integration (Borgmann *et al.* 2016; McAvoy *et al.* 2016), the synaptic connections generated *de novo* in the hippocampus could displace pre-existing, outdated connections, which would eventually degrade retrograde memories (Frankland *et al.* 2013; Akers *et al.* 2014). Although we cannot rule out that AHN also modulated motivational factors, such as cocaine *craving*, the seminal work of the Frankland laboratory (Akers *et al.* 2014; Epp *et al.* 2016) established the role of AHN in forgetting non-drug-related hippocampal memories [i.e., associative memories (contextual fear conditioning and odor–context pairings) and spatial navigation], suggesting that the reduced CPP behavior in the LPA-treated mice can be explained in terms of weakened contextual memories.

Our results also confirmed the *in vivo* pro-AHN action of central LPA administration, as was shown in a previous study (Walker *et al.* 2016). Indeed, mice exposed to repeated i.c.v. injections of LPA displayed a higher number of hippocampal cells that proliferated during cocaine withdrawal and/or survived until the end of the behavioral protocol and that were also more likely to express a mature neuronal phenotype. Importantly, acute LPA infusion increased cell proliferation, and this pro-proliferative effect was suppressed in mice lacking the LPA_1 receptor that, interestingly, are characterized by reduced basal levels of AHN and do not benefit from environmental pro-AHN strategies (Matas-Rico *et al.* 2008). Considering that the LPA_1 receptor is highly expressed in both proliferating and nonproliferating neural precursors in the DG subgranular zone (Walker *et al.* 2016), this evidence supports the effectiveness of exogenous LPA administration as a pharmacological strategy to promote *in vivo* AHN through the critical role of the LPA_1 receptor. Multiple pro-neurogenic actions are likely involved because the downstream signaling pathways that are coupled to the LPA_1 receptor may eventually upregulate cell proliferation [Rho-associated protein kinase (Rho-ROCK) and phospholipase C/protein kinase C (PLC-PKC) pathways], survival [phosphatidylinositol 3-kinase/Akt (PI3K-AKT) pathway], and differentiation/maturation [Ras/mitogen-activated protein kinase (Ras-MAPK) pathway] (Choi & Chun 2013; Walker *et al.* 2016). However, this is the first attempt to assess

how chronic LPA administration may modulate behavioral processes. Despite their reduced cocaine-CPP behavior, the animals chronically treated with LPA showed unaltered locomotor/exploratory activity as well as normal emotional responses in the EPM and OF tasks and preserved spatial working memory (Y-maze continuous spontaneous alternations). These outcomes clearly differ from the actions of an acute i.c.v. LPA infusion, which consistently alters exploration and induces anxiety-like behaviors in both mice and rats (Castilla-Ortega *et al.* 2014; Yamada *et al.* 2015). Acute and chronic LPA are likely to trigger different neurobiological mechanisms because repeated LPA exposure induces significant hippocampal neuroadaptations, such as the AHN increase reported here. Importantly, acute LPA effects were prevented in this study since the pharmacological treatment was never administered on a behavioral testing day.

Finally, we also tested the effects of $LPA_{1/3}$ receptor blockade by the central administration of ki16425 on CPP behavior and AHN. While the *in vitro* blockade of $LPA_{1/3}$ receptor signaling consistently inhibits the trophic cellular responses induced by LPA (Noguchi *et al.* 2009; Choi & Chun 2013; Walker *et al.* 2016), the effects of LPA receptor antagonists in the absence of LPA co-administration are largely unknown. It was therefore surprising that chronic ki16425 treatment decreased CPP retention after cocaine withdrawal similarly to LPA treatment without affecting general exploratory or anxiety-like behaviors. Certainly, agonist and antagonist drugs might converge to the same outcome through different neurochemical processes (e.g., Woods *et al.* 2012). Nevertheless, the resemblance between the COC-LPA and COC-ki16425 mice was only transient because the ki16425-treated group displayed abnormal behavior through the extinction sessions by progressively increasing their CPP response as they were exposed to the apparatus. Overall, the neurobiological mechanisms by which chronic ki16425 reduces initial long-term memory retrieval and hinders its subsequent extinction remain to be elucidated. Repeated antagonism of the $LPA_{1/3}$ receptors could dysregulate intracellular signaling pathways in hippocampal or extra-hippocampal regions involved in memory processing. For example, PI3K signaling is coupled to $LPA_{1/3}$ receptors and is required for both retrieval and extinction of contextual associative memories (Chen *et al.* 2005). In any case, our results show that, contrary to LPA, chronic ki16425 actions seem to not be mediated by AHN. In fact, the AHN-related markers were not influenced by acute or chronic ki16425 administration. It is then possible for LPA_1–mediated signaling to be required for stimulating AHN in supraphysiological conditions, such as after LPA

Author manuscript published in final edited form as: Addiction Biology, 2019. 24(3):458-470. doi: 10.1111/adb.12612

administration but not necessarily for basal AHN maintenance. Another possibility is that our chronic ki16425 treatment did not last long enough to exert significant changes considering that LPA_1–null mice show both AHN impairments and hippocampal-dependent behavioral deficits (Matas-Rico *et al.* 2008; Santin *et al.* 2009; Castilla-Ortega *et al.* 2010). Nevertheless, profound neurodevelopmental alterations in these mice (Estivill-Torrús *et al.* 2008; García-Díaz *et al.* 2015) prevent conclusions regarding the extent to which their phenotype is attributed to the absence of LPA_1 signaling in adulthood.

Overall, the results of this study highlight the usefulness of increasing AHN during cocaine withdrawal as a strategy to promote the forgetting of retrograde cocaine memories, such as the cocaine-context associations that eventually trigger recurrent drug-seeking and relapse. We used a pharmacological approach (central LPA administration), but other pro-AHN strategies, such as environmental manipulations, could be useful to achieve this outcome. Furthermore, potentiating AHN may have additional therapeutic implications for cocaine addiction. DG restructuring and renewal by adult-born neurons may eliminate memories of previous cocaine experiences, but at the same time, they would also facilitate the updating of memories and the learning of new, adaptive information (Epp *et al.* 2016), thus ameliorating the defective cognition frequently displayed by those addicted to cocaine (Vonmoos *et al.* 2014; Castilla-Ortega *et al.* 2017).

Acknowledgments

The authors acknowledge the IBIMA's common research support structure of Animal Experimentation (Animal Facility at the University of Málaga) and their staff for their valuable assistance during the behavioral experiments and maintenance of the mice, IBIMA's common research support structure of Imaging for the use of the white-light microscope, the University of Málaga's Research Support Central Services (SCAI) and David Navas Fernández for the confocal microscopy, and Lorena Moreno Campos for her contribution to the analysis of the behavioral measures.

This study was funded by grants from the Spanish Ministry of Economy and Competitiveness (Agencia Estatal de Investigación), which is cofunded by the European Research Development Fund AEI/FEDER, UE- (PSI2013-44901-P and PSI2017-82604-R to LJS and PSI2015-73156-JIN to ECO);

by the National System of Health-Instituto de Salud Carlos III, which is co-funded by AEI/FEDER, UE (Red de Trastornos Adictivos; RD16/0017/0001 to FRdF); and by the Andalusian R&D&I Programme, Regional Ministry of Economy and Knowledge (PAIDI CTS643 to GET).

DLGM and RDMF hold FPU grants from the Spanish Ministry of Education, Culture and Sports (FPU13/04819 and FPU14-01610, respectively). CRV received a 'Plan Propio' grant from the University of Malaga. FJP and AS hold 'Miguel Servet' grants (CP14/00212 and CP14/00173, respectively) from the National System of Health-Instituto de Salud Carlos III, which is co-funded by AEI/FEDER, UE. FRdF and GET are supported by Nicolas Monardes Programme, from the Andalusian Regional Ministry of Health. ECO holds a 'Jóvenes Investigadores' grant (code: PSI2015-73156-JIN) from the Spanish Ministry of Economy and Competitiveness (Agencia Estatal de Investigación) which is co-funded by the European Research Development Fund (AEI/FEDER, UE).

The authors declare no conflicts of interest.

Authors contribution

LJS and ECO were responsible for the study concept and design. DLGM, RDMF, and SGR performed the animal experiments and histology. CRV, GET, AS, and FJP contributed to the data collection and/or interpretation. DLGM performed the statistical analyses. DLGM, LJS, and ECO wrote the manuscript that was reviewed by FRdF for important intellectual content. All authors critically reviewed the content and approved the final version for publication.

REFERENCES

Akers KG, Martinez-Canabal A, Restivo L, Yiu AP, De Cristofaro A, Hsiang HL, Wheeler AL, Guskjolen A, Niibori Y, Shoji H, Ohira K, Richards BA, Miyakawa T, Josselyn SA, Frankland PW (2014) Hippocampal neurogenesis regulates forgetting during adulthood and infancy. *Science* 344:598–602.

Baron RM and Kenny DA (1986) The moderator-mediator variable distinction in social psychological

research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 51:1173–1182.

Blanco E, Bilbao A, Luque-Rojas MJ, Palomino A, Bermúdez-Silva FJ, Suárez J, Santín LJ, Estivill-Torrús G, Gutiérrez A, Campos-Sandoval JA, Alonso-Carrión FJ, Márquez J, De Fonseca FR (2012) Attenuation of cocaine-induced conditioned locomotion is associated with altered expression of hippocampal glutamate receptors in mice lacking LPA1 receptors. *Psychopharmacology (Berl)* 220:27–42.

Borgmann KF, Gräff J, Mansuy I, Toni N, Jessberger S (2016) Enhanced plasticity of mature granule cells reduces survival of newborn neurons in the adult mouse hippocampus. *Matters Sel*.

Burgdorf CE, Schierberl KC, Lee AS, Fischer DK, Van Kempen TA, Mudragel V, Huganir RL, Milner TA, Glass MJ, Rajadhyaksha AM (2017) Extinction of contextual cocaine memories requires Cav1.2 within D1R-expressing cells and recruits hippocampal Cav1.2-dependent signaling mechanisms. *J Neurosci* 37:11894–11911.

Castilla-Ortega E, Blanco E, Serrano A, Ladrón De Guevara-Miranda D, Pedraz M, Estivill-Torrús G, Pavón FJ, Rodríguez De Fonseca F, Santín LJ (2016a) Pharmacological reduction of adult hippocampal neurogenesis modifies functional brain circuits in mice exposed to a cocaine conditioned place preference paradigm. *Addict Biol* 21:575–588.

Castilla-Ortega E, Escuredo L, Bilbao A, Pedraza C, Orio L, Estivill-Torrús G, Santín LJ, De Fonseca FR, Pavón FJ (2014) 1-Oleoyl lysophosphatidic acid: a new mediator of emotional behavior in rats. *PLoS One* 9: e85348.

Castilla-Ortega E, Ladrón de Guevara-Miranda D, Serrano A, Pavón FJ, Suárez J, Rodríguez de Fonseca F, Santín LJ (2017) The impact of cocaine on adult hippocampal neurogenesis: potential neurobiological mechanisms and contributions to maladaptive cognition in cocaine addiction disorder. *Biochem Pharmacol* 14:100–117.

Castilla-Ortega E, Pedraza C, Estivill-Torrús G, Santín LJ (2011) When is adult hippocampal neurogenesis necessary for learning? Evidence from animal research. *Rev Neurosci* 22:267–283.

Castilla-Ortega E, Sánchez-López J, Hoyo-Becerra C, Matas-Rico E, Zambrana-Infantes E, Chun J, De Fonseca FR, Pedraza C, Estivill-Torrús G, Santin LJ (2010) Exploratory, anxiety and spatial memory impairments are dissociated in mice lacking the LPA1 receptor. *Neurobiol Learn Mem*

94:73–82.

Castilla-Ortega E, Serrano A, Blanco E, Araos P, Suárez J, Pavón FJ, Rodríguez de Fonseca F, Santín LJ (2016b) A place for the hippocampus in the cocaine addiction circuit: potential roles for adult hippocampal neurogenesis. *Neurosci Biobehav Rev* 66:15–32.

Chen X, Garelick MG, Wang H, Li V, Athos J, Storm DR (2005) PI3 kinase signaling is required for retrieval and extinction of contextual memory. *Nat Neurosci* 8:925–931.

Choi J and Chun J (2013) Lysophospholipids and their receptors in the central nervous system. *Biochim Biophys Acta* 1831:20–32.

Dash PK, Orsi SA, Moody M, Moore AN (2004) A role for hippocampal Rho-ROCK pathway in long-term spatial memory. *Biochem Biophys Res Commun* 322:893–898.

Deng W, Saxe MD, Gallina IS, Gage FH (2009) Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. *J Neurosci* 29:13532–13542.

Epp JR, Silva-Mera R, Köhler S, Josselyn SA, Frankland PW (2016) Neurogenesis-mediated forgetting minimizes proactive interference. *Nat Commun* 7:10838.

Estivill-Torrús G, Llebrez-Zayas P, Matas-Rico E, Santín L, Pedraza C, De Diego I, Del Arco I, Fernández-Llebrez P, Chun J, De Fonseca FR (2008) Absence of LPA1 signaling results in defective cortical development. *Cereb Cortex* 18:938–950.

Everitt BJ (2014) Neural and psychological mechanisms underlying compulsive drug seeking habits and drug memories - indications for novel treatments of addiction. *Eur J Neurosci* 40:2163–2182.

Fotros A, Casey KF, Larcher K, Verhaeghe JA, Cox SM, Gravel P, Reader AJ, Dagher A, Benkelfat C, Leyton M (2013) Cocaine cue-induced dopamine release in amygdala and hippocampus: a high-resolution PET [18F] fallypride study in cocaine dependent participants. *Neuropsychopharmacology* 38:1780–1788.

Frankland PW, Köhler S, Josselyn SA (2013) Hippocampal neurogenesis and forgetting. *Trends Neurosci* 36:497–503.

García-Díaz B, Riquelme R, Varela-Nieto I, Jiménez AJ, de Diego I, Gómez-Conde AL, Matas-Rico E, Aguirre JÁ, Chun J, Pedraza C, Santín LJ, Fernández O, Rodríguez de Fonseca F, Estivill-Torrús G (2015) Loss of lysophosphatidic acid receptor LPA1 alters oligodendrocyte differentiation and myelination in the mouse cerebral cortex. *Brain Struct Funct* 220:3701–3720.

Hayes AF (2013) *Introduction to mediation, moderation, and conditional process analysis: a*

regression-based approach. New York, NY: Guilford Press.

Hernández-Rabaza V, Hontecillas-Prieto L, Velázquez-Sánchez C, Ferragud A, Pérez-Villaba A, Arcusa A, Barcia JA, Trejo JL, Canales JJ (2008) The hippocampal dentate gyrus is essential for generating contextual memories of fear and drug-induced reward. *Neurobiol Learn Mem* 90:553–559.

Kutlu MG and Gould TJ (2016) Effects of drugs of abuse on hippocampal plasticity and hippocampus-dependent learning and memory: contributions to development and maintenance of addiction. *Learn Mem* 23:515–533.

Ladrón de Guevara-Miranda D, Millón C, Rosell-Valle C, Pérez-Fernández M, Missiroli M, Serrano A, Pavón FJ, Rodríguez de Fonseca F, Martínez-Losa M, Álvarez-Dolado M, Santín LJ, Castilla-Ortega E (2017) Long-lasting memory deficits in mice withdrawn from cocaine are concomitant with neuroadaptations in hippocampal basal activity, GABAergic interneurons and adult neurogenesis. *Dis Model Mech* 10:323–336.

Ladrón de Guevara-Miranda D, Pavón FJ, Serrano A, Rivera P, Estivill-Torrús G, Suárez J, Rodríguez de Fonseca F, Santín LJ, Castilla-Ortega E (2016) Cocaine-conditioned place preference is predicted by previous anxiety-like behavior and is related to an increased number of neurons in the basolateral amygdala. *Behav Brain Res* 298:35–43.

Lazic SE (2012) Using causal models to distinguish between neurogenesis-dependent and -independent effects on behaviour. *J R Soc Interface* 9:907–917.

Lazic SE, Fuss J, Gass P (2014) Quantifying the behavioural relevance of hippocampal neurogenesis. *PLoS One* 9: e113855.

López-Quintero C, Pérez de los Cobos J, Hasin DS, Okuda M, Wang S, Grant BF, Blanco C (2011) Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: results of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *Drug Alcohol Depend* 115:120–130.

Matas-Rico E, García-Diaz B, Llebrez-Zayas P, López-Barroso D, Santín L, Pedraza C, Smith-Fernández A, Fernández-Llebrez P, Tellez T, Redondo M, Chun J, De Fonseca FR, Estivill-Torrús G (2008) Deletion of lysophosphatidic acid receptor LPA1 reduces neurogenesis in the mouse dentate gyrus. *Mol Cell Neurosci* 39:342–355.

McAvoy KM, Scobie KN, Berger S, Russo C, Guo N, Decharatanachart P, Vega-Ramirez H, Miake-

Lye S, Whalen M, Nelson M, Bergami M, Bartsch D, Hen R, Berninger B, Sahay A (2016) Modulating neuronal competition dynamics in the dentate gyrus to rejuvenate aging memory circuits. *Neuron* 91:1356–1373.

Mustroph ML, Merritt JR, Holloway AL, Pinardo H, Miller DS, Kilby CN, Bucko P, Wyer A, Rhodes JS (2015) Increased adult hippocampal neurogenesis is not necessary for wheel running to abolish conditioned place preference for cocaine in mice. *Eur J Neurosci* 41:216–226.

Mustroph ML, Pinardo H, Merritt JR, Rhodes JS (2016) Parameters for abolishing conditioned place preference for cocaine from running and environmental enrichment in male C57BL/6J mice. *Behav Brain Res* 312:366–373.

Mustroph ML, Stobaugh DJ, Miller DS, Deyoung EK, Rhodes JS (2011) Wheel running can accelerate or delay extinction of conditioned place preference for cocaine in male C57BL/6J mice, depending on timing of wheel access. *Eur J Neurosci* 34:1161–1169.

Noguchi K, Herr D, Mutoh T, Chun J (2009) Lysophosphatidic acid (LPA) and its receptors. *Curr Opin Pharmacol* 9:15–23.

Noonan MA, Bulin S, Fuller DC, Eisch AJ (2010) Reduction of adult hippocampal neurogenesis confers vulnerability in an animal model of cocaine addiction. *J Neurosci* 30:304–315.

Otis JM, Fitzgerald MK, Mueller D (2014) Inhibition of hippocampal β-adrenergic receptors impairs retrieval but not reconsolidation of cocaine-associated memory and prevents subsequent reinstatement. *Neuropsychopharmacology* 39:303–310.

Pedraza C, Sánchez-López J, Castilla-Ortega E, Rosell-Valle C, Zambrana-Infantes E, García-Fernández M, Rodriguez de Fonseca F, Chun J, Santín LJ, Estivill-Torrús G (2014) Fear extinction and acute stress reactivity reveal a role of LPA(1) receptor in regulating emotional-like behaviors. *Brain Struct Funct* 219:1659–1672.

Poltyrev T and Yaka R (2013) Influence of abstinence and intervals between extinction trials on the expression of cocaine-conditioned place preference in adolescent rats. *Psychopharmacology (Berl)* 226:699–706.

Ramirez-Amaya V, Marrone DF, Gage FH, Worley PF, Barnes CA (2006) Integration of new neurons into functional neural networks. *J Neurosci* 26:12237–12241.

Santin LJ, Bilbao A, Pedraza C, Matas-Rico E, López-Barroso D, Castilla-Ortega E, Sánchez-López J, Riquelme R, Varela-Nieto I, De La Villa P, Suardíaz M, Chun J, De Fonseca FR, Estivill-Torrús G

(2009) Behavioral phenotype of maLPA1-null mice: increased anxiety-like behavior and spatial memory deficits. *Genes, Brain Behav* 8:772–784.

Shrout PE and Bolger N (2002) Mediation in experimental and nonexperimental studies: new procedures and recommendations. *Psychol Methods* 7:422–445.

Smith MA, Iordanou JC, Cohen MB, Cole KT, Gergans SR, Lyle MA, Schmidt KT (2009) Effects of environmental enrichment on sensitivity to cocaine in female rats: importance of control rates of behavior. *Behav Pharmacol* 20:312–321.

Solinas M, Chauvet C, Thiriet N, El Rawas R, Jaber M (2008) Reversal of cocaine addiction by environmental enrichment. *Proc Natl Acad Sci U S A* 105:17145–17150.

Tashiro A, Makino H, Gage FH (2007) Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. *J Neurosci* 27:3252–3259.

United Nations Office of Drug and Crime (2016). *World Drug Report*. United Nations publication, Sales No. E.16.XI.7.

Vonmoos M, Hulka LM, Preller KH, Minder F, Baumgartner MR, Quednow BB (2014) Cognitive impairment in cocaine users is drug-induced but partially reversible: evidence from a longitudinal study. *Neuropsychopharmacology* 39:1–11.

Walker TL, Overall RW, Vogler S, Sykes AM, Ruhwald S, Lasse D, Ichwan M, Fabel K, Kempermann G (2016) Lysophosphatidic acid receptor is a functional marker of adult hippocampal precursor cells. *Stem Cell Reports* 6:552–565.

Woods S, Clarke NN, Layfield R, Fone KCF (2012) 5-HT6 receptor agonists and antagonists enhance learning and memory in a conditioned emotion response paradigm by modulation of cholinergic and glutamatergic mechanisms. *Br J Pharmacol* 167:436–449.

Yamada M, Tsukagoshi M, Hashimoto T, Oka JI, Saitoh A, Yamada M (2015) Lysophosphatidic acid induces anxiety-like behavior via its receptors in mice. *J Neural Transm* 122:487–494.

FIGURE LEGENDS

Figure 1. Impact of chronic intracerebroventricular (i.c.v.) treatments on conditioned place preference (CPP) retention and extinction. (A) Design of Experiment I. The boxes represent weeks. (B, C) Despite similar CPP acquisition, chronic treatments with both lysophosphatidic acid (LPA) and ki16425 attenuated the long-term retention of CPP, and only the cocaine (COC)-vehicle (VEH) (COC-VEH) mice differed from the saline group and maintained a significant preference for the cocaine-paired compartment after 23 days of CPP withdrawal. (E, F) Compared to the other cocaine-treated groups, the COC-ki16425 mice progressively increased their preference for the cocaine-paired compartment during CPP extinction. In C and F, normalized habituation and retention data, respectively, are represented in the graphs but not included in the statistical analyses. (D, G) No between-group differences were found in locomotion across the CPP testing. (H-J) Chronic pharmacological treatments did not alter the mice's anxiety-like and exploratory behaviors. The results in H and I are expressed as behavioral dimensions resulting from a factorial analysis performed on the registered variables (**supporting information**).

Post hoc least significant difference (LSD) comparisons: difference vs. the other three groups: $*P \leq 0.05$; difference vs. COC-LPA and saline (SAL)-VEH: $\$P \leq 0.05$; difference vs. COC-VEH: $\&P \leq 0.05$. All data are represented as the mean ± standard error of the mean (SEM).

Figure 2. Increases in adult hippocampal neurogenesis induced by chronic LPA treatment. (A, B) The COC-LPA mice showed approximately 50% more adult-born hippocampal cells compared to the other groups. The images correspond to representative bromodeoxyuridine (BrdU) staining of the dentate gyrus (DG) in each group; the arrows indicate examples of positive cells. (C, D) LPA treatment also enhanced the differentiation of cells into mature neurons. The image displays an example of a BrdU-neuronal nuclei (NeuN) co-labelling in the DG (arrow), which was observed with confocal microscopy. The results were similar for both the suprapyramidal DG (SupraDG) and infrapyramidal DG (InfraDG) blades.

Post hoc LSD differences vs. the other three groups: $*P \leq 0.05$; $**P \leq 0.001$. The data are expressed as the mean ± SEM.

Author manuscript published in final edited form as: Addiction Biology, 2019. 24(3):458-470. doi: 10.1111/adb.12612

Figure 3. Increased adult hippocampal neurogenesis (AHN) led to the reduction of cocaine-CPP retention in the LPA-treated group. (A) LPA-induced AHN was inversely correlated with CPP retention. (B) Mediation model for the LPA condition. The causal steps analysis revealed that LPA treatment reduced the long-term retention of CPP by increasing the number of newborn neurons in the DG (paths *a* and *b*). The results indicated a complete mediation of AHN because the total effect of LPA on behavior (path *c*) became nonsignificant when the contribution of AHN was eliminated (path *c'*). (C) Bootstrapping testing confirmed that enhancing AHN via LPA significantly explained the reduction observed in CPP retention. (D) There was no correlation between AHN and CPP retention for the COC-ki16425 mice. (E) The mediation model for the ki16425 condition showed that the effect of the treatment was not mediated by AHN because the *a* and *b* paths were nonsignificant. (F) Testing for the significance of effects indicated that the contribution of AHN to the reduction of CPP experienced by the COC-ki16425 mice was nonsignificant. The data of (B) and (E) are displayed as coefficient (standard error), and the signs indicate the direction of the correlations. The categorical values for the treatments were 0 (COC-VEH) and 1 (COC-LPA or COC-ki16425). Significant correlation values: $*P \leq 0.05$; $**P \leq 0.01$. The gray lines in (C) and (F) represent the 95% confidence intervals (CIs), and the sign of the standardized effects indicates whether they were a reducer (–) or an enhancer (+) of the CPP-Score. In (C, F), significant effects (i.e., 95% CI excluding 0) are indicated by (*).

Figure 4. LPA increased hippocampal cell proliferation. (A) Design of Experiment II. (B, C) A single i.c.v. injection of LPA increased the number of proliferating cells in the DG 24 h after the treatment, and this effect was dependent on the presence of the LPA_1 receptor. The images show representative proliferating cell nuclear antigen (PCNA) staining in the DG of each group. (D) Representative immunostaining of LPA_1 receptor expression in the subgranular zone of the DG in wild-type (WT) and maLPA_1-null mice. The antibody was rabbit anti-LPA_1 (diluted 1:500; Cayman Chemical, Ann Arbor, MI, USA).

The arrows indicate examples of positive cells. Post hoc LSD differences vs. all other groups: $*P \leq 0.05$. All data are displayed as the mean ± SEM.

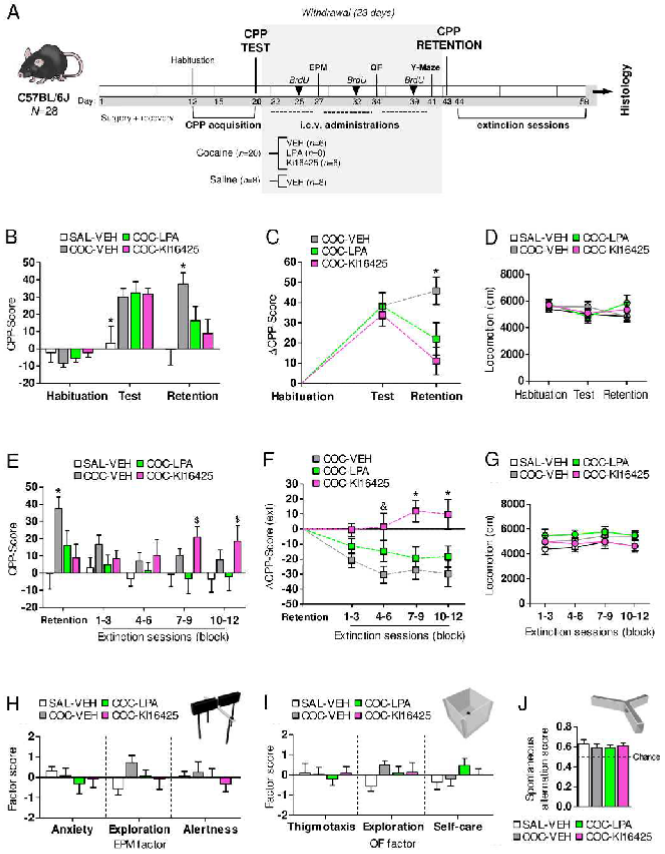

Figure 1

297x420mm (300 x 300 DPI)

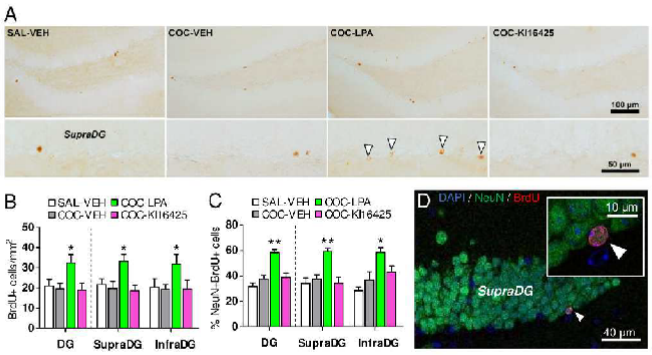

Figure 2

297x420mm (300 x 300 DPI)

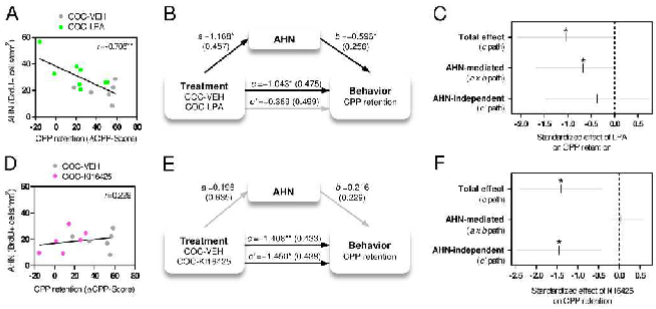

Figure 3

297x420mm (300 x 300 DPI)

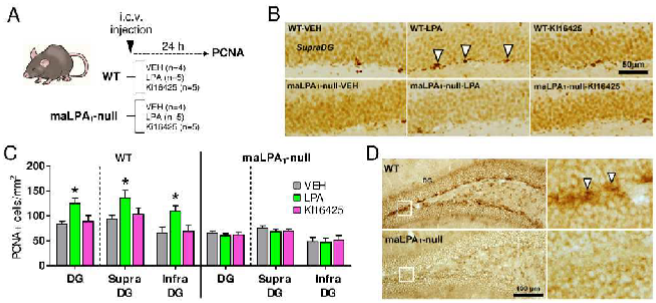

Figure 4

297x420mm (300 x 300 DPI)