Palmitoylethanolamide attenuates cocaine-induced behavioral sensitization and conditioned place preference in mice

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Abstract

Cocaine addiction is a chronically relapsing disorder characterized by compulsive drug-seeking and drug-taking ~~behaviours~~behaviors. Previous studies have demonstrated that cocaine, as well as other drugs of abuse, alters the levels of lipid-based ~~signalling~~signaling molecules, such as N-acylethanolamines (NAEs). Moreover, brain levels of NAEs have shown sensitivity to cocaine self-administration and extinction training in rodents. Given this background, the aim of this study was to investigate the effects of repeated or acute administration of palmitoylethanolamide (PEA), an endogenous NAE, on psychomotor sensitization and cocaine-induced contextual conditioning. To this end, the potential ability of repeated PEA administration (1 or 10 mg/kg, i.p.) to modulate the acquisition of cocaine-induced ~~behavioural~~behavioral sensitization (BS) and conditioned place preference (CPP) was assessed in male C57BL/6J mice. In addition, the expression of cocaine-induced BS and CPP following acute PEA administration were also studied. Results showed that repeated administration of both doses of PEA were able to block the acquisition of cocaine-induced BS. Furthermore, acute administration of both doses of PEA was able to abolish the expression of BS, while the highest dose also abolished the expression of cocaine-induced CPP. Taken together, these results indicate that exogenous administration of PEA attenuated psychomotor sensitization, while the effect of PEA in cocaine-induced CPP depended on whether PEA was administered repeatedly or acutely. These findings could be relevant to understand the role that NAEs play in processes underlying the development and maintenance of cocaine addiction.

Abbreviations: AEA, anandamide; BS, ~~behavioural~~behavioral sensitization; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CL, conditioned locomotion; Coc, cocaine; CPP, conditioned place preference; CS+, positive conditioned stimulus; CS-, neutral conditioned stimulus; EPM, elevated plus maze test; FAAH, fatty acid amide hydrolase; GPR119, orphan G protein-coupled receptor GPR119; GPR55, orphan G protein-coupled receptor GPR55; HB, hole-board test; i.p., intraperitoneally; NAE, *N*-acylethanolamines; NAPE, *N*-acylphosphatidylethanolamines; OEA, oleoylethanolamide; OF, open field; PEA, palmitoylethanolamide; PPARα, nuclear peroxisome proliferator-activated receptor alpha; TRPV1, transient receptor potential vanilloid 1; URB597, FAAH inhibitor; Veh, vehicle; WY-14643, selective PPARα agonist

Keywords: Cocaine; Palmitoylethanolamide; Locomotor sensitization; Conditioned locomotion; Conditioned place preference

~~1.~~1 Introduction

N-acylethanolamines (NAEs) are endogenous bioactive lipid mediators that are involved in a wide range of physiological activities. These lipid transmitters are synthesized on demand from membrane phospholipids called *N*-acylphosphatidylethanolamines (NAPEs) in the brain, and they are not stored in intracellular compartments (Hansen, 2010; Rodríguez de Fonseca et al., 2005). These compounds include oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), which bind to the nuclear peroxisome proliferator-activated receptor alpha (PPARα), the transient receptor potential vanilloid 1 (TRPV1), and the orphan G protein-coupled receptors GPR55 and GPR119 (Godlewski et al., 2009; Hansen, 2010). OEA and PEA act peripherally, through PPARα, as satiety signals that modulate lipid metabolism, feeding behavio~~u~~r and body weight (Fu et al., 2003; Rodríguez de Fonseca et al., 2001). Substantial evidence has also shown that OEA and PEA possess antinociceptive and anti-inflammatory effects (D'Agostino et al., 2009; LoVerme et al., 2006; Suardíaz et al., 2007), as well as neuroprotective activity trough activation of PPARα (Lombardi et al., 2007; Scuderi et al., 2014; Sun and Bennett, 2007). In addition to these multiple physiological activities, there is increasing evidence suggesting that OEA and PEA participate in the regulation of reward-related ~~behaviours~~behaviors (Fu et al., 2008; Hansen and Diep, 2009), such as those involved in addictive behavio~~u~~r to several drugs of abuse (Bilbao et al., 2015; Bystrowska et al., 2014; Mascia et al., 2011; Melis et al., 2008). For instance, cocaine self-administration and extinction training alter the levels of NAEs, including OEA and PEA, in specific regions of the brain reward system (Bystrowska et al., 2014). It has also been shown that inhibition of fatty acid amide hydrolase (FAAH) (Luchicchi et al., 2010), which increases the bioavailability of NAEs, and exogenous administration of OEA and PEA (Melis et al., 2008) block nicotine-induced activation of neurons in the nucleus accumbens shell and ventral tegmental area. These effects are mediated by type-1 cannabinoid receptors (CB1) (Luchicchi et al., 2010) and PPARα receptors, while PPARα agonists have also been shown to modulate nicotine rewarding effects and reinstatement (Mascia et al., 2011). Regarding cocaine-related ~~behaviours~~behaviors, recent studies have demonstrated that OEA is able to block the expression of cocaine conditioned responses induced after repeated administration of the drug (Bilbao et al., 2013). Nevertheless, as far as we know, there are no studies on the potential role of exogenous administration of PEA in ~~behavioural~~behavioral processes related to cocaine addiction.

In the field of addiction, animal models are frequently employed to study the cognitive processes related to drug abuse and relapse. ~~Behavioural~~Behavioral sensitization (BS) and conditioned place preference (CPP) are two paradigms extensively used to study the rewarding effects of different drugs of abuse in mice or rats (Sanchis-Segura and Spanagel, 2006). BS to cocaine, and to other psychomotor stimulant drugs, is characterized by a progressive increment of drug-induced ~~behavioural~~behavioral responses (e.g. motor activity, stereotyped behavio~~u~~r) throughout the repetitive administration of the drug (Robinson and Berridge, 1993). Sensitization is often composed of an initial induction phase (also called acquisition phase), in which the drug is administrated repeatedly, followed by a withdrawal period, and finally a re-exposure to the drug that allows the expression of the sensitized response (Pierce and Kalivas, 1997; Robinson and Berridge, 1993). On the other hand, when vehicle-treated animals are re-exposed to the environment (e.g. an open field) where repeated drug administration had taken place, they show an increased locomotor activity compared with those animals that did not receive repeated drug administration. This phenomenon, known as conditioned locomotion (CL), indicates that animals have learned to associate the environment (conditioned stimulus) with the drug-induced motor response (unconditioned response) developing an implicit memory (Galeano et al., 2013). The CPP paradigm has been extensively used to study the conditioned rewarding effects of addictive drugs by repeated pairing drug effects with an initially neutral context, resulting in an explicit memory of the remembered place as preferred or ~~favourite~~favorite (Tzschentke, 2007).

In the present study, we aimed to investigate whether repeated and acute PEA administrations were able to modulate acquisition and/or expression of cocaine-induced BS, CL and CPP. Furthermore, in order to evaluate whether the acute administration of PEA was able to induce other ~~behavioural~~behavioral effects that could influence the cocaine-related ~~behaviours~~behaviors studied, exploratory activity, anxiety-like ~~behaviours~~behaviors and general neurological status were also assessed.

~~2.~~2 Materials and methods

~~2.1.~~2.1 Animals

Three-month-old C57BL/6 male mice (25–30 g; Charles River, Barcelona, Spain) were employed for this study. Animals were single-housed under controlled environmental conditions [temperature (20 ± 2 °C); humidity (40 ± 5%), 12:12 h light/dark cycle (lights on at 08:00 p.m.)]. Food (standard diet, A04 SAFE Panlab, Barcelona, Spain) and tap water were provided ad libitum. To minimize the potential stress induced by injections, each mouse was handled, in the testing room, for 5 min along five consecutive days before the beginning of each experiment. All experiments were carried out in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments)

guidelines and in accordance with the European Communities Council Directives 2010/63/UE, 90/219/CEE, Regulation (EC) N° 1946/2003 and Spanish National and Regional Guidelines for Animal Experimentation (Real Decreto 53/2013). Experimental protocols were approved by the Local Ethical Committee for Animal Research of the University of Málaga (Cod. 2014-0006).

~~2.2.~~2.2 Drugs

Cocaine hydrochloride (Sigma-Aldrich Chemie GmbH, Munich, Germany) was dissolved in physiological saline solution (0.9~~-~~% NaCl) and it was daily prepared before its use. The dose of cocaine applied was 20 mg/kg, except for the "priming" injection when 10 mg/kg were employed. PEA (Tocris Bioscience, Bristol, UK) was dissolved in 5~~-~~% Tween-80 and physiological saline solution. The doses of PEA administered were 1 or 10 mg/kg. Since, as far as we know, the effects of PEA on psychostimulant-induced behaviors were not previously reported, the doses of PEA were chosen based on previous works where these doses showed to possess anti-inflammatory (Di Paola et al., 2012; Esposito et al., 2012), anti-nociceptive (Luongo et al., 2013) and anti-epileptic (Sheerin et al., 2004) effects; being the first two the most well-known properties of PEA.

Both drugs (cocaine and PEA) were injected intraperitoneally (i.p.) in a final volume of 5 ml/kg of body weight and injections were administered on the left or right side of the peritoneum alternatively. In every experiment (but see Section 2.5.1), PEA was administered 30 min before each trial while cocaine was administered immediately before the beginning of the trial.

~~2.3.~~2.3 Cocaine-induced ~~behavioural~~behavioral sensitization protocol

The ~~behavioural~~behavioral sensitization protocol was a modified version of the previously described by Blanco et al. (2016) and Galeano et al. (2013). The apparatuses used were four open field (OF) arenas made of grey Plexiglas with the following dimensions: 40~~ x~~ x 40~~ x~~ x 30 cm. (Panlab, Barcelona, Spain). Light intensity in the centre of the arenas was 60~~-~~–75~~ lux~~lx, similar to those employed in one our previous studies (Galeano et al., 2013). Mice were daily habituated to the testing room for at least 30 min. Immediately after cocaine or vehicle (saline solution) administration, mice were individually placed in the centre of the OF and allowed to freely explore the apparatus for 30 min (trial duration). Every trial was digitally recorded (Sony DCR-SX22E) and later ~~analysed~~analyzed using a video-tracking system (Ethovision XT 5.0., Noldus Information Technology, Wageningen, The Netherlands). The dependent variable was the total distance ~~travelled~~traveled. The cocaine sensitization protocol consisted of four phases: 1) Basal locomotion phase. On the first day, each mouse was injected with vehicle and received one trial (30 min) in the OF to measure basal locomotion; 2) Cocaine conditioning phase. From the second to the sixth day, each mouse received a daily injection of cocaine (20 mg/kg) or vehicle and the total distance ~~travelled~~traveled in the OF during 30 min was assessed. This phase was considered as the pre-treatment; 3) Cocaine conditioned locomotion phase. On the eighth day, following one day of withdrawal (when mice were left undisturbed in their home cage), mice received a saline injection and were immediately re-exposed to the OF for 30 min. The total distance ~~travelled~~traveled was considered as an operationalized measurement of the cocaine-induced CL; 4) Cocaine sensitization phase. On the twelfth day, mice received a priming injection of cocaine (10 mg/kg) and the total distance ~~travelled~~traveled was measured during 30 min to assess BS.

~~2.3.1.~~2.3.1 Experiment 1. Effects of repeated PEA administration on the acquisition of cocaine-induced conditioned locomotion and ~~behavioural~~behavioral sensitization

The objective of this first experiment was to evaluate the effects of repeated administrations of PEA on the acquisition of cocaine-induced conditioned locomotion (CL) and ~~behavioural~~behavioral sensitization (BS). To this end, thirty naive male mice were randomly assigned to four groups (n = 7~~-~~–8 mice per group) and the cocaine sensitization protocol described in Section 2.3 was carried out with two modifications: 1) In the basal locomotion phase, mice were administered vehicle or PEA (1 or 10 mg/kg) (Fig. 1A); 2) During the cocaine conditioning phase, mice were injected with vehicle, cocaine (20 mg/kg) or cocaine (20 mg/kg) plus PEA (1 or 10 mg/kg) (Fig. 1A). The rest of the protocol remained the same.

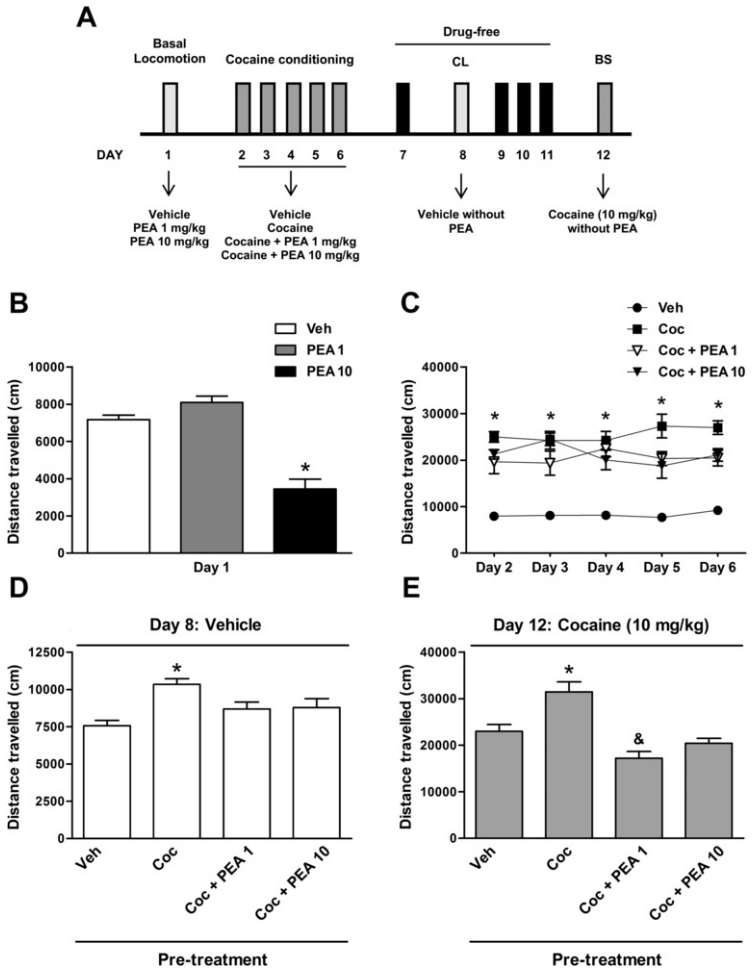

Fig. 1 Acquisition of conditioned locomotion and ~~behavioural~~behavioral sensitization to cocaine is blocked by repeated pre-treatment with PEA. (A) Schematic representation of the conditioned locomotion (CL) and ~~behavioural~~behavioral sensitization (BS) protocol employed (for a detaile description see Sections 2.3 and 2.3.1.). (B) During the basal locomotion phase, mice administered with 10 mg/kg of PEA ~~travelled~~traveled a shorter distance than mice administered with either vehicle or 1 mg/kg of PEA. (C) In the cocaine conditioning phase, all groups administered with cocaine ~~travelled~~traveled longer distances than the group of mice administered with vehicle. (D) In the CL session, the only group of mice that showed a CL response was that pre-treated with cocaine, indicating that pre-treatment with both doses of PEA blocked the acquisition o the CL to cocaine. (E) In the BS session, mice treated with a priming injection of cocaine (10 mg/kg) and pre-treated with cocaine (20 mg/kg), ~~travelled~~traveled a longer distance than the rest of groups, demonstrating that repeated pre-treatment with PEA blocked the acquisition of cocaine-induce BS. Data are expressed as the mean ± SEM of 7–8 mice per group. *p < ~~0~~.05 vs. all other groups (in B, D and E) and vs. mice administered with vehicle (in C); &p < ~~0~~.05 vs. vehicle-pre-treated group (in E).

alt-text: Fig. 1

~~2.3.2.~~2.3.2 *Experiment 2. Effects of acute PEA administration on the expression of cocaine-induced conditioned locomotion and ~~behavioural~~behavioral sensitization*

The aim of this second experiment was to assess the effects of the acute administration of PEA on the expression of BS to cocaine. In addition, the effects of acute PEA administration on cocaine-induced CL were assessed. Forty-one naive male mice were randomly assigned to four groups (n = 8–11 mice per group) and subjected to the cocaine sensitization protocol described in 2.3 with the following modifications: 1) During the cocaine conditioned locomotion phase, mice pre-treated with cocaine in the previous phase (cocaine conditioning phase) were injected with either vehicle or PEA (1 or 10 mg/kg) (Fig. 2A); 2) In the cocaine sensitization phase, mice pre-treated with cocaine during the cocaine conditioning phase were challenged with

either a “priming” dose of cocaine (10 mg/kg) or a “priming” dose of cocaine (10 mg/kg) plus PEA (1 or 10 mg/kg); while every mouse pre-treated with vehicle received an acute cocaine injection at the same dose used in the “priming” injection (10 mg/kg) (Fig. 2A).

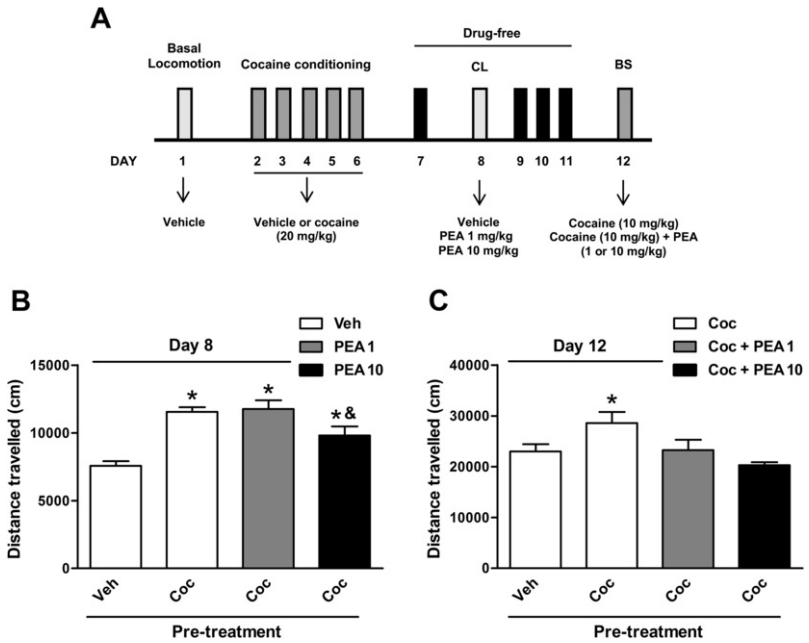

Fig. 2 Acute PEA treatment reduced the expression of cocaine conditioned locomotion and abolished the expression of cocaine sensitization. (A) Schematic representation of the conditioned locomotion (CL) and ~~behavioural~~behavioral sensitization (BS) protocol employed (for a detailed description see Sections 2.3 and 2.3.2.). (B) In the CL session, all groups of mice pre-treated with cocaine (20 mg/kg) ~~travelled~~traveled longer distances than the one pre-treated with vehicle. Moreover, the group pre-treated with cocaine and acutely treated with 10 mg/kg of PEA, ~~travelled~~traveled a shorter distance than the other two groups, revealing that the acute treatment with the highest dose of PEA reduced the expression of cocaine-induced CL. (C) In the BS session, the group pre-treated with cocaine (20 mg/kg) that were administered an acute priming injection of cocaine (10 mg/kg) without PEA, ~~travelled~~traveled a longer distance that the rest of groups, indicating that acute PEA treatments were able to abolish the expression of cocaine-induced BS. Data are expressed as the mean ± SEM of 8~~-~~11 mice per group. *p<~~0~~.05 vs. vehicle pre-treated group (in B) and vs. the rest of groups (in C); $^{\&}p$<~~0~~.05 vs. groups pre-treated with cocaine and treated with either acute injection of vehicle or acute injection of 1 mg/kg of PEA.

alt-text: Fig. 2

~~2.4.~~2.4 Cocaine-induced conditioned place preference

The procedures to assess the acquisition and expression of cocaine-induced CPP were based on previous reports (Bilbao et al., 2013; Castilla-Ortega et al., 2015; Ribeiro Do Couto et al., 2009; Solinas et al., 2008). All experiments were conducted in three spatial place preference apparatuses (Panlab, Barcelona, Spain) each one consisting of a box with two equally sized compartments (20~~ x~~ x 18~~ x~~ x 25 cm) interconnected by a rectangular corridor. All the compartments were separated by manually operated guillotine doors for cocaine conditioning phase. The compartments were differentiated by motifs painted on the walls (dots or stripes), ~~colours~~colors (different shades of grey tones, light or dark), and combinations of three-dimensional polygons placed in the corners. Mice were habituated to the testing room for 30 min prior to ~~behavioural~~behavioral testing. The CPP paradigm consisted of three different phases over six days: 1) Day 1: pre-conditioning phase (one session). Each mouse was injected with vehicle and immediately placed in the central corridor with free access to explore both compartments for 20 min; 2) Day 2~~-~~5: conditioning phase (four sessions with two conditioning trials per session). Mice were daily injected with 20 mg/kg of cocaine and immediately confined in the cocaine-paired compartment for 30 min (Positive Conditioned Stimulus, CS+). After an interval of 3~~ hours~~h, mice were injected with vehicle and confined for 30 min in the opposite compartment (Neutral Conditioned Stimulus, CS-) (Ribeiro Do Couto et al., 2009; Solinas et al., 2008). This phase was considered as the pre-treatment; 3) Day 6: Test phase (one session). Mice were injected with vehicle and allowed to explore the entire apparatus for 20 min under the same conditions as in the pre-conditioning phase (Fig. 3A). Throughout the four conditioning sessions, CS+ trials always took place before CS- trials, and treatments (cocaine or vehicle) were counterbalanced between compartments. The time spent and the distance ~~travelled~~traveled in each compartment was determined during the pre-conditioning and test sessions. The CPP score was defined as the time spent in the cocaine-paired compartment minus the time spent in the saline-paired compartment (Bilbao et al., 2013). Each trial/session

was digitally recorded (Sony DCR-SX22E) and behaviour was later ~~analysed~~analyzed using Ethovision XT 5.0 (Noldus Information Technology, Wageningen, The Netherlands).

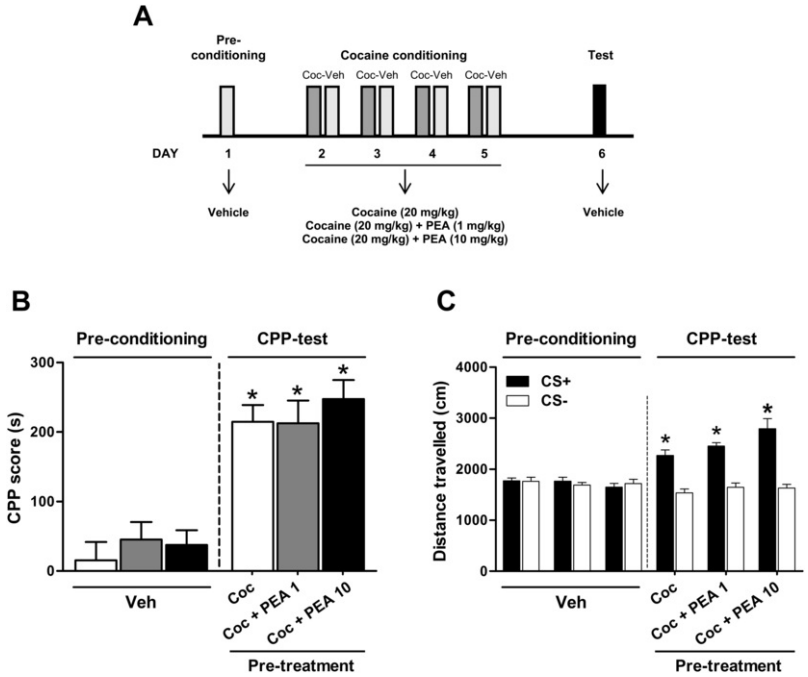

Fig. 3 Acquisition of cocaine-induced conditioned place preference was not affected by repeated PEA administration. (A) Schematic representation of the cocaine conditioned place preference (CPP) protocol employed (for a detailed description see Sections 2.4. and 2.4.1.). (B) All groups of mice pre-treated with cocaine (20 mg/kg), regardless whether they were administered with PEA or not, showed higher CPP scores in the test session than in the pre-conditioning phase. (C) All groups of mice pre-treated with cocaine (20 mg/kg), regardless whether they were administered with PEA or not, ~~travelled~~traveled longer distances in the CS+ compartment during the test session than during the pre-conditioning session. Data are expressed as the mean ± SEM of 9–10 mice per group. *p < 0.05 vs. pre-conditioning phase.

alt-text: Fig. 3

~~2.4.1.~~2.4.1 *Experiment 3. Effects of repeated PEA administration on the acquisition of cocaine-induced CPP*

To study the effects of repeated PEA administration on the acquisition of cocaine-induced CPP, twenty-eight naive male mice were randomly assigned to three groups (n = 9–10 mice per group). Thereafter, animals were submitted to the CPP protocol described in 2.4 with the following modification: during the conditioning phase, mice were injected with either cocaine (20 mg/kg) or cocaine (20 mg/kg) plus PEA (1 or 10 mg/kg) and immediately confined in the corresponding compartment for 30 min (CS+ trial). CS- trial was carried out 3 h later as described in Section 2.4 (Fig. 3A). The rest of the protocol remained the same.

~~2.4.2.~~2.4.2 *Experiment 4. Effects of acute PEA administration on the expression of cocaine-induced CPP*

In order to assess the effects of acute PEA administration on the expression of cocaine-induced CPP, in this fourth experiment thirty-five naive male mice were submitted to the same CPP protocol described in Section 2.4, except that 24 h after the test session (test 1), a second test session was performed (test 2) (Fig. 4A). Once test 1 had finished, mice were randomly assigned to three groups (n = 10–13 mice per group). Twenty-four hours later, during test 2, one group of mice was treated with vehicle while the other two groups were treated with vehicle plus PEA (1 or 10 mg/kg) (Fig. 4A). The CPP score was calculated for both test sessions.

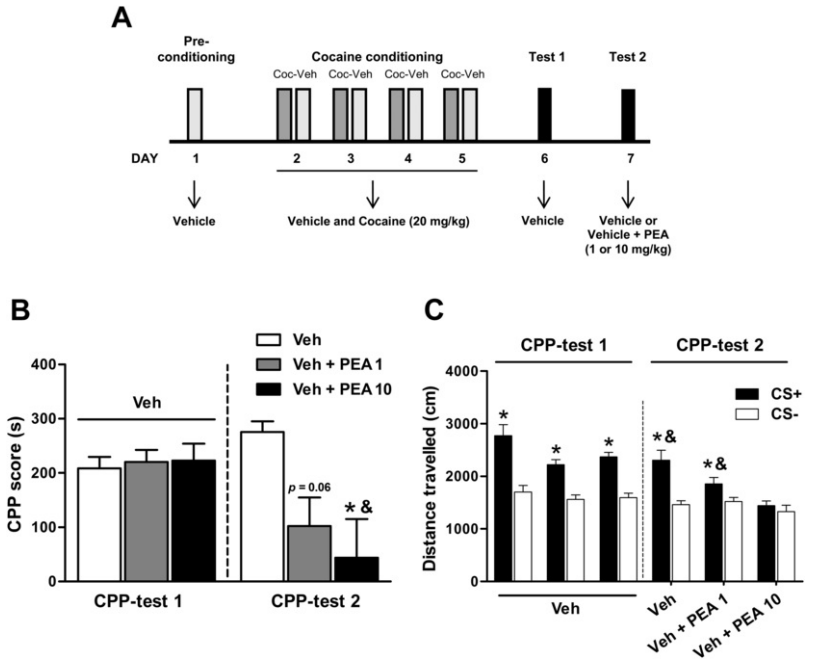

Fig. 4 Expression of cocaine-induced conditioned place preference was abolished by acute PEA administration. (A) Schematic representation of the cocaine conditioned place preference (CPP) protocol employed (for a detailed description see Sections 2.4. and 2.4.2.). (B) The group o mice acutely treated with vehicle plus 10 mg/kg of PEA during test 2, displayed a lower CPP score than during test 1. Moreover, during test 2, this same group exhibited a lower CPP score that the vehicle-treated group. Furthermore, a strong tend toward significance was observed when the CPP scores from test 1 and test 2 sessions were compared for the group of mice that was acutely injected with vehicle plus 1 mg/kg of PEA. (C) When distances ~~travelled~~traveled were compared between compartments and test sessions, it was revealed that during test 1, all groups of mice ~~travelled~~traveled longer distances in the CS+ than in the CS- compartment. The same pattern was observed during test 2 for groups of mice acutely treated with vehicle or vehicle plus 1 mg/kg of PEA. On the contrary, the group of mice treated with vehicle plus 10 mg/kg of PEA ~~travelled~~traveled similar distances in both compartments during test 2, being the distance ~~travelled~~traveled in the CS+ compartment shorter than those ~~travelled~~traveled by the other two groups in the same compartment. Data are expressed as the mean ± SEM of 10–13 mice per group. *p < ~~0~~.05 vs. test 1 (in B) and vs. CS- compartment in test 2 (in C). &p < ~~0~~.05 vs. group of mice injected with vehicle during test 2 (in B) and vs. the mean distance ~~travelled~~traveled in the CS+ compartment by the group acutely treated with vehicle plus 10 mg/kg of PEA during test 2.

alt-text: Fig. 4

~~2.5.~~2.5 Experiment 5. Effects of PEA administration on neurological functions, exploratory activity and anxiety-like behaviour

~~2.5.1.~~2.5.1 *Neurological screening*

The neurological screening was performed in a testing room in which animals were previously habituated for 30 min. Twenty-four naive male mice were randomly divided into three groups (n = 7–9 mice per group). Mice were treated with either vehicle or PEA (1 or 10 mg/kg) and a neurological screening was carried out 5 and 30 min later (Fig. 5A). To test sensorimotor orientation, coordinated limb movements and neurological function, mice were subjected to a battery of previously described tests (Björklund et al., 1980; Bures et al., 1983; Marshall and Teitelbaum, 1974; Santín et al., 2009). The following sensory reflexes were assessed: (1) whisker touch, in which a toothpick was brought close to the animal from the lower rear so as to avoid the visual field, and then lightly brushed against the vibrissae; (2) head shaking, where the mouse was placed on a small, elevated platform and tested for reaction to a puff of air gently released through a narrow rubber tube (internal diameter, 1 mm) to its pinna; (3) somesthesis, in which a pin prick was applied to six sites on the lateral surface of the animal body, combining dorsal and ventral placements at rostral, middle and caudal levels; (4) olfaction, where a small cotton swab dipped in ammonia solution was slowly brought close to the mouse's nose in a lateral-medial direction; (5) corneal reflex, in which the animal was restrained with a hand while the cornea was superficially stimulated with a fine, hair-tipped probe; (6) auditory startle, in which an unexpected, loud acoustic stimulus was applied. Limb reflexes and limb coordination were assessed using the following tests: (1) surface righting reflexes, in which the animal was placed on its back onto a flat surface, and the time for the animal to right itself was measured; (2) Inclined plane test, in which the mouse was placed facing downwards on a wiremesh platform tilted 30°, after which it was turned to face up the slope and then was finally placed on a horizontal wooden bar (diameter, 2 cm; length, 30 cm) suspended 50 cm above the floor, and its ability to stay on the bar was assessed; (3) extension reflex, where the mouse was suspended by its tail and was displaced so that it hung over the edge. Neurological tests were rated on a three-point scale

(0 = absent, 1 = weak or 2 = strong) presented as a percentage of incidences for each treatment (Table 1). Use of this test battery allowed us to determine whether PEA affected a particular brain region, interfered with a specific function or affected the central nervous system as a whole (Bures et al., 1983).

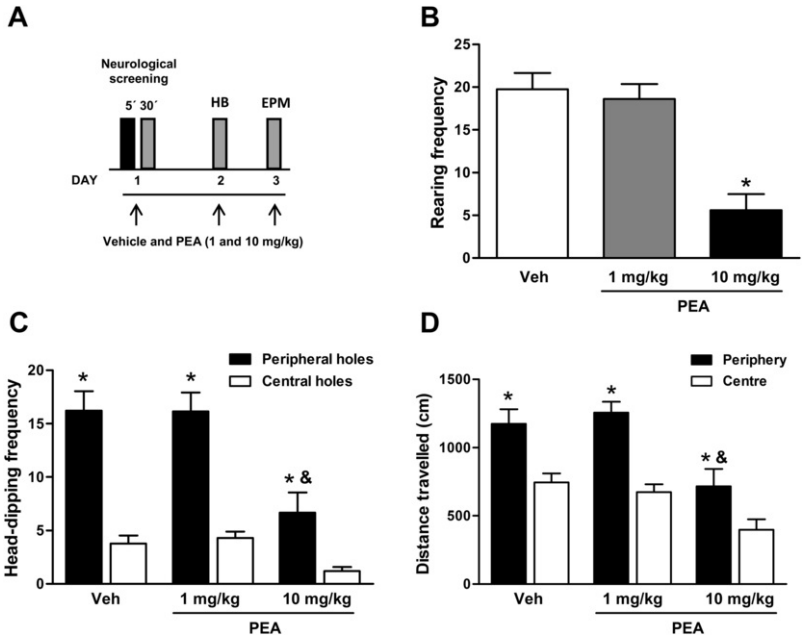

Fig. 5 The highest dose of PEA reduced spontaneous exploratory activity. (A) Chronological sequence of the tests administered. Results from neurological screening and elevated plus maze (EPM) are showed in Tables 1 and 2, respectively. (B) In the hole board (HB) test, groups of mice treated with vehicle plus 10 mg/kg of PEA showed a lower rearing frequency than groups treated with vehicle or vehicle plus 1 mg/kg of PEA. (C) All groups of mice exhibited higher head-dipping frequencies in peripheral than in central holes in the HB test. Nevertheless, the group of mice treated with vehicle plus 10 mg/kg of PEA exhibited a lower head-dipping frequency in peripheral holes in comparison with those shown by the other groups. (D) The same pattern observed for head-dipping frequency was revealed when distances ~~travelled~~traveled were analyzed. Data are expressed as the mean ± SEM of 13–15 mice per group. *$p < 0.05$ vs. the other two groups (in B) and vs. head-dipping frequency in central holes (in C) or distance ~~travelled~~traveled in the centre (in D). $^{\&}p < 0.05$ vs. head-dipping frequency in peripheral holes or distance ~~travelled~~traveled in periphery zone shown by groups acutely treated vehicle or vehicle plus 1 mg/kg of PEA.

alt-text: Fig. 5

Table 1 Neurological screening 5 and 30 min after PEA administration.

alt-text: Table 1

Neurological test	Treatment	5 ~~minutes~~min post-administration			30 ~~minutes~~min post-administration		
		Absence deficit (0)	Weak deficit (1)	Strong deficit (2)	Absence deficit (0)	Weak deficit (1)	Strong deficit (2)
Whisker touch	Vehicle	100%	–	–	100%	–	–
	PEA 1 mg/kg	100%	–	–	100%	–	–
	PEA 10 mg/kg	100%	–	–	100%	–	–
Head shaking	Vehicle	100%	–	–	100%	–	–
	PEA 1 mg/kg	100%	–	–	100%	–	–
	PEA 10 mg/kg	100%	–	–	75%	25%	–

Somesthesis	Vehicle	89 %	11 %	–	89 %	–	11 %
	PEA 1 mg/kg	100 %	–	–	86 %	14 %	–
	PEA 10 mg/kg	100 %	–	–	63 %	–	37 %
Olfaction test	Vehicle	100 %	–	–	100 %	–	–
	PEA 1 mg/kg	100 %	–	–	100 %	–	–
	PEA 10 mg/kg	100 %	–	–	100 %	–	–
Corneal reflex	Vehicle	100 %	–	–	100 %	–	–
	PEA 1 mg/kg	100 %	–	–	100 %	–	–
	PEA 10 mg/kg	100 %	–	–	100 %	–	–
Auditory startle	Vehicle	89 %	–	11 %	100 %	–	–
	PEA 1 mg/kg	86 %	14 %	–	100 %	–	–
	PEA 10 mg/kg	89 %	11 %	–	75 %	–	25 %
Righting réflex	Vehicle	100 %	–	–	100 %	–	–
	PEA 1 mg/kg	100 %	–	–	100 %	–	–
	PEA 10 mg/kg	100 %	–	–	100 %	–	–
Inclined plane test	Vehicle	89 %	11 %	–	100 %	–	–
	PEA 1 mg/kg	100 %	–	–	72 %	28 %	–
	PEA 10 mg/kg	75 %	25 %	–	75 %	–	25 %
Extension réflex	Vehicle	100 %	–	–	100 %	–	–
	PEA 1 mg/kg	100 %	–	–	86 %	14 %	–
	PEA 10 mg/kg	100 %	–	–	75 %	12.5 %	12.5 %

Results are expressed as percentage of mice.

~~2.5.2.~~2.5.2 Assessment of exploratory and anxiety-like ~~behaviours~~behaviors

To study whether PEA has any effect on spontaneous exploratory activity and unconditioned anxiety-like ~~behaviours~~behaviors, forty-one naive male mice (not employed for neurological assessment) were randomly assigned to one of three experimental groups (vehicle, PEA 1 or 10 mg/kg) (n = 13–15 mice per group) and subjected to the hole-board (HB) test, followed twenty-four hours later by the elevated plus maze (EPM) test (Fig. 5A). PEA was administrated 30 min before each test.

~~2.5.2.1.~~2.5.2.1 Hole-board test The HB apparatus consisted of an open box (40 ~~x~~ × 40 cm) surrounded by clear Plexiglas walls (20 cm in height), virtually divided into a peripheral zone (with a virtual limit 6.5 cm away from the walls) and central zone that contained 16 equidistant holes (5.5 cm apart, 2.5 cm diameter, 3 cm depth). Mice were placed in the centre of the HB and their behaviour was digitally recorded for 5 min. Distance ~~travelled~~traveled (cm) in peripheral and central zones wa registered using Ethovision XT 5.0. The frequency of head-dipping (the mouse introduced its nose in a hole) and rearing (the mouse stood on its hind paws, with forelegs supported or unsupported on the walls), in the peripheral and central zones, wer assessed observationally by a blind observer.

~~2.5.2.2.~~2.5.2.2 Elevated plus maze test The EPM apparatus consisted of two open arms (30 × 10 cm), two enclosed arms (30 × 10 cm ~~x~~ × 12.5 cm), and a connecting central platform (5 × 5 cm). The maze was raised to a height of 57 cm abov the floor. Mice were placed in the intersection of the four arms and allowed to explore freely the entire apparatus during 5 min. An arm entry was counted when the four legs of the mouse entered the zone. Behaviour was digitally recorded and analyze observationally by a blind observer to treatments or using Ethovision XT 5.0. The following parameters were measured: frequency of entries into both the open and closed arms; time spent in open arms; latency to enter the open arms; total distanc

travelledtraveled; mean velocity; rearing, grooming, head-dipping and stretching frequency. Anxiety was assessed by comparing activity in open vs. closed arms using the following index: time spent in open arms / (time spent in open arms + time spent i closed arms) (Malleret et al., 1999; Santín et al., 2009). Low values denoted high anxiety levels.

2.6.2.6 Statistical analysis

Results were expressed as the mean ± SEM. Data were analysedanalyzed by one-way ANOVA tests or by two- or three-way mixed ANOVA tests. Post-hoc multiple comparisons were conducted using Newman-Keuls tests. The total distanced travelledtraveled and the CPP score in the test 1 of the experiment 4 were analysedanalyzed by paired sample t-tests. Data obtained from the neurological assessment were analysedanalyzed by non-parametric Kruskal-Wallis tests. The probability to reject the null hypothesis was set at 5-%. All statistical analyses were performed with STATISTICA software for Windows.

3.3 Results

3.1.3.1 Experiment 1. Repeated PEA administration abolished acquisition of cocaine-induced conditioned locomotion and behaviouralbehavioral sensitization

When basal locomotion was analysedanalyzed during the first day of the cocaine sensitization protocol, a one-way ANOVA test revealed that the group of mice treated with 10 mg/kg of PEA showed a significantly reduced locomotor activity compared to those groups treated with either vehicle or 1 mg/kg of PEA ($F_{(2,27)} = 39.79$, $p < 0.001$; Newman–Keuls *post-hoc* tests, $p < 0.05$) (Fig. 1B). During the cocaine conditioning phase, a two-way mixed ANOVA test, with Day (2 to 6) as the within-subject factor and Treatment [(vehicle, cocaine, cocaine plus PEA (1 or 10 mg/kg)] as the between-subject factor, showed that the main effect of Treatment and the interaction Day × Treatment were significant ($F_{(3,26)} = 34.14$; $p < 0.001$; $F_{(12,104)} = 2.10$, $p < 0.05$, respectively), while the main effect of Day was not ($F_{(4,104)} = 0.54$, $p =$ n.s.). Newman–Keuls *post-hoc* tests indicated that, during each day, groups that were administered cocaine travelledtraveled significantly longer distances than the group that was administered with vehicle, regardless whether PEA was administered or not ($p < 0.05$ for all comparisons) (Fig. 1C). These results revealed that PEA did not modify the locomotor activity induced by repeated administrations of cocaine. During the cocaine conditioned locomotion phase, cocaine pre-treated group exhibited a significantly longer distance travelledtraveled than all other groups (one-way ANOVA test, $F_{(3,26)} = 6.38$, $p < 0.01$; Newman–Keuls *post-hoc* tests, $p < 0.05$) (Fig. 1D). These results indicated that the group pre-treated with cocaine acquired cocaine-induce CL, while pre-treatment with both doses of PEA was able to block the acquisition of this conditioned response. Finally, when mice were challenged with a priming dose of cocaine (10 mg/kg) (cocaine sensitization phase), the only group that showed a significantly longer distance travelledtraveled was the one pre-treated with cocaine (one-way ANOVA test: $F_{(3,26)} = 14.56$, $p < 0.001$; Newman–Keuls *post-hoc* tests, $p < 0.05$) (Fig. 1E). This denoted that pre-treatment with both doses of PEA was also able to block acquisition of BS to cocaine. Moreover, mice pre-treated with cocaine plus 1 mg/kg of PEA displayed a significantly reduced distance travelledtraveled compared to vehicle pre-treated mice (Newman-Keuls *post-hoc* test, $p < 0.05$) (Fig. 1E).

3.2.3.2 Experiment 2. Acute PEA administration reduced the expression of cocaine induced conditioned locomotion and blocked the expression of cocaine sensitization

During the cocaine conditioning phase, a two-way mixed ANOVA test revealed that only the main effect of Treatment (vehicle or cocaine) was significant ($F_{(1,39)} = 134.05$, $p < 0.001$). Newman–Keuls *post-hoc* tests showed that mice daily injected with cocaine travelledtraveled significantly longer distances than vehicle-injected mice during the five days ($p < 0.05$ for all comparisons) (Supplementary Fig. S1). On the eighth day, a one-way ANOVA test revealed that cocaine pre-treated groups travelledtraveled significantly longer distances than the vehicle pre-treated group ($F_{(3,37)} = 11.16$; $p < 0.001$; Newman–Keuls *post-hoc* tests, $p < 0.05$) (Fig. 2B). Interestingly, *post-hoc* tests also revealed that the cocaine pre-treated group that was acutely administered with 10 mg/kg of PEA travelledtraveled a significantly shorter distance than other cocaine pre-treated groups, although it did not reach the levels showed by the group pre-treated with vehicle ($p < 0.05$ for all comparisons) (Fig. 2B). These results indicated that all groups pre-treated with cocaine displayed CL, while the highest dose of PEA was able to reduce its expression. Finally, during the cocaine sensitization phase, a one-way ANOVA test showed that the cocaine pre-treated group challenged with a priming dose of cocaine (10 mg/kg) travelledtraveled a significantly longer distance than all other groups ($F_{(3,37)} = 4.28$; $p < 0.01$; Newman–Keuls *post-hoc* tests, $p < 0.05$) (Fig. 2C). Moreover, groups pre-treated with cocaine and challenged with a priming dose of cocaine (10 mg/kg) plus PEA (1 or 10 mg/kg) showed similar travelledtraveled distances than the group pre-treated with vehicle and challenged with cocaine (Newman–Keuls *post-hoc* tests, $p < 0.05$) (Fig. 2C). These results demonstrated that the acute administration of either dose of PEA, 30 min before the challenge cocaine injection, was able to abolish the expression of cocaine-induced BS.

3.3.3.3 Experiment 3. Repeated PEA administration did not interfere with acquisition of cocaine-induced conditioned place preference

To analyseanalyze the conditioning phase of the CPP, a three-way mixed ANOVA test, with Session (Day 2 to 5) and Type of trial (CS+ or CS-) as within-subject factors and Treatment [cocaine (20 mg/kg) and cocaine (20 mg/kg)

plus PEA (1 or 10 mg/kg)] as between-subject factor, was performed. The analysis revealed that Type of trial was the only main effect that resulted to be significant ($F_{(7,175)} = 80.81$, $p <$ 0.001). Newman–Keuls *post-hoc* tests showed that in every session of the conditioning phase, all groups of mice ~~travelled~~traveled a significantly longer distance during the CS+ trial than during the CS- trial, regardless of PEA administration ($p <$ 0.05 for all comparisons) (Supplementary Fig. S2).

To study whether repeated PEA administration during the conditioning phase could affect the CPP acquisition, CPP scores were calculated for the pre-conditioning and test sessions, and then a two-way mixed ANOVA test, with Session (pre-conditioning or test) as the within-subject factor and Pre-treatment [(the type of treatment received during the conditioning phase in the CS+ compartment (cocaine, cocaine plus 1 mg/kg of PEA or cocaine plus 10 mg/kg of PEA)] as the between-subject factor, was carried out. Results indicated that the main effect of Session was significant ($F_{(1,25)} = 125.75$, $p <$ 0.001), while the main effect of Pre-treatment and the interaction Session × Pre-treatment were not ($F_{(2,25)} = 0.55$, $p =$ n.s.; $F_{(2,25)} = 2.72$, $p =$ n.s., respectively). Newman–Keuls *post-hoc* tests confirmed that during the test session all groups of mice displayed significantly higher CPP scores than during the pre-conditioning session ($p <$ 0.05 for all comparisons) (Fig. 3B). These results indicated that all groups of mice acquired the CPP regardless whether they were repeatedly pre-treated with PEA during the conditioning phase or not.

To further explore whether repeated PEA administration could modulate the CPP acquisition, the distances ~~travelled~~traveled in the CS+ and CS- compartments during the pre-conditioning and test sessions were calculated. A three-way mixed ANOVA test with Session (pre-conditioning or test) and Compartment (CS+ or CS-) as within-subject factors, and Pre-treatment (cocaine, cocaine plus 1 mg/kg of PEA or cocaine plus 10 mg/kg of PEA) as the between-subject factor, revealed that of mice ~~travelled~~traveled significantly longer distances in the CS+ compartment during the test session than during the pre-conditioning session ($p <$ 0.05 for all comparisons) (Fig. 3C).

~~3.4.~~3.4 Experiment 4. Acute PEA administration abolished the expression of cocaine-induced conditioned place preference

During the conditioning phase, a two-way repeated measured ANOVA test [within-subject factors: Session (Day 2 to 5) and Type of trial (CS+ or CS-)] revealed that the main effect of Type of trial was significant ($F_{(1,68)} = 83.57$, $p <$ 0.001). Newman-Keuls *post-hoc* tests indicated that in every session mice ~~travelled~~traveled significantly longer distances during the CS+ trial than during the CS- trial ($p <$ 0.05 for all comparisons) (Supplementary Fig. S3A). In order to ~~analyse~~analyze whether mice correctly acquired cocaine-induced CPP and CL, CPP-scores and total distances ~~travelled~~traveled were compared between pre-conditioning and test 1 session. Student's paired *t*-tests confirmed that all mice showed a significantly higher CPP score and ~~travelled~~traveled a significantly longer distance during test 1 than during the pre-conditioning session ($t =$ 9.48, *d.f.* 34, $p <$ 0.001; $t =$ 5.04, *d.f.* 34, $p <$ 0.001, respectively) (Supplementary Fig. S3B–C).

To assess the effects of acute PEA administration on the expression of cocaine-induced CPP, a two-way mixed ANOVA test, with Test session (test 1 or test 2) as the within-subject factor and Treatment (vehicle, vehicle plus 1 mg/kg of PEA or vehicle plus 10 mg/kg of PEA) as the between-subject factor, was performed. Results revealed that the main effect of Test session as well as the interaction Test session × Treatment were both significant ($F_{(1,32)} = 6.90$, $p <$ 0.05; $F_{(2,32)} = 6.08$, $p <$ 0.01, respectively). Newman-Keuls *post-hoc* tests indicated that the group of mice that were administered 10 mg/kg of PEA during test 2, showed a significantly lower CPP score compared to the one showed during test 1 ($p <$ 0.05) (Fig. 4B), while a strong trend toward significance ($p =$ 0.06) was observed for the group of mice treated with 1 mg/kg of PEA. Moreover, CPP score displayed by mice treated with 10 mg/kg of PEA was also significantly lower than that displayed by the vehicle-treated group during the same session (test 2) ($p <$ 0.05) (Fig. 4B). In addition, distances ~~travelled~~traveled in each compartment (CS+ and CS-) during test 2 were compared to those ~~travelled~~traveled during test 1 for the different treatments. The three-way mixed ANOVA test [within subject factors: Test Session (test 1 or test 2) and Compartment (CS+ or CS-); between-subject factor: Treatment (vehicle, vehicle plus 1 mg/kg of PEA or vehicle plus 10 mg/kg of PEA] revealed that the main effect of Compartment and the second order interaction Test session × Compartment × Treatment were significant ($F_{(1,32)} = 124.43$, $p <$ 0.001; $F_{(2,32)} = 4.18$, $p <$ 0.05, respectively). Newman-Keuls *post-hoc* tests indicated that all groups of mice ~~travelled~~traveled significantly longer distances in the CS+ compartment than in the CS- compartment during the test 1 session ($p <$ 0.05 for all comparisons) (Fig. 4C). On the contrary, during the test 2 session the group of mice acutely treated with 10 mg/kg of PEA ~~travelled~~traveled a similar distance in both compartments ($p =$ n.s.) (Fig. 4C). Finally, groups acutely treated with either vehicle or 1 mg/kg of PEA ~~travelled~~traveled significantly longer distances in the CS+ compartment than the group treated with the highest dose of PEA ($p <$ 0.05 for both comparisons) (Fig. 4C).

Taken together, these results indicate that the highest dose of PEA (10 mg/kg) administered acutely was able to abolish the expression of cocaine-induced CPP.

~~3.5.~~3.5 Experiment 5. Acute administration of the highest dose of PEA slightly affected some neurological functions and reduced exploratory activity

~~3.5.1.~~3.5.1 *Effects of acute PEA administration on neurological functions*

At 5 and 30 min after acute administration of PEA (1 or 10 mg/kg), a high preservation of neurological functions was observed, since groups of mice that received the different treatments (vehicle, 1 mg/kg of PEA or 10 mg/kg of PEA) did not differ significantly in any of the sensorimotor and coordinated limb reflexes assessed ($p =$ n.s. for all Kruskal-Wallis tests) (Table 1). Nevertheless, although not significantly, a slight affectation in somesthesis ($H_{(2, N=24)} = 2.29$, $p =$ n.s.), auditory startle ($H_{(2, N=24)} = 4.18$, $p =$ n.s.), equilibrium (inclined plane test) ($H_{(2, N=24)} = 2.73$, $p =$ n.s.) and extension reflex ($H_{(2, N=23)} = 2.37$, $p =$ n.s.) was observed 30 min after the acute administration of 10 mg/kg of PEA (Table 1).

3.5.2.3.5.2 *Effects of acute PEA administration on exploratory activity and anxiety-like behaviours behaviors*

One-way ANOVA test followed by *post-hoc* tests revealed that acute administration of 10 mg/kg of PEA significantly reduced the rearing frequency in the HB test ($F_{(2,38)} = 18.77$, $p < 0.001$; Newman-Keuls, $p < 0.05$ for all comparisons) (Fig. 5B). Moreover, two-way mixed ANOVA tests, with Zone (periphery or centre) as the within-subject factor and Treatment (vehicle, 1 mg/kg or 10 mg/kg of PEA) as the between-subject factor, indicated that, for both head-dipping frequency and distance travelled traveled, the main effects of Zone ($F_{(1,38)} = 118.73$, $p < 0.001$; $F_{(1,38)} = 43.44$, $p < 0.001$, respectively), Treatment ($F_{(2,38)} = 10.82$, $p < 0.001$; $F_{(2,38)} = 11.51$, $p < 0.001$, respectively), and the interaction Zone × Treatment ($F_{(2,38)} = 6.31$, $p < 0.01$; $F_{(2,38)} = 3.34$, $p < 0.05$, respectively) were all significant. Newman–Keuls *post-hoc* tests revealed that all groups of mice showed significantly higher head-dipping frequency and longer distance travelled traveled in the peripheral zone than in the central zone ($p < 0.05$ for all comparisons) (Fig. 5C–D). Nevertheless, the group of mice treated with 10 mg/kg of PEA showed significantly lower head-dipping frequency and shorter distance travelled traveled than the other groups in the peripheral zone ($p < 0.05$ for all comparisons) (Fig. 5C–D).

Regarding anxiety-like behaviours behaviors, none of the variables that operationalized anxiety in the EPM test differed between treatments (Table 2). However, one-way ANOVA tests, followed by Newman-Keuls *post-hoc* tests, revealed that the group of mice treated with 10 mg/kg of PEA showed a shorter distance travelled traveled ($F_{(2,37)} = 7.13$, $p < 0.01$; Newman-Keuls tests, $p < 0.05$), a lower velocity ($F_{(2,37)} = 7.14$, $p < 0.01$; Newman-Keuls tests, $p < 0.05$) and a lower rearing frequency ($F_{(2,37)} = 6.09$, $p < 0.01$; Newman-Keuls tests, $p < 0.05$) than the remaining groups (Table 2). Finally, stretching frequency differed only between vehicle and 10 mg/kg of PEA-treated groups ($F_{(2,37)} = 4.78$, $p < 0.01$; Newman-Keuls test, $p < 0.05$) (Table 2).

Table 2 Elevated plus maze test.

alt-text: Table 2

Treatment	Elevated plus maze: behavioral parameters (Mean ± SEM)				
	Open arms			Anxiety	Velocity
	Time (s)	Latency (s)	Entries (n°)	Index	(cm/s)
Vehicle	41.09 ± 4.46	12.86 ± 5.40	12.44 ± 1.56	0.17 ± 0.02	7.74 ± 0.58
PEA 1 mg/kg	39.75 ± 5.27	2.87 ± 2.65	10.29 ± 1.48	0.16 ± 0.02	7.29 ± 0.27
PEA 10 mg/kg	35.98 ± 5.60	9.65 ± 3.44	10.50 ± 1.10	0.14 ± 0.02	6.85 ± 0.35*#

	Locomotion (cm)	Rears (n°)	Grooming (n°)	Dips (n°)	Stretches (n°)
Vehicle	2266.28 ± 163.76	10.44 ± 1.41	1.22 ± 0.22	10.33 ± 1.41	15.11 ± 1.12
PEA 1 mg/kg	2329.42 ± 123.16	14.43 ± 1.13	1.29 ± 0.36	12.43 ± 1.89	12.71 ± 1.13
PEA 10 mg/kg	1782.42 ± 123.67*#	7.40 ± 1.48*#	1.47 ± 0.34	7.53 ± 1.37	9.07 ± 0.71*

* $p < 0.05$ vs. vehicle.

# $p < 0.05$ vs. 1 mg/kg of PEA.

Overall, these results indicated that the treatment with the highest dose of PEA reduced general exploratory activity while neither dose affected anxiety-related behaviours behaviors.

4.4 Discussion

The major findings of the present study are that repeated administration of PEA was able to block the acquisition of BS and CL to cocaine, while its acute administration reduced/abolished the expression of both behavioural behavioral responses. Regarding the CPP paradigm, it was found that when PEA was administrated repeatedly did not alter the acquisition of cocaine-induced CPP. Finally, acute administration of PEA was able to abolish the expression of cocaine-induced CPP.

4.1.4.1 The blocking effects of PEA administration on acquisition and expression of cocaine-induced BS and CL

BS is the expression of a series of brain neuroadaptations as a consequence of repeated cocaine administration (Blanco et al., 2012; De Vries et al., 1998; Robinson and Berridge, 1993). Since this process underlie to the development

and maintenance of cocaine addiction, promoting craving and relapse (Blanco et al., 2016; De Vries et al., 1998), finding compounds with the ability to modulate it could be important for the development of potential therapeutic agents.

Experimental evidence suggests that neuroinflammation of the central nervous system (CNS) is involved in the sensitizing effects of psychostimulants (Maeda et al., 2007; Nakajima et al., 2004; Zalcman et al., 1999). On the other hand, PEA has shown to possess potent anti-inflammatory effects in the CNS. Therefore, we could hypothesize that the blocking effect of PEA on cocaine-induced BS could be, in part, due to its anti-inflammatory effects. In fact, our group has previously shown that OEA, another *N*-acylethanolamine with anti-inflammatory effects, was able to attenuate cocaine-induced BS in a dose-dependent manner (Bilbao et al., 2013). Whether PEA modulates the cocaine-induced BS through its anti-inflammatory actions remains to be tested in further studies.

Interestingly, PEA and OEA has a common main target: PPARα receptor (Mattace Raso et al., 2014). As it was mentioned above, in a previous work from our group, OEA was able to attenuate cocaine-induced BS. However, this effect seemed to be through a PPARα receptor-independent mechanism, since OEA administration was still able to attenuated BS to cocaine in PPARα knock-out mice (Bilbao et al., 2013). This experimental evidence leads us to conclude that the PEA blocking effect on cocaine-induced BS observed in the present study is not very likely to be mediated by PPARα-dependent mechanisms, although this hypothesis should be tested in future studies.

The fact that PEA was able to affect acquisition and expression of cocaine-induced CL suggest that PEA may also potentially affect cocaine-mediated associative learning processes underlying this conditioned response. In this regard, we have also recently demonstrated that repeated administration of OEA reduces cocaine-induced CL, being this effect also PPARα-independent (Bilbao et al., 2013).

In conclusion, further studies are needed to determine by which specific mechanisms PEA and OEA modulate cocaine-induced BS and CL.

~~4.2.~~4.2 The blocking effect of PEA administration on expression of cocaine-induced CPP

Dosage, frequency and route of administration are important factors modulating cocaine effects, although associative learning mechanisms also play a key role in cocaine addiction processes (for example, drug-environment associations). The involvement of conditioned responses in cocaine-seeking ~~behaviours~~behaviors can be observed in different processes, being one of the most studied the CPP paradigm (Tzschentke, 2007).

Our group has previously reported the ability of the analogous structurally lipid OEA to reduce or suppress the acquisition of cocaine-induced CPP (Bilbao et al., 2013). Interestingly, these effects were not mediated by PPARα-dependent mechanism. However, in the present study we have demonstrated that PEA did not affect the acquisition of cocaine-induced CPP. It is important to notice that OEA and PEA exert their effects acting on many different receptors, ion channels and enzymes (for instance, vanilloid receptors, K+ channels, cannabinoid-like G-coupled receptors GPR55 and GPR119, etc.) (Godlewski et al., 2009; Hansen, 2010; Syed et al., 2012), which also may explain their discrepancy effects on acquisition of cocaine-induce CPP. Moreover, OEA and PEA have also shown to differ in the modulation of some addiction-related mood states, being OEA able to block stress-induced anhedonia while PEA fails to block it (Sayd et al., 2014). Therefore, although OEA and PEA act through some shared receptor and have both anti-inflammatory effects, they seem to exert differential actions on addicted-related ~~behaviours~~behaviors.

Regarding the blocking effect of PEA on cocaine-induced CPP expression observed in the present study, it was previously reported that the administration of a selective antagonist of TRPV1 in the nucleus accumbens was able to abolish the expression of morphine-induced CPP (Heng et al., 2014). Therefore, it seems unlikely that the activation of TRPV1 by PEA could underlie the abolishment of the CPP expression by PEA. However, this hypothesis should be taken cautiously due to the different drugs employed in both studies.

~~4.3.~~4.3 Effects of PEA administration on exploratory activity, anxiety-like ~~behaviours~~behaviors and neurological functions

In the present study, we have observed that acute PEA administration did not affect anxiety-like ~~behaviours~~behaviors. On the contrary, previous studies have reported that PEA induced anxiolytic effects, although this reduction of anxiety was observed only after a repeated administration (Crupi et al., 2013; Guida et al., 2015; Yu et al., 2011). Regarding locomotor activity, PEA administration did not modify the normal exploratory pattern, whereas the spontaneous locomotor activity was significantly reduced by the highest dose of PEA. This is consistent with previous studies using PEA congeners. For instance, acute administration of high doses of AEA reduced a number of responses mediated by activation of TRPV1 (de Lago et al., 2004; Panlilio et al., 2009), such as horizontal locomotor activity, rearing and grooming frequency (Bruijnzeel et al., 2016; Romero et al., 1995; Scherma et al., 2008). In addition, OEA and the PPARα agonist, WY-14643, have also been shown to reduce locomotor activity, rearing and grooming (Proulx et al., 2005; Rodríguez de Fonseca et al., 2001). It is important to note that in the present study, the highest dose of PEA also reduced spontaneous locomotor activity during the basal locomotion phase in experiment one. Therefore, the capacity of acute PEA administration to reduce the expression of cocaine-induced CL in experiment two might be better explained by PEA-induced reduction of spontaneous locomotor activity. However, this did not seem to be the case, since mice injected with an acute dose of 10 mg/kg of PEA ~~travelled~~traveled longer distances than vehicle-treated animals. In addition, the blocking effect of acute administration of PEA on the expression of cocaine-induced BS could not be ascribed to reduced spontaneous locomotor activity induced by 10 mg/kg of PEA because this blocking effect was also observed in mice treated with 1 mg/kg of PEA. Finally, the blocking effects of the acquisition of cocaine-induced CL and BS observed in experiment one could not be due to a hypothetical hypolocomotor response elicited by repeated PEA administration, since it has been previously shown that chronic treatment with 10 mg/kg of PEA increases spontaneous locomotor activity (Crupi et al., 2013). Furthermore, the abolishment of the expression of cocaine-induced CPP after acute PEA administration, could not be better explained by the decreased distance ~~travelled~~traveled in the HB, since the acute injection of 10 mg/kg of PEA did not alter distance ~~travelled~~traveled in the CS- compartment.

Regarding neurological functions, our results showed a preservation of the evaluated reflexes. However, when the highest dose of PEA was administered 30 min before neurological assessment, slight changes in somesthesis, auditory startle, equilibrium and extension reflexes was observed, although treatments did not differ statistically. The effects on somesthesis could be a consequence of the analgesic effects of PEA previously reported in rats and humans (Gatti et al., 2012; Paladini et al., 2016). On the other hand, the effects on limb reflexes could be ascribed to the reduced spontaneous locomotor activity induced by the highest dose of PEA.

~~5.~~5 Conclusions

As far as we know, this is the first study showing that PEA modulates cocaine-induced ~~behavioural~~behavioral effects. Given that BS and CPP are two processes relevant to the induction and maintenance of drug abuse and dependence, PEA may be potentially useful to modulate ~~behavioural~~behavioral processes related to cocaine addiction. Further studies are needed to explore the neurobiological mechanisms underlying these ~~behavioural~~behavioral effects.

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Conflicts of interest

None.

~~Appendix A.~~Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2018.01.002.

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~~Appendix A.~~Appendix A. Supplementary data

Multimedia Component 1

Supplementary figures

alt-text: Image 1

Highlights

- Repeated PEA administration blocks acquisition of cocaine-conditioned locomotion.
- Repeated PEA administration blocks acquisition of cocaine sensitization.
- Acute PEA administration abolishes cocaine sensitization expression.
- Acquisition of cocaine-induced CPP is not modulated by repeated PEA administration.
- Expression of cocaine-induced CPP is blocked by acute PEA administration.

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