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Pre-training anandamide infusion within the basolateral amygdala impairs plus-maze discriminative avoidance task in rats

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ABSTRACT

Endocannabinoids (eCBs) modulate a variety of brain functions via activation of the widely expressed CB1 receptor. One site of high density of this receptor is the basolateral amygdala (BLA), a structure involved in the formation of aversive memories. The activation and blockade of CB1 receptors by systemic or hippocampal drug administrations have been shown to modify memory processing. However, little is known about the role of the BLA endocannabinoid system in aversive memories. Additionally, BLA endocannabinoid transmission seems to be related to emotional states, but the relevance of these effects to memory formation is still unknown. In this study we investigated the effects of the eCB anandamide (AEA) and the CB1 antagonist/inverse agonist AM251 infused into the BLA on the acquisition of an aversive memory task, concomitantly evaluating basal anxiety levels in rats. Male rats received pre-training micro-injection of AEA, AM251 or vehicle bilaterally into the BLA, and were studied with the plus-maze discriminative avoidance task (a paradigm that allows concomitant and independent evaluation of anxiety-like behavior and the memory of an aversive task). Our results showed that AEA into the BLA before training prevented memory retrieval 24 h later, as evaluated by exploration of the aversive arm of the maze, while AM251 into the BLA did not interfere with animals' performance. In addition, AEA had no effect on anxiety-like behavior (as evaluated by open arm exploration and risk assessment), while AM251 induced an anxiogenic effect. Our data indicate an important role of BLA CB1 receptors in aversive memory formation, and suggest that this involvement is not necessarily related to a possible modulation of anxiety states.

1. Introduction

Endocannabinoids (eCBs), represented by the two main compounds anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are endogenous lipids produced on demand that act as retrograde messengers through the activation of the cannabinoid receptors CB1 (Alger, 2002). CB1 receptors are G_i-protein-coupled receptors whose activation has shown to decrease neuronal excitability and neurotransmitter release (Freund, Katona, & Piomelli, 2003). They are widely distributed in the central nervous system (Herkenham et al., 1991), mainly in the basal ganglia, cerebellum, neocortex, hippocampus (Bisogno et al., 1999; Herkenham et al., 1991), pre-frontal cortex and amygdala (Katona, Rancz, & Acsady, 2001; McDonald & Mascagni, 2001).

The endocannabinoid system has been shown to participate in a variety of brain functions, such as pain, locomotion, feeding, and learning and memory (Ameri, 1999; Freund et al., 2003). In that regard, eCBs have emerged as important modulators of memory processing (Heifets & Castillo, 2009) although the exact mechanisms underlying this regulation are not completely understood. Indeed, systemic blockade of CB1 receptors in rodents has either failed to affect memory acquisition (Hampson & Deadwyler, 1998; Marsicano et al., 2002) or induced memory improvement (Lichtman, 2000; Takahashi, Pamplona, & Fernandes, 2005). Conversely, pre-training systemic administration of CB1 receptor agonists has shown to impair memory performance in rats (Fride, 2005; Hampson & Deadwyler, 1998; Lichtman & Martin, 1996).

The participation of the eCBs within specific brain regions on memory modulation has recently started to be elucidated. It has been observed, for example, that intra-hippocampal pre-training infusions of either AM251, a CB1 receptor antagonist/inverse agonist, or AEA did not influence acquisition of the step-down inhibitory avoidance task (de Oliveira Alvares, Genro, Diehl, & Quillfeldt, 2008). Nevertheless, AM251 micro-injections into the medial prefrontal cortex blocked learning of an olfactory fear-conditioning procedure, while CB1 receptor activation enabled associative learning when a subthreshold level of footshock was applied (Laviolette & Grace, 2006). Moreover, it has been suggested that

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, anandamide; BLA, basolateral amygdala; eCBs, endocannabinoids.

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different effects on memory following endocannabinoid system activation or blockade could be related to the emotional salience of the sensory stimuli of the task. In this respect, while non-aversive memories do not seem to be regulated by the endocannabinoid system (de Oliveira Alvares et al., 2006; Hölter et al., 2005; Niyuhire et al., 2007; Pamplona & Takahashi, 2006), strong evidences support the involvement of this system on memory processing for emotional stimuli (Viveros, Marco, Llorente, & López-Gallardo, 2007). This idea is further supported by the fact that the amygdala, known for its crucial role in associating emotionally charged stimuli (for a review Phelps & LeDoux, 2005), presents high CB1 receptors density (Katona et al., 2001; McDonald & Mascagni, 2001) and also exhibits increased levels of AEA and 2-AG after presentation of stress and aversive stimuli (Marsicano et al., 2002; Rademacher et al., 2008).

The amygdala has been divided into various cytoarchitecturally and functionally distinct nuclei, being the complex of the basolateral amygdala (BLA) one of the main regions responsible for integrating sensory inputs and modulating emotions, as well as acquisition and expression of emotional memories (Maren, Aharonov, & Fanselow, 1996; Phelps & LeDoux, 2005). BLA outputs have shown to be related to the induction of a variety of endocrine and emotional responses (Pitkanen, Savander, & LeDoux, 1997) by regulating the hypothalamus–pituitary–adrenal (HPA) axis during responses to anxiety and fear (LeDoux, 2000). In this respect, recent research from Ganon-Elazar and Akirav (2009) has shown that infusion of a CB1 agonist into the BLA before exposure to a stressor has reversed the stress-induced memory enhancement and decreased corticosterone levels in rats.

Besides modulating emotional memory formation, the eCBs have also been shown to play an important role in the control and regulation of anxiety states (Lutz, 2009; Viveros et al., 2007). This idea is supported by studies showing that systemic administration of a cannabinoid antagonist induces anxiety-like responses in rats (Navarro et al., 1997), along with CB1 receptor knockout (KO) mice studies reporting increased anxiety-like behaviors in a variety of tests (Uriguen, Perez-Rial, Ledent, Palomo, & Manzanares, 2004). Conversely, systemic studies enhancing CB1 receptors activation have shown biphasic effects, with low doses being anxiolytic (Kathuria et al., 2003) and high doses, anxiogenic (Scherma et al., 2008). Yet, a recent study has observed that, depending on the structure, activation of the endocannabinoid system may have different effects upon anxiety modulation (Rubino et al., 2008). In that respect, it is important to note that although the endocannabinoid transmission within the BLA seems to be related to emotional states, the relevance of this relationship to endocannabinoid effects on memory is still unknown.

In view of the fact that the BLA contains not only more CB1 receptors, but also more abundant CB1 mRNA compared to the other amygdalar nuclei (Katona et al., 2001) and also plays an important role in the modulation of emotional behaviors (Davis, Rainnie, & Cassell, 1994; Maren, Aharonov, & Fanselow, 1996), the present study investigated the effects of intra-BLA pre-training infusions of AEA or AM251 in the plus-maze discriminative avoidance task in order to simultaneously evaluate anxiety-like behavior, learning and memory (Silva & Frussa-Filho, 2000).

2. Material and methods

2.1. Subjects

Three-month-old male Wistar rats (250–300 g) were housed under conditions of controlled temperature (20–23 °C) under a 12 h light/12 h dark (lights on 06:30 am). Animals were housed in groups of 4–5 until surgery, when they were individually housed. Food and water were available *ad libitum* throughout the experiment. Rats were handled in accordance with the guidelines of the Brazilian Society for Neuroscience and Behavior for the use of animals in research, and all procedures were approved by the local ethical committee. All efforts were made to minimize animal pain, suffering or discomfort.

2.2. Surgery

Rats were anesthetized with ketamine and xylazine (i.p., 100 and 50 mg/kg, respectively) and two 23-gauge stainless-steel guide cannulae were bilaterally implanted into the BLA. The coordinates were AP −2.8 mm from bregma, LL ±4.9 mm, DV-6.4 mm (2.0 mm above BLA), according to Paxinos and Watson (2009). The cannulae were permanently fixed to the skull with dental acrylic and two stainless-steel screws. To prevent clogging, a stainless-steel stylus (30-gauge) was inserted into the guide cannulae and kept until the time of infusion. After surgery, each rat was individually housed and a recovery period of 5–7 days was given before testing. After two days of recovery, each animal was handled 10 min once a day until the day of the experiment.

2.3. Drugs and administration

AM251, a CB1 receptor antagonist/inverse agonist (Pertwee, 2006), and AEA, an endogenous cannabinoid, from Tocris Bioscience, were dissolved in a vehicle solution of 8% dimethylsulfoxide (DMSO) in phosphate buffered saline. Vehicle (DMSO 8%, $n = 11$), AM251 (10 ng per side, $n = 10$) or AEA (1 ng per side, $n = 9$) were infused bilaterally in the BLA in a volume of 0.2 μL at a rate of 0.1 μL/min via a microsyringe pump using Hamilton syringes connected to polyethylene tubing. Infusions were given 15 min before training session of the behavioral task. The concentrations and interval of injections chosen have been shown to be effective in the hippocampus (de Oliveira Alvares et al., 2008) and similarly in the BLA (Ganon-Elazar & Akirav, 2009). The infusion cannulae were left for 1 min more to allow total diffusion.

2.4. Plus-maze discriminative avoidance task (PM-DAT)

The PM-DAT is an aversive task paradigm which allows the concomitant and independent evaluation of aversive memory and anxiety-like behavior in rodents (Ribeiro et al., 2010; Silva & Frussa-Filho, 2000). The apparatus employed was a modified plus-maze, containing two open arms (49 × 13 cm) opposite to two enclosed arms (49 × 13 × 40 cm). A lamp and two loudspeakers were placed over one of the enclosed arms (aversive enclosed arm). In the training session, each rat was individually placed in the center of the apparatus (facing the space between both open arms) and, over a period of 10 min, every time the animal entered with the four paws in the aversive enclosed arm, the aversive stimuli were given until the animal left it. The aversive stimuli were the light (100 W, 600 lx) and a digitally produced 80 dB noise. Test session was performed 24 h after the conditioning, during which the animals were again placed in the apparatus for 3 min, without receiving the aversive stimulation.

Both sessions (training and test) were performed in the same room with a controlled intensity of light. On each side of the plus-maze discriminative avoidance apparatus there were different extra-maze visual cues that rats could use to distinguish the location of the different arms of the maze. All behavioral sessions were recorded using a camera fixed on the ceiling above the apparatus. During both sessions, behavior was tracked and registered by the Any-Maze software (Stoelting), and an observer monitored the session by a computer screen placed at another room. After each session, the apparatus was cleaned with 5% alcohol solution.

Learning was evaluated by the decrease in the percent time spent in the aversive enclosed arm [(time spent in the aversive enclosed arm/time spent in both enclosed arms) × 100] throughout the training session. Memory was evaluated by the percent time spent in the aversive enclosed arm in the test session, as well as the discrimination between the aversive and non-aversive enclosed arms. Anxiety-like behavior was evaluated by the percent time spent in the open arms [(time spent in open arms/time spent in both open and enclosed arms) × 100)], percentage of entries in the open arms [(open arms entries/total entries in all arms) × 100] and time performing risk assessment, which consists of postures and behaviors performed by the rodents when evaluating/avoiding the open arms (Carobrez & Bertoglio, 2005). Distance traveled was used to evaluate motor activity.

2.5. Histology

After behavioral procedures, rats were anesthetized with thiopental and perfused intracardially with phosphate-buffered saline (PBS) with NaCl 0.9% (pH 7.4), followed by 4.0% paraformaldehyde in 1.0 M PBS. Injection sites were marked by microinjection of methylene blue (0.2 μL per side) into the guide cannulae. Brains were removed and postfixed for two days in a 4.0% paraformaldehyde solution and then were put in sucrose 30% for 2–3 days. All brains were sectioned on a microtome and sections (100 μm) were stained with Neutral red. The location of cannulae tips was microscopically verified using the coordinates and limits defined by Paxinos and Watson (2009). Only rats with both cannulae tips within the boundaries of BLA were included in the data analysis (Fig. 1).

2.6. Statistical analysis

Percent time throughout the training session was compared by two-way analysis of variance (ANOVA) with treatment as a between subject factor and time (minutes of observation) as a repeated measure. Distance traveled, percentage of time spent in the aversive enclosed arm, percent time spent in open arms, percentage of entries in the open arms and risk assessment were compared by the one-way ANOVA followed by Bonferroni's test. The discrimination between the aversive and non-aversive enclosed arms was compared by the paired samples Student's *t*-test. A probability of $p < 0.05$ was considered to show significant differences for all comparisons made.

3. Results

3.1. Learning and memory

In the training session, two-way ANOVA, revealed a time effect for the percent time spent in the aversive arm [$F(9,243) = 20.66$, $p < 0.001$] but no treatment effect [$F(2,27) = 0.65$, $p = 0.53$] or treatment *x* time interaction [$F(18,243) = 0.66$, $p = 0.84$]. This result indicates that all groups learned the task, as seen by the decrease in percent time spent in the aversive arm throughout the training session (Fig. 2A). One-way ANOVA considering the whole training session revealed no difference among groups [$F(2,27) = 0.52$, $p = 0.6$] (Fig. 2B).

In the test session, as shown in Fig. 2C, one-way ANOVA followed by Bonferroni's test revealed that the group receiving AEA showed increased percent time spent in the aversive arm when compared to control [$F(2,27) = 4.3$, $p = 0.02$], suggesting that pretraining micro-infusion of AEA into the BLA caused memory impairment. The AM251 group showed no differences when compared to control group. Accordingly, when discrimination between the enclosed arms (aversive arm vs. non-aversive arm, Fig. 2D) was analyzed, vehicle and AM251-treated groups spent significantly more time in the non-aversive arm [($t = 4.52$, $p = 0.001$), ($t = 4.03$, $p = 0.003$), respectively], indicating memory retrieval, whereas AEA-treated animals showed no discrimination between enclosed arms ($t = 1.14$, $p = 0.29$).

3.2. Anxiety-like behavior

When the percent time spent in the open arms during the whole session were evaluated, no differences were found among the groups [$F(2,27) = 1.53$, $p = 0.24$ – Fig. 3A]. On the other hand, as shown in Fig. 3B, one-way ANOVA for the percentage of entries in the open arms during the whole training session revealed a difference among the groups [$F(2,27) = 4.44$, $p = 0.02$]. However, post hoc analysis with Bonferroni's test did not reach significance, although suggesting that AM251-treated animals showed increased anxiety-like behavior (decreased open-arm entries) when compared to control group.

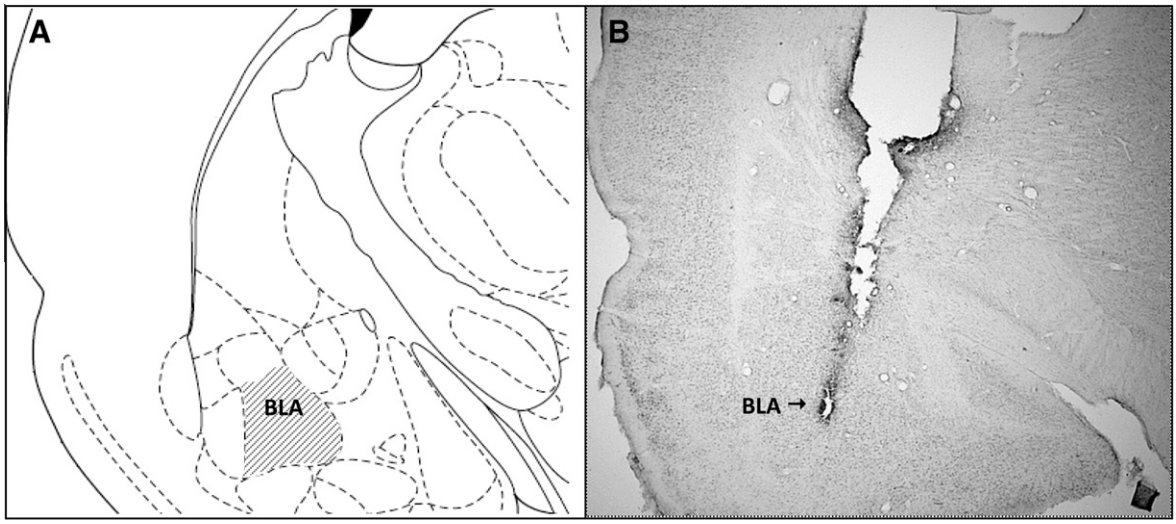

Fig. 1. (A) Schematic drawing illustrating the injection cannulae placement for rats included in statistical analysis (Paxinos & Watson, 2009). (B) Representative microphotography showing the injector cannula mark.

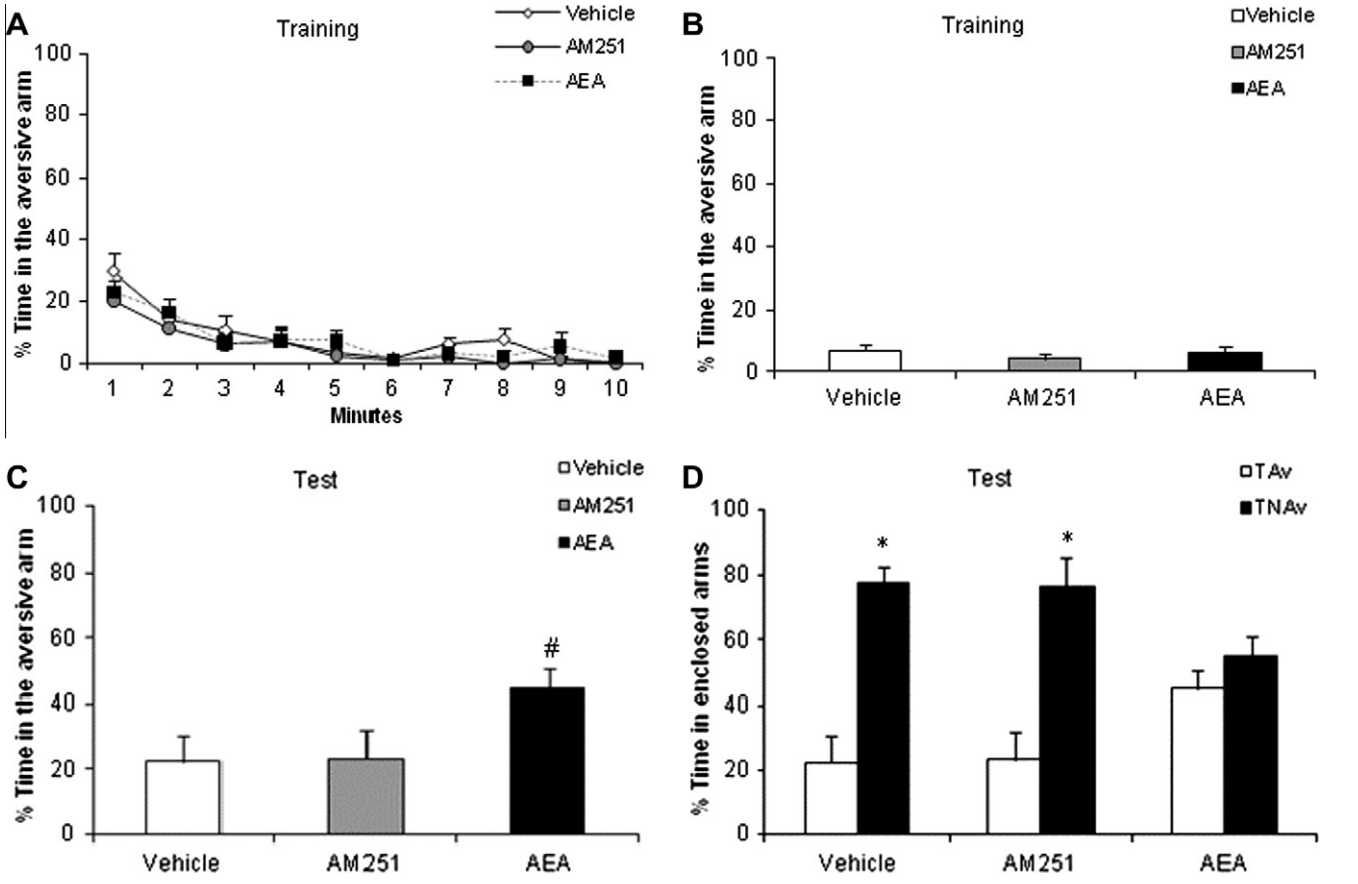

Fig. 2. Effects of pre-training micro-injection of AM251 or AEA in the BLA on learning (A and B) and retrieval (C and D) of discriminative avoidance task in rats (VEH, $n = 11$; AM251, $n = 10$; AEA, $n = 9$). Mean ± S.E. for (A) percent time spent in the aversive arm throughout training session (two-way ANOVA with repeated measures, revealing time effect, (B) percent time spent in the aversive enclosed arm (TAv) during the whole training, (C) percent time spent in the aversive enclosed arm during test session and (D) percent time spent in each of the enclosed arms (TAv and TNAv) during test session. $^{\#}p < 0.05$ compared to vehicle; $^{*}p < 0.01$ compared to TAv (paired samples t-test).

The time animals spent performing risk assessment behaviors was also evaluated (Fig. 3C), since it has been suggested that this parameter would be a more sensitive measure of anxiety-like behavior than the traditional measures (Carobrez & Bertoglio, 2005). During training session, one-way ANOVA for risk assessment showed a significance difference [$F(2,27) = 3.61$, $p = 0.04$] with post hoc indicating an anxiogenic effect of AM251 ($p = 0.04$), seen by a reduction of the time performing risk assessment.

One-way ANOVA revealed no differences between the groups when evaluating the percent time spent in the open arms (Fig. 3A), percentage of entries in the open arms (Fig. 3B) or risk assessment (Fig. 3C) during test session [$F(2,27) = 0.37$, $p = 0.58$; $F(2,27) = 0.87$, $p = 0.43$; $F(2,27) = 0.5$, $p = 0.61$, respectively].

3.3. Locomotion

No differences were found in distance traveled during training [$F(2,27) = 1.28$, $p = 0.29$] or test [$F(2,27) = 1.26$; $p = 0.3$] sessions (Table 1).

4. Discussion

In summary, our results showed that (1) during training session, all groups learned the task by progressively avoiding the aversive arm throughout the session (Fig. 2A and B); (2) AEA given before training into the BLA disrupted memory formation – shown by the lack of discrimination between the two enclosed arms in the test session, whereas AM251 did not interfere with memory formation (Fig. 2C and D); and (3) AM251 into the BLA induced an anxiogenic effect – shown by the reduced percentage of entries in the open arms and risk assessment during training, while AEA had no effects on anxiety levels (Fig. 3).

Endocannabinoid release has been shown to occur in the amygdala when animals are exposed to stressful stimuli (Marsicano et al., 2002; Rademacher et al., 2008). As acquisition of a new aversive memory evidently requires an unpleasant emotional context, it is possible that eCBs are recruited during acquisition of emotional-charged information, in order to reduce the stress-induced arousal, as proposed by Lutz (2009). It is widely accepted that the amygdala plays a crucial role in acquisition of aversive memories. In humans, it is known to be recruited during early phases of aversive conditioning (LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998). Similarly, several animal studies show impaired acquisition of aversive memory after BLA inactivation (for a review see Phelps & LeDoux, 2005). In this respect, there is evidence that canabinnoids not only alter BLA function by reducing neuronal firing rates (Pistis et al., 2004), but also induce long-term depression (LTD) at excitatory synapses (Marsicano et al., 2002). It has also been shown that infusion of the cannabinoids THC and CP55940 in the hippocampus disrupted the temporal coordination of cell assemblies and impaired physiological oscillations, being this impairment associated with memory deficits (Robbe et al., 2006).

In the present study, although successful conditioning was shown by all groups, it is possible that the weak fearful nature of non-painful stimuli would require the BLA to integrate the different sensory inputs (LeDoux, 1990; Turner, 1981) further needed for subsequent memory consolidation. Therefore, the disrupted re-

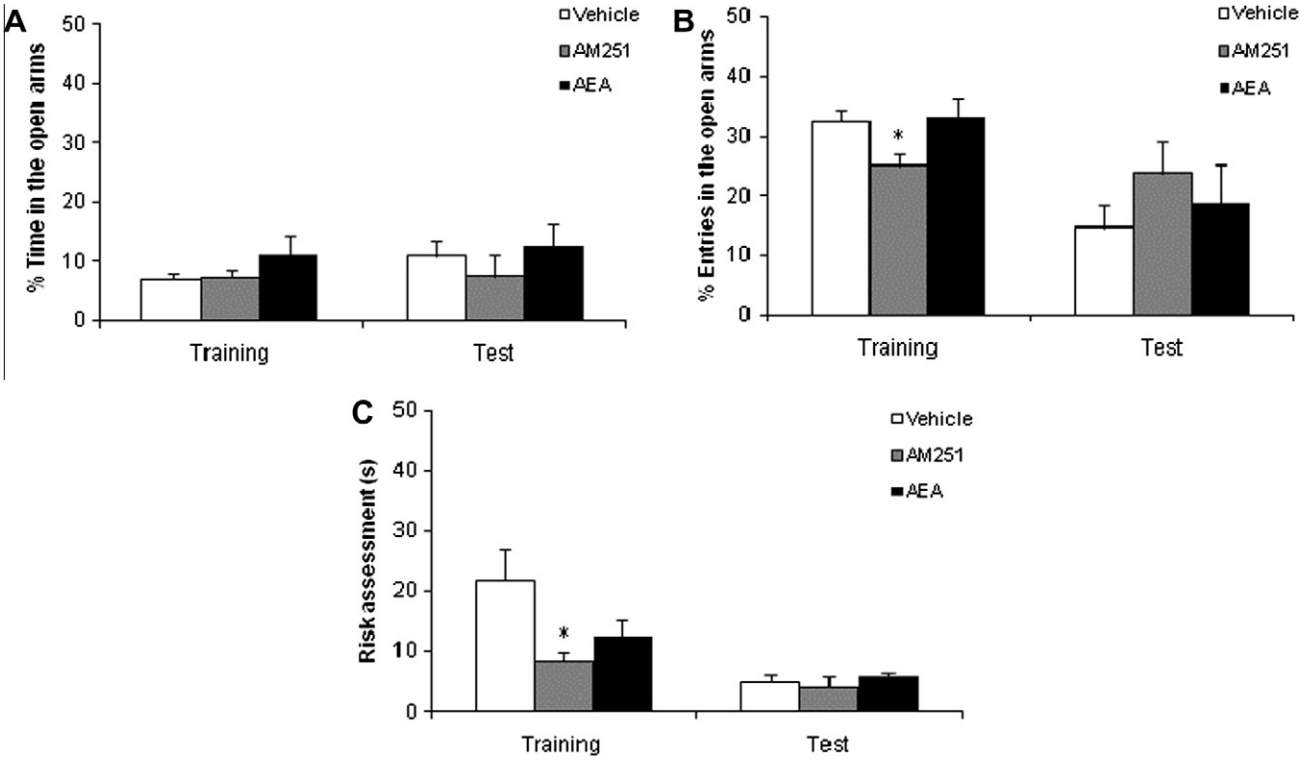

Fig. 3. Effects of pre-training micro-injection of AM251 or AEA in the BLA on anxiety-like behavior in discriminative avoidance task in rats (VEH, $n = 11$; AM251, $n = 10$; AEA, $n = 9$). Mean ± S.E. for (A) percent time spent in the open arms, (B) percent of entries in the open arms and (C) time performing risk assessment during training session. $^{*}p < 0.05$ compared to vehicle.

Table 1
Effects of pre-training micro-injection of vehicle, AEA or AM251 in the BLA on locomotion in discriminative avoidance apparatus in rats (VEH, $n = 11$; AM251, $n = 10$; AEA, $n = 9$). Mean ± S.E. for distance traveled (m) in the training and test sessions. No significant differences were found (one-way ANOVA).

		Training	Test
Distance	Vehicle	13.7 ± 2.2	4.6 ± 0.8
	AM251	8.8 ± 1.7	4.1 ± 0.8
	AEA	12.9 ± 3.1	5.9 ± 1.1

trieval due to pre-training administration of AEA could be related to amygdala dysfunction during training, with consequent loss of its capacity to recognize and integrate cues of threat and danger (Adolphs, Tranel, & Damasio, 1998), leading to inefficient aversive stimuli processing and memory formation.

On the other hand, because micro-infusions have been given before training, drugs would remain acting after training session and could modulate memory consolidation as it takes place during the first hours after training (Izquierdo et al., 2006; McGaugh, 2000). However, Campolongo and collaborators et al. (2009) have recently shown that post-training micro-injection of WIN-55,212-2, a CB1 receptor agonist, within the BLA has, in fact, improved memory consolidation of inhibitory avoidance task. Thus, although different task and CB1 agonist were used in the present study, the memory deficit following AEA administration in the BLA before training is most likely related to a disruption in BLA function during the acquisition of the information rather than an action during subsequent memory consolidation.

Corroborating the present data, Phan and collaborators (2008) have recently reported reduced amygdala reactivity to threatening stimuli after an acute dose of THC in humans. Furthermore, a recent fMRI study proposed that amygdala interactions with other brain regions would enhance memory formation by increasing attention and perceptual processing of emotional information (Murty, Ritchey, Adcock, & Labar, 2010), which is in accordance with several human studies showing attention deficits (Curran, Brignell, Fletcher, Middleton, & Henry, 2002; Marks & MacAvoy, 1989), as well as memory impairments (Curran et al., 2002; Lewеke et al., 1998; Ranganathan & D'Souza, 2006) after acute THC consumption.

In the present study, blockade of CB1 receptors with AM251 within the BLA did not interfere with memory formation. Accordingly, some studies with systemic CB1 antagonist administrations were also unsuccessful in modulating memory acquisition (Hampson & Deadwyler, 2000; Marsicano et al., 2002) although others have shown an enhancement on memory acquisition (Lichtman, 2000; Takahashi et al., 2005). Moreover, a recent study showed that AM251 in the BLA (5 and 50 ng) was not sufficient to alter acquisition of conditioned fear memories (Tan, Lauzon, Bishop, Bechard, & Laviolette, 2010). On the other hand, comparing the effects of AM251 (CB1 antagonist/inverse agonist) with AM4113 (CB1 antagonist) on contextual fear conditioning, Sink, Segovia, Collins et al. (2010) showed that systemic administration of AM251 before conditioning increased memory formation, while AM4113 had no effects, suggesting that the effects of AM251 could be specifically related to its inverse agonist activity. Nevertheless, although only AM251 was tested in the present study, it seems that the blockade of CB1 receptors in the BLA would not alter plus-maze discriminative avoidance acquisition despite the activation of these receptors by AEA would impair it. In this respect, it is important to note that CB1 receptor activation is not the only mechanism of action of AEA (Pertwee, 2006). This eCB also targets, for example, the vanilloid receptor TRVP1 (Tognetto et al., 2001), as well as potassium channels (Maingret, Patel, Lazdunski, & Honore, 2001), and a possible role of these other mechanisms in the effect of AEA on aversive memory could not be ruled out.

As mentioned above, the endocannabinoid transmission is not only involved in memory processing, being considered an important modulator of emotional states (Lutz, 2009; Viveros et al., 2007). Accordingly, knockout mice for CB1 receptors show increased anxiety levels in several behavioral tasks (Haller, Bakos, Szirmay, Ledent, & Freund, 2002; Uriguen et al., 2004) and increased anxiety levels have also been reported after a systemic CB1 receptor antagonist administration (Navarro et al., 1997). Moreover, a systemic AM251 administration caused enhanced c-Fos expression in the amygdala, which was also associated with an anxiogenic effect (Sink, Segovia, Sink et al., 2010). In general, our results corroborate those findings, since AM251 administration in the BLA increased anxiety-like behavior (although a statistically significant difference was found only for one of the parameters measured – see Fig. 3). Furthermore, Rubino and collaborators (2008) have recently reported that THC at higher concentrations in the BLA had no effects on anxiety levels, which is in line with our results, since AEA did not alter anxiety-like behaviors. It has also been seen that the GABAA agonist muscimol (which might cause an inactivation of BLA neurons similar to AEA), when infused within the BLA was also ineffective in altering anxiety levels of rats submitted to the PM-DAT (Ribeiro et al., in press). In contrast, however, studies with systemic potentiation of the endocannabinoid signaling have shown anxiolytic effects in low doses (Kathuria et al., 2003), with higher doses being anxiogenic (Scherma et al., 2008).

The mechanisms underlying the role of eCBs in the amygdala in anxiety states is not completely understood. Interestingly, a high co-localization has been observed in BLA GABAergic interneurons containing CB1 receptors and the anxiogenic neuropeptide cholecystokinin (CCK) (Katona et al., 1999; Marsicano & Lutz, 1999; McDonald & Mascagni, 2001). In addition, activation of CB1 receptors has shown to reduce CCK release (Beinfeld & Connolly, 2001; Burdyga et al., 2004; Fride, 2005). Higher CCK levels would, in turn, excite BLA interneurons (Chung & Moore, 2009a), as well as enhance its GABAergic transmission (Chung & Moore, 2007). Although it may be argued that this increase of GABAergic transmission would in fact reduce BLA activity, leading to anxiolytic states, it has been recently reported that CCK also excites glutamatergic projections in the BLA by modulation specific subsets of interneurons (Chung & Moore, 2009b). Along with that idea, Truitt, Johnson, Dietrich, Fitz, and Shekhar (2009) have shown that discrete subpopulations of interneurons in the rat BLA are sufficient to modulate anxiety states.

Alternatively, corticosteroids have an important role in regulating anxiety states, as well as in response to stressful stimuli. In accordance, glucocorticoids were shown to enhance BLA principal neurons excitability (Duvarci & Pare, 2007), which could generate anxiogenic effects (Davis et al., 1994). Recently, glucocorticoids have been found to act together with the endocannabinoid system in the modulation of stress responses (Hill et al., 2010; Patel, Roelke, Rademacher, Cullinan, & Hillard, 2004), as well as memory processing influenced by stressful factors (de Oliveira Álvares et al., 2010) as would be the case of aversive memory tasks.

In summary, our data show that intra-amygdala AEA infused before conditioning impaired memory formation for the discriminative avoidance task, as seen by deficient memory retrieval during test. Although not discarding a possible interference with subsequent memory consolidation, we propose that the enhancement of AEA levels within the BLA may act on the acquisition of aversive information by altering the integration of relevant sensory inputs or even by impairing the evaluation of the threat nature of the stimuli. Importantly, the antagonist/inverse agonist of CB1 receptors AM251 did not interfere with memory, inducing, however, an anxiogenic effect. Taken together, the findings presented here suggest that, although drugs that act on the endocannabinoid system within the BLA may alter aversive memory as well as anxiety-like behaviors, these modulations do not seem to be necessarily related, since alteration of anxiety-like states and memory formation occurred independently in rats tested in the PM-DAT.

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