Basolateral amygdala inactivation impairs learned (but not innate) fear response in rats

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ABSTRACT

Numerous studies have suggested that the amygdala is involved in the formation of aversive memories, but the possibility that this structure is merely related to any kind of fear sensation or response could not be ruled out in previous studies. The present study investigated the effects of bilateral inactivation of the amygdaloid complex in rats tested in the plus-maze discriminative avoidance task. This task concomitantly evaluates aversive memory (by discrimination of the two enclosed arms) and innate fear (by open-arm exploration). Wistar rats (3–5 months-old) were implanted with bilateral guide cannulae into basolateral amygdala. After surgery, all subjects were given 1 week to recover before behavioral experiments. Afterwards, in experiment 1, 15 min prior to training, 0.5 μl of saline or muscimol (1 mg/ml) was infused in each side via microinjection needles. In experiment 2 the animals received injections immediately after the training session and in experiment 3 rats were injected prior to testing session (24 h after training). The main results showed that (1) pre-training muscimol prevented memory retention (evaluated by aversive arm exploration in the test session), but did not alter innate fear (evaluated by percent time in open arms); (2) post-training muscimol impaired consolidation, inducing increased percent in aversive arm exploration in the test session and (3) pre-testing muscimol did not affect retrieval (evaluated by aversive enclosed arm exploration in the test session). The results suggest that amygdala inactivation specifically modulated the learning of the aversive task, excluding a possible secondary effect of amygdala inactivation on general fear responses. Additionally, our data corroborate the hypothesis that basolateral amygdala is not the specific site of storage of aversive memories, since retention of the previously learned task was not affected by pre-testing inactivation.

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1. Introduction

The amygdaloid complex (specifically the basolateral portion) is considered to play a key role in the neurobiological mechanisms involved in aversive memory. Two main hypothesis are the core of the investigations on this issue: (1) the amygdala is the site of the plastic process that underlie acquisition and consolidation of aversive information (see LeDoux, 2000; Maren & Quirk, 2004 for reviews) and (2) this structure exerts a modulatory role on acquisition and consolidation that take place in other brain regions (see Cahill & McGaugh, 1998; McGaugh, 2004; Packard & Teather, 1998; Vazdarjanova, Cahill, & McGaugh, 2001).

The results that give support to the first hypothesis were obtained mainly in a rodent model of fear conditioned response, performed by pairing a neutral stimulus with an electric shock

delivered to animaĺs feet. Upon reexposure to the neutral stimulus, the animal presents a conditioned freezing response. In short, these studies have shown that lesions or temporary inactivation of basolateral amygdala impair the acquisition, retrieval and extinction of the conditioned fear response (Davis, 1994; Falls, Miserendino, & Davis, 1992; Fanselow & Gale, 2003; Fanselow & LeDoux, 1999; LeDoux, 1996, 2000; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; Maren & Fanselow, 1996; Muller, Corodimas, Fridel, & LeDoux, 1997; Quirk, Armony, & LeDoux, 1997; Sacchetti, Lorenzini, Baldi, Tassoni, & Bucherelli, 1999; Wilensky, Schafe, & LeDoux, 1999). Studies have also shown long-lasting modifications in synaptic efficiency in amygdala pathways (Clugnet & LeDoux, 1990; LeDoux, 2000; Maren & Fanselow, 1995; Rogan, Stäubli, & LeDoux, 1997; Schroeder & Shinnick-Gallagher, 2005). In addition, the expression of genes related to plasticity (Shumyatsky et al., 2002; Yeh, Mao, Lin, & Gean, 2006) was demonstrated in the amygdala of rats submitted to fear conditioning.

Nevertheless, it has been argued that conditioned freezing response is insufficient as an experimental model to study emotional influence on memory, because it is restricted to a conditioned

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emotional response (Cahill & McGaugh, 1998). Conversely, the second hypothesis is largely based in studies with the passive avoidance paradigm. In this task, rats are placed in a different location from that in which aversive stimulation will be applied and will receive a foot shock only when voluntarily moving to the stimulus chamber. The use of this paradigm addresses some critical points compared to the studies with conditioned fear (evaluation of memory by a response that is not freezing and the supposed declarative nature of the task). In summary, these studies have pointed to a modulatory (but not critical) role of the amygdala in aversive memory, since: (1) amygdala inactivation attenuates but not prevent task retention; (2) impairment of passive avoidance performance only occurs if amygdala inactivation is performed before or shortly after the training procedure; (3) participation of other structures in memory consolidation such as insular cortex or hippocampus are modulated by amygdala activity and (4) basolateral amygdala activity modulates plasticity-related proteins expression in the hippocampus (Cahill & McGaugh, 1998; Cahill, Vazdarjanova, & Setlow, 2000; LaLumiere, Nawar, & McGaugh, 2005; Liang et al., 1982; Malin & McGaugh, 2006; McGaugh, 2004; McGaugh, Cahill, & Roozendaal, 1996; McIntyre et al., 2005; Miranda & McGaugh, 2004; Roozendaal & McGaugh, 1997; Roozendaal, McReynolds, & McGaugh, 2004; Roozendaal, Portillo-Marquez, & McGaugh, 1996; Vazdarjanova & McGaugh, 1998, 1999).

However, there are still critical issues when using this model to study the role of amygdala on aversive memory. First, the increase in motor activity usually induced by amygdala inactivation (Blanchard & Blanchard, 1972; Burns, Annett, Kelley, Everitt, & Robbins, 1996) could induce per se a decrement in latency to move to stimulus chamber (used to evaluate animaĺs performance). More important, a reduced basal (innate) fear due to manipulations in amygdala could soften the relevance of the aversive stimulus (Bellgowan & Helmstetter, 1996; Kemble, Blanchard, & Blanchard, 1990; Mesches, Bianchin, & McGaugh, 1996; Sanders & Shekhar, 1995; Vazdarjanova & McGaugh, 1998; Walker & Davis, 1997). In this respect, studies have shown that the amygdaloid complex is part of the neural circuits which modulate innate fear responses (Blanchard & Blanchard, 1972; Canteras, Ribeiro-Barbosa, & Comoli, 2001). Importantly, a differential role of amygdala central and basolateral sub-regions in innate fear has been proposed (Moreira, Masson, Carvalho, & Brandão, 2007). It is still unclear, however, if the role of amygaloid circuits in organizing innate response is similar to the participation of this structure in processing learned fear responses (Antoniadis & McDonald, 2001; Canteras et al., 2001; Muller & Fendt, 2006).

Despite important data has risen from the use of the animal models mentioned above, none of these tasks allows the separate evaluation of aversive learning/memory and innate fear response. Additionally, although differential results were obtained with the two tasks, they are contextually very similar. Indeed, the same kind of aversive stimulation is used, neither responses depend on spatial clues, and the presence or absence of freezing response is not addressed when rats are submitted to the passive avoidance paradigm.

The plus-maze discriminative avoidance task allows the simultaneous evaluation of mnemonic and emotional aspects during memory process. This paradigm is based on the simultaneous presentation of a context-paired aversive situation and a naturally aversive situation (to which the fear response is innate). Because these responses (learned and innate) are evaluated by different parameters, it is possible to address if the effects of experimental manipulations on aversive memory are related specifically to acquisition, consolidation or retrieval processes or to modifications in the emotional response to aversive situations regardless the cognitive component. Importantly, mild aversive stimulation (light and noise) is used and spatial information (extra-maze cues) is present (Calzavara, Lopez, Abílio, Silva, & Frussa-Filho, 2004; Kameda et al., 2007; Silva & Frussa-Filho, 2000; Silva et al., 2002).

We evaluated the effects of temporary inactivation of the BLA in rats tested in the plus-maze discriminative avoidance task. Inactivation was accomplished via local infusion of the GABA$_A$ agonist muscimol, which is widely used with this purpose (e.g. Krupa & Thompson, 1997; Lomber, 1999; Muller et al., 1997). In this context, the BLA contains a high density of GABA neurons (Nitecka & Ben-Ari, 1987) and electrophysiological studies have suggested the existence of inhibitory intra-amygdaloid connections (Le Gal La Salle, 1976). Thus, the behavioral effects of intra-amygdaloid muscimol on learning, consolidation and retrieval of an aversive task were addressed, simultaneously evaluating possible alterations in innate fear response in rats.

2. Materials and methods

2.1. Subjects

Forty-nine three-month old male Wistar rats were individually housed in a climate controlled room, under a 12 h light/12 h dark cycle and at constant temperature of 25 ± 1 °C. Rats were kept under standard laboratory conditions with *ad libitum* access to food and water. All animals were submitted to handling for 6 days during 10 min before the beginning of the experimental procedures, which were conducted during the light phase of the cycle (1:00–5:00 p.m.). All procedures were performed in accordance with the Brazilian Society for Neuroscience and Behavior guidelines for the use of animals in research. All efforts were made to minimize animal pain, suffering or discomfort, and diminish the number of rats used.

2.2. Surgery

Prior to surgery rats were anesthetized with an intraperitoneal injection of a ketamine (100 mg/kg) plus xylazine (50 mg/kg) and fixed in a stereotaxic frame (Insight, Brazil). A stainless steel guide cannulae (25 gauge, 20 mm length) was implanted bilaterally in the amygdaloid complex (Basolateral amygdala nuclei). Stereotaxic coordinates for the guide cannulae placements were anterior–posterior (AP) = −2.8 mm from bregma, medial–lateral (ML) = ±5.0 mm, and dorsal–ventral (DV) = −6.2 mm (Paxinos & Watson, 2009). Guide cannulae were anchored to the skull with screws and dental acrylic. At the end of the surgery each cannulae was temporarily sealed with a stainless steel wire to protect it from obstruction. Each rat was then given an intramuscular injection of penicillin (60.000 UI/ml) and sodium diclofenac (10 mg/25 μl) to minimize post-surgical discomfort. Animals were given 7 days of post-operative recovery prior to the start of behavioral training.

2.3. Drug and injection procedures

The selective GABA$_A$ receptor agonist muscimol (Sigma, USA) was dissolved in physiological saline. Bilateral intra-amydala infusions of muscimol (0.5 μg in 0.5 μl per side) or saline (0.5 μl) were performed over a period of 2 min via a microsyringe pump using Hamilton syringes connected to polyethylene tubing. Injection needles were left in the guide cannulae for an additional 60 s following the infusions to allow for diffusion of the drug from the needle tip. After injection, rats were placed back into their home cages. The dose and volume of muscimol was selected on the basis of previous studies investigating the effect of muscimol infusion in the BLA on fear memory (e.g. Muller, Corodimas, Fridel, & LeDoux, 1997). This concentration (1 μg/1 μl) induces a complete inhibition