Neurobiology of Learning and Memory 93 (2010) 196–202

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Enhancement of spatial learning by predator odor in mice: Involvement of amygdala and hippocampus

Emmanuel Galliot *, Maryline Levaillant, Elidie Beard, Jean-Louis Millot, Gregory Pourié

Laboratoire de Neurosciences, Université de Franche-Comté, 1 Place Leclerc, F-25030 Besançon Cedex, France

ARTICLE INFO

Article history:
Received 27 July 2009
Revised 3 September 2009
Accepted 25 September 2009
Available online 1 October 2009

Keywords:
Spatial learning
Hippocampus
Amygdala
Predator odor
Mice

ABSTRACT

Olfaction has particular links with learning and memory compared with other sensory cues, due to the interrelations between their neural circuitry. The present study deals with the effects of a putative stressor (i.e. a predator odor) on visuo-spatial learning in mice. Firstly, the results show that a predator odor spread during the Morris water maze task led to learning enhancement. In addition, a stereotaxic approach was used to investigate the involvement of the amygdala in this hippocampus-dependent type of learning. Thus, the performance of mice in visuo-spatial learning under predator odor conditions was dramatically reduced by an ibotenate bilateral amygdala lesion. The involvement of the amygdala was confirmed by a reduced expression of c-fos in the CA1 hippocampus of amygdala-lesioned mice at the end of the learning procedure.

Mild exposure to a predator odor during hippocampus-dependent learning therefore leads to an enhancement of performance through the co-activation of the amygdala, probably by a stress mediated mechanism.

1. Introduction

Olfaction is generally defined as a primitive sense involved in either inter- or intra-specific relationships or in basic environmental explorations. However, whether or not this sense is involved in complex neurobehavioral acquisitions is still unclear. Moreover, numerous studies have shown that the olfactory system and the circuitry of learning and memory share some links. Thus, via the primary olfactory cortex, the olfactory inputs project to the entorhinal cortex, which in turn projects to the hippocampus. Projections from the primary olfactory cortex also directly reach the amygdala in the lateral nucleus (Lledo, Gheusi, & Vincent, 2005). The amygdala itself projects to several hippocampal regions, including the CA1 area (Aggleton, 1986; Krettek & Price, 1977) and can therefore modulate hippocampal functions such as learning (Kim, Lee, Han, & Packard, 2001; McGaugh, 2004). Learning and memory involve numerous neural structures, two of which are believed to have major encoding functions. The hippocampal formation is described as essential for relational memory (i.e. spatial and episodic memory), whereas the parahippocampal formation, including the entorhinal and the perirhinal cortices, appears to be more concerned with non-relational memory (i.e. stimulus recognition or association between stimuli) (Hanson, Bunsey, & Riccio, 2002).

Odors have been extensively studied in rodents in relation to non-relational memory (Bodyak & Slotnick, 1999; Brennan, Schellinck, de la Riva, Kendrick, & Keverne, 1998; Gall, Hess, & Lynch, 1998; Kim & Ragozzino, 2005), but little is known of the potential contribution of odors to spatial learning. Lavenex and Schenk (1997) have shown that proximal olfactory cues potentiate learning of distant visuospatial information. In addition, bulbectomy impairs Morris water maze learning, but only as a transient deficit (Van Rijzingen, Gispen, & Spruijt, 1995).

Several studies indicate that predator odors appear to be relevant signals (especially for species such as rodents), which elicit innate fearful behavioral and physiological responses (Dielenberg & McGregor, 2001; Kats & Dill, 1998; Masini, Sauer, & Campeau, 2005; Wallace & Rosen, 2000). Some of these studies used 2,4,5 trimethylthiazoline (TMT), a component of fox feces, to induce unconditioned fear in rodents (Fendt, Endres, Lowry, Apfelbach, & McGregor, 2005).

It is well documented that acute or chronic stress impairs cognitive performances like learning and memory (Kim et al., 2001; Song, Che, Min-Wei, Murakami, & Matsumoto, 2006). Several studies have shown that acute stressors such as electric foot shock or tail shock induce long-term potentiation (LTP) and learning modulation. However, the results of studies investigating the effects of stress on learning performances appear inconsistent, especially because a variety of stress inducing methods are used. It is known that a predator odor can modulate fear-related behaviors which can influence learning (Takahashi, Nakashima, Hong, & Watanabe,

* Corresponding author. Fax: +33 381665746.
E-mail address: emmanuel.galliot2@univ-fcomte.fr (E. Galliot).

1074-7427/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2009.09.011

2005), and spatial learning deficit was also apparent in mice repetitively exposed to rats (Grootendorst, de Kloet, Vossen, Dalm, & Oitzl, 2001). However if the stress is induced during the learning procedure, it can enhance learning and memory processes by a corticosterone up-regulation (Joels, Pu, Wiegert, Oitzl, & Krugers, 2006). Moreover, the hippocampus which is involved in LTP (Eichenbaum, Otto, & Cohen, 1992; Squire & Zola, 1996) is rich in corticosterone receptors, and several studies have already shown that an elevation of corticosterone activates several central structures that regulate the stress response, mood, learning and memory (Nishi & Kawata, 2006). One particular study on birds by Pravosudov (2003) reported that moderate chronic elevation of corticosterone can enhance spatial learning performance.

The amygdala is a brain structure which plays a key role in emotional processing (Kim et al., 2001). Amygdala lesions critically disrupt the development and expression of conditioned fear in rodents (Davis, 1992). In addition, the direct involvement of the amygdala in emotional learning has been suggested (Davis, 2000; LeDoux, 2000; McGaugh, 2004). Finally, recent studies have also highlighted that the amygdala is involved in the modulation of hippocampal functions, since amygdala lesions could impair unconditioned fear-related behaviors (Blanchard, Canteras, Markham, Pentkowski, & Blanchard, 2005; Takahashi, Hubbard, Lee, Dar, & Sipes, 2007). However, the direct influence of the amygdala has not been reported for hippocampus-dependent spatial learning.

The present study aims to investigate the potential effects of a predator odor (2,4,5 trimethylthiazoline: TMT) on spatial learning in mice. TMT is a predator (fox) odor, known to be repulsive (Endres, Apfelbach, & Fendt, 2005), and is usually used in high doses to induce fear in rodents (King, De Oliveira, & Patel, 2005; McGregor, Schrama, Ambermoon, & Dielenberg, 2002; Redmond, Morrow, Elsworth, & Roth, 2002). In this study we investigated the hypothesis that a mild fear might enhance learning performances through the activation of the amygdala. To address this objective, spatial learning performance was assessed in a Morris water maze test with and without the presence of TMT. We attempted to determine the involvement of ibotenic lesions in the amygdala in a group of mice. Finally, we used immunochemical detection of Fos protein in the hippocampus as an inducible transcription factor which leads to neuronal modifications underlying learning processes (Chaudhuri, Zangenehpour, Rahbar-Dehgan, & Ye, 2000; Guzowski, Setlow, Wagner, & McGaugh, 2001; Kaczmarek, 2002). Fos detection has been successfully used to investigate brain activation in various learning experiments (Ferreira, Ferry, Meurisse, & Levy, 2006; Navarro, Spray, Cubero, Thiele, & Bernstein, 2000) and in studies on olfactory memory (Datiche, Roullet, & Cattarelli, 2001).

2. Materials and methods

2.1. Animals

Adult female OF1 mice (Charles River, France) were used in this study. They were single-housed in standard-size Plexiglas cages, allowing them free access to food and water and maintaining them in a climate controlled environment (22 °C) on a 12 h light/dark cycle (light onset at 7 a.m.). The animals were 3 months old at the beginning of the experiment and had no previous experience in any behavioral study.

2.2. Odorants

Trimethylthiazoline (PheroTech, Delta, Canada) was used as a predator odor (fox feces). Distilled water was used as a control odorant.

2.3. Surgery

The surgery was conducted under chloral anesthesia (0.5 g/kg), using standard stereotaxic procedures. Injection sites were determined with the aid of a mouse brain atlas (Franklin & Paxinos, 1997). The injection was performed with a Hamilton 1 μL microsyringe that was implanted bilaterally at the following coordinates (AP = −1.50 mm from the bregma; ML = ± 3.25 mm; DV = −5 mm from the skull surface). A solution of ibotenic acid (5 mg/ml) in phosphate buffer saline (PBS) (pH 7.4) was bilaterally injected. 1 μL was injected at the rate of 200 nl/min. Ibotenic acid is a neurotoxin (glutamate agonist) that selectively kills cells in the affected area, but spares fibers of passage (Schwarcz et al., 1979). A peculiarity of the ibotenic acid is that it do not spread to surrounding tissues and thus create discrete lesions (Jarrard, 1989). Following the injection, the needle was left in place for another 2 min before retraction. Mice in the sham-lesioned groups underwent the same surgical procedures, except that PBS was neurotoxic-free. All operated mice were allowed to recover in their home cages in the animal room for at least 7 days before the behavioral part of the experiment began.

2.4. Behavioral tests

2.4.1. Preference test

We proceeded with preliminary tests in order to check the basic effects of TMT on the general activity of mice. We firstly performed a preference test in a Y-shaped maze. The apparatus was 6.5 cm wide, the walls were 12 cm high, and each arm was 25 cm long. Filter papers (1 × 1 cm) were placed at the end of the two arms of the Y-maze as substrates for compounds. Five micro litre of TMT or water (control odor) were deposited on each of these two filter papers with a random distribution. For each test, the mouse was placed in the starting arm (arm opposite odors) of the maze and was allowed to move freely for 3 min. During the test, the maze was covered by glass to avoid odorant dissipation in the room and the time spent by the mouse in each arm was measured. The maze was cleaned with 50% ethanol and the filter papers were changed after each trial. Ten intact adult females were used for this test, and they were not used for any other tests.

2.4.2. Open field

An open-field test was conducted in a circular arena (48 cm in diameter, walls 15 cm high), virtually divided into four quadrants. 1.25 μL of odorants (TMT or water) were deposited on each of the four filter paper strips fixed at the top of the wall, one paper strip in each of the four quadrants of the circular arena. Mice were allowed to move freely for 5 min. The arena was covered with glass to avoid odorant dissipation in the room and the distance moved was recorded. The maze was cleaned with 50% ethanol and the filter papers were changed after each trial. Fourteen intact adult females were used for this test, seven in each odorant condition, and they were not used for any other tests.

2.4.3. Morris water maze task

The learning procedure was performed using a classic hidden platform water maze task (Morris, 1981). Animals underwent four massed training trials in which they had to find a submerged platform (diameter: 5 cm; depth: 1 cm) in a fixed place and thus escape from a circular tank (diameter: 60 cm; height: 15 cm) filled with grayish water to a depth of 10 cm. The water temperature was maintained at 25 °C. Four colored Plexiglas geometric forms (black, red, gray and brown squares or circles) were fixed on the top of the inner side of the maze to give the mice additional salient spatial reference markers. The starting point for the mice was always in the centre of the tank, but the initial orientation of the