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Medial prefrontal cortex is a crucial node of a rapid learning system that retrieves recent and remote memories

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ABSTRACT

The neocortex is thought to be a distributed learning system that gradually integrates semantic information into the initial mnemonic representation rapidly formed by the hippocampus after acquisition. Nevertheless, an emerging view suggests that some cortical regions, in particular the medial prefrontal cortex (mPFC), may also have a role during the initial steps of memory consolidation as well as in the recall of recent memories. Here, we show that mPFC plays a critical role during the first few hours of inhibitory avoidance memory consolidation and is necessary for the normal retrieval of both recent and remote memories, supporting the idea that involvement of neocortical areas in memory processing is not restricted to the late post-training consolidation phase.

1. Introduction

In order to last further than few minutes or hours, long-term memory (LTM) must undergo a protein synthesis-dependent process known as cellular consolidation (Lechner, Squire, & Byrne, 1999; McGaugh, 2000; Müller & Pilzecker, 1900). For permanent storage, LTM seems to undergo an additional process denominated systems consolidation. It supposes the gradual reorganization of the mnemonic trace in the cortex, requiring a dynamic dialogue between temporal lobe structures and neocortical regions (Frankland & Bontempi, 2005; Morris, 2006; Squire & Zola, 1996). Supporting this idea, it has been shown that, while the hippocampus is involved in consolidating and recalling recent episodic-like memories, some cortical regions, including prelimbic, orbitofrontal, and anterior cingulate areas, are implicated in remote memory processing (Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004; Lesburguères et al., 2011; Maviel, Durkin, Menzaghi, & Bontempi, 2004; Shan, Chan, & Storm, 2008).

The medial prefrontal cortex (mPFC) plays a central role in controlling executive functions such as working memory, decision-making, attentional selection and behavioral inhibition (Brown & Bowman, 2002; Dalley, Cardinal, & Robbins, 2004; Granon, Vidal, Thinus-Blanc, Changeux, & Poucet, 1994; Kesner & Churchwell, 2011; Rich & Shapiro, 2007). It has been proposed that this cortex supports cognitive control (Miller & Cohen, 2001) and several authors postulate that mPFC is essential for recalling remote memories that initially were hippocampus-dependent (Frankland et al., 2004; Maviel et al., 2004; Takehara, Kawahara, & Kirino, 2003; Teixeira, Pomedli, Maei, Kee, & Frankland, 2006). Recent findings suggest that, besides this, mPFC is also necessary for cellular consolidation and retrieval of newly acquired spatial memory (Leon, Bruno, Allard, Nader, & Cuello, 2010), acquisition and consolidation of overlapping associations (DeVito, Lykken, Kanter, & Eichenbaum, 2010), as well as for integration of information into pre-existing schemas (Tse et al., 2011).

In the present study we sought to determine whether mPFC is also involved in inhibitory avoidance (IA) memory processing, studying the participation of mPFC in IA memory formation and retrieval. The IA paradigm is suitable for studying time-dependent memory consolidation because it is a one-trial and rapidly learned task that leaves a consistent long-term memory. We evaluated the effect of muscimol-induced reversible inactivation of mPFC and the effect of two different protein synthesis inhibitors, emetine and

Abbreviations: LTM, long-term memory; IA, inhibitory avoidance; mPFC, medial prefrontal cortex.

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anisomycin, on memory formation. We also determined the effect of temporal inactivation of the mPFC 15 min before a test session carried out 2, 14, 28 or 42 days after training. To determine whether the temporal profile of the amnesia induced by mPFC inactivation is similar to that obtained with hippocampal inactivation, we studied the effect on memory retrieval of infusing muscimol into the dorsal hippocampus 15 min before a test session carried out 2, 14, 28 or 42 days after training.

2. Materials and methods

2.1. Subjects

Experiments were conducted in male Wistar rats (UBA, Argentina and FEPPS, Brazil) weighing 220–250 g. Animals were housed five to a cage and kept at a constant temperature of 23 °C, with water and food *ad libitum*, under a 12-h light/dark cycle (lights on at 7:00 a.m.). Experimental procedures followed the guidelines of the USA National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees of the University of Buenos Aires (CICUAL) and Pontifical Catholic University or Rio Grande do Sul (PUCRS).

2.2. Surgery

Rats were bilaterally implanted under deep ketamine/xylacine (100 and 5 mg/kg, respectively) anesthesia with 22-g guide cannulas aimed to the mPFC (AP + 3.20 mm/LL ± 0.75 mm/DV −3.20 mm) or the CA1 region of the dorsal hippocampus (AP – 3.9/LL ± 3.0/DV 3.0 (Paxinos & Watson, 1997). Cannulas were fixed to the skull with dental acrylic. At the end of surgery, animals were injected with a single dose of meloxicam (0.2 mg/kg) as analgesic. Behavioral procedures commenced 5–7 days after surgery.

2.3. Inhibitory avoidance training and testing

After recovery from surgery, animals were handled once a day for 2 days and then trained in IA as described previously (Bekinschtein et al., 2007). Briefly the apparatus was a 50 × 25 × 25 cm acrylic box whose floor was a grid made of 1 mm-caliber bronze bars. The left end of the grid was covered by a 7 cm-wide, 5.0-cm high platform. For training, animals were gently placed on the platform and, as they stepped down onto the grid, received a single 3-s, 0.7 mA scrambled footshock (strong training) or a 3-s, 0.3 mA scrambled foot-shock (weak training). Rats were tested for retention either at 1.5 h or 2, 7, 14, 28 or 42 days after training, depending on the experiment. All animals were tested only once. In the test sessions the footshock was omitted. For each experiment, the number of animals per group is detailed in Section 3.

For the immunohistochemistry experiments, three experimental groups were analyzed: (1) IA-trained group, rats were trained as described above with the strong protocol (IA-TR, $n = 5$). (2) Shocked group, rats were put directly on the grid of the training box and received a 0.7 mA scrambled foot-shock (Sh, $n = 4$). (3) Context group, animals were placed on the platform and they stepped down onto the grid, but did not received the scrambled footshock (Context, $n = 4$). (4) Naïve group, rats were left in their home cages without receiving any specific behavioral stimulation (Naïve, $n = 5$). Animals were anaesthetized and perfused 90 min after the end of the behavioral manipulations.

2.4. Drug infusions

For memory consolidation experiments, rats received at different time points (15 min pre-training or 0, 6 or 24 h post-training) bilateral infusions of saline or emetine (50 μg/side, Sigma Aldrich) (Lima et al., 2009), anisomycin (80 μg/side, Sigma Aldrich) (Igaz, Vianna, Medina, & Izquierdo, 2002), or muscimol (0.1 μg/side, Sigma Aldrich) (Majchrzak & Di Scala, 2000). For memory retrieval experiments, rats received bilateral infusions of saline or muscimol (0.1 μg/side, Sigma Aldrich) 15 min previous the test session. Infusions were delivered through an injector cannula extending 1 mm beyond the tip of the guide cannula. The volume infused was 1 μl/side and the infusion rate was 1 μl/min. Injectors were left in place for an additional minute following infusion before they were removed.

2.5. Immunohistochemistry

Animals were deeply anaesthetized ketamine/xylacine (100 and 20 mg/kg, respectively) 90 min after the training session, and perfused intracardially with 4% paraformaldehyde in 0.1 M PBS. Brains were removed, fixed in 4% paraformaldehyde for 2 days and transferred to 30% sucrose solution for overnight storage at 4 °C. Using a cryostat (Leica CM1950), brains were cut in 50 μm coronal sections and stored at −20 °C in a cryoprotectant solution (glycerol 50% in 0.1 M PBS). Peroxidase-immunohistochemical staining was performed on free-floating consecutive sections. Sections were washed twice in phosphate-buffered saline (PBS 0.1 M, pH 7.4) and treated with 0.6% H_2O_2 in PBS for 30 min, washed four times in PBS, and followed by incubation in blocking solution (2% normal goat serum, 0.1% BSA in PBS with 0.4% Triton X-100) for 1 h. Then, the sections were incubated with anti-c-Fos polyclonal rabbit antibody (1:3500; Santa Cruz Biotechnology, # sc-52) in the blocking solution for 48 h at 4 °C. Sections were then washed three times in PBS/Triton, incubated for 2 h with biotinylated goat anti-rabbit IgG (1:1000; Bio-Rad), washed again and incubated for 1 h at room temperature in streptavidin–biotin-peroxidase complex (Vector Labs). The reaction product was visualized using the nickel-DAB technique (Vector Labs).

Positive nuclei quantitative analysis was performed as described by Sacco and Sacchetti (2010). Prelimbic cortex was anatomically defined according to the atlas of Paxinos & Watson (Paxinos & Watson, 1997). Images were obtained using an Axiophot microscope (Zeiss; X10 objective) equipped with a digital camera interfaced with QCapture imaging software. c-Fos-positive nuclei were analyzed bilaterally using serial sections of mPFC (AP from = +3.72 mm to +2.50). c-Fos-positive nuclei were counted by an experimenter blind to experimental conditions using Image J software (http://rsb.info.nih.gov/ij/). For graphic representation of the data, the mean count of each animal was normalized to the mean value of the naive group.

A note of caution is needed. Although the method used to quantify c-Fos positive cells has been utilized by many studies, it has limitations. Two-dimensional counting could be subject to artifacts, such us overprojection or truncation (Peterson, 1999), among others.

2.6. Histology

Histological examination of cannula placement was performed. Briefly, 24 h after the end of the behavioral procedures, 1 μl of 4% methylene blue in saline was infused as indicated above. Animals were killed by decapitation 15 min later, and histological localization of the infusion sites was established. Infusions spread with a radius of about 1 mm^3 (Fig 1). Only data from animals with cannulas located in the intended site were included in the final analysis. When infused outside the intended target brain areas drugs did not affect retention. For instance, muscimol did not impair retention performance when infused in the ventral part of the infralimbic