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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

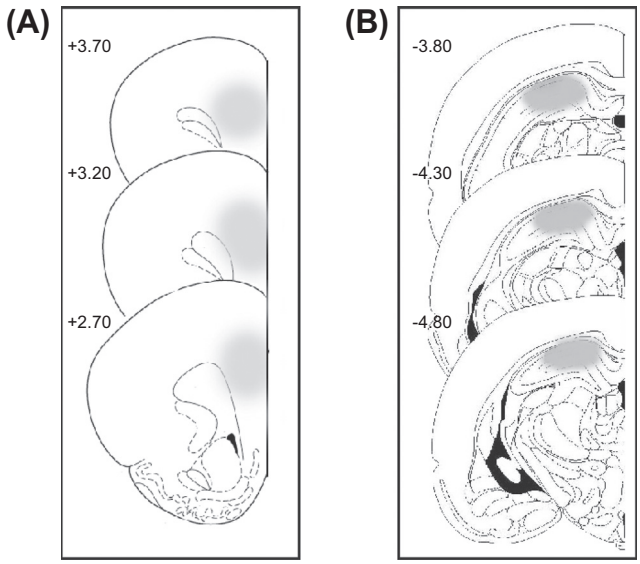

Fig. 1. Schematic representations of drug infusion areas. (A) Schematic representations of rat brain sections at three rostrocaudal planes (+3.70, +3.20 and +2.70 from bregma) taken from the atlas of Paxinos and Watson (1997). In stippling, the extension of the area reached by the infusions in mPFC. (B) Schematic representations of rat brain sections at three rostrocaudal planes (−3.80, −4.30 and −4.80 from bregma) taken from the atlas of Paxinos and Watson (1997). In stippling, the extension of the area reached by the infusions in the dorsal hippocampus.

cortex ($n = 3$) or in brain regions 1–2 mm lateral to the mPFC ($n = 2$).

2.7. Data analysis

In all behavioral experiments statistical analysis was performed by unpaired Student's t test or, when required, one-way ANOVA followed by Newman–Keuls multiple comparison test, comparing mean step-down latencies of the drug-treated groups and vehicle-treated groups at each time point studied. Immunohistochemistry data were analyzed by Newman–Keuls Multiple Comparison Test after one-way ANOVA, before normalization of the data.

3. Results

IA training produces a fear-motivated, hippocampus-dependent memory. This paradigm permits the uncontaminated analysis of the different stages of memory processing initiated by a single training experience without the interference from retrieval of the learned behavior that occurs in multi-trial tasks (Bekinschtein et al., 2007; Izquierdo & Medina, 1997; Taubenfeld, Milekic, Monti, & Alberini, 2001). To determine if the mPFC is involved in IA memory processing, we studied the expression of the immediate early gene *c-fos* 90 min after training. Immediate early genes are well-known markers of short-term neuronal activity resulting from experience (Feldman, Shapiro, & Nalbantoglu, 2010; Herrera & Robertson, 1996). mPFC showed an increase in c-Fos expression soon after IA training (Fig 2, $^{**}p < 0.01$, vs. Naïve; $^{*}p < 0.05$, vs. Context or Sh, Newman–Keuls Multiple Comparison Test after ANOVA). No changes were observed between Sh, Context and Naïve groups ($p > 0.05$). Previous studies demonstrated the requirement of protein synthesis for the consolidation of IA memory in the amygdala and hippocampus (Alberini, 2008; Bekinschtein et al., 2007; Igaz et al., 2002). Therefore, we wondered whether protein synthesis in mPFC was also needed for IA memory formation. We found that intra-mPFC infusion of the protein synthesis inhibitor emetine 15 min before training (TR) impaired LTM retention measured 2 days later (Fig 3A, $^{**}p < 0.01$, $n = 7–10$ per group). No effect was seen when emetine was given 6 or 24 h after training, indicating that the amnesia induced by this inhibitor is not attributable to retrieval failure or to nonspecific behavioral effects (Fig 3A, $p > 0.05$, $n = 8–11$ per group). LTM impairment was also observed in a different group of IA trained rats given intra-mPFC emetine 15 min before training and tested for retention 7 days later (Fig 3B, $^{*}p < 0.05$, $n = 10$ per group), demonstrating that the amnesic effect of intra-mPFC emetine is long-lasting. When injected in mPFC

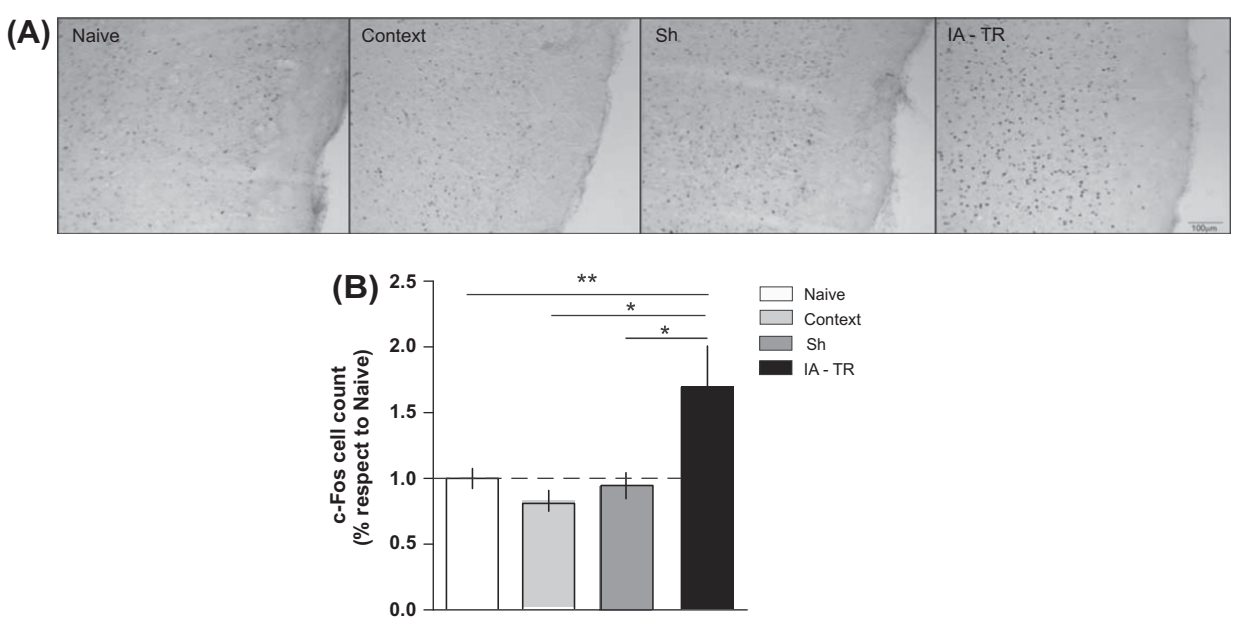

Fig. 2. c-Fos expression is induced in mPFC 90 min after IA training. (A) Representative photomicrographs (10× magnification) of the mPFC region (+3.20 from bregma; from Paxinos & Watson, 1997) showing c-Fos immunoreactivity 90 min after the different behavioral manipulation. Scale bar, 100 μm. (B) c-Fos-positive cell counts in the mPFC. mPFC show an increase in c-Fos expression soon after IA training (IA-TR; $^{**}p < 0.01$ vs. Naïve; $^{*}p < 0.05$ vs. Context or Sh group; $n = 3–5$ per group). c-Fos counts are similar between Naïve, Context and Sh groups ($p > 0.05$; $n = 3–5$ per group).

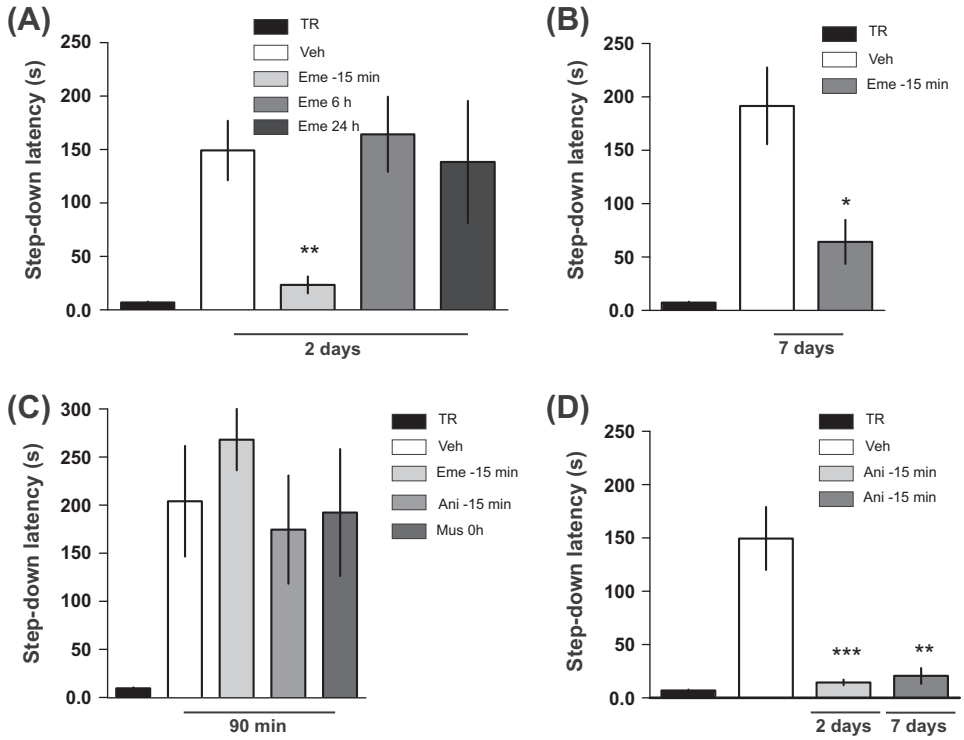

Fig. 3. Protein synthesis around IA training is required in mPFC for long-term memory consolidation. (A) Animals were trained in IA using a strong footshock and 15 min before or either 6 or 24 h after training received bilateral intra-mPFC infusions of vehicle (Veh) or emetine (Eme; 50 μg/μl). LTM retention was evaluated 2 days later. Data are expressed as mean ± SEM of training (TR, black bars) or test session step-down latency; $n = 7–10$ per group; $^{**}p < 0.01$ in Student's *t* test. (B) Animals received bilateral intra-mPFC infusions of Veh or Eme and 15 min later were trained in IA using a strong footshock. LTM retention was evaluated 7 days later. Data are expressed as mean ± SEM of training (TR, black bars) or test session step-down latency; $n = 10$ per group; $^{*}p < 0.05$ in Student's *t* test. (C) Animals received bilateral intra-mPFC infusions of vehicle (Veh), emetine (Eme; 50 μg/μl; 15 min pre-training), anisomycin (Ani; 80 μg/μl; 15 min pre-training) or muscimol (Mus; 0.1 μg/μl; immediately post-training) and were trained in IA using a strong footshock. STM retention was evaluated 90 min later. Data are expressed as mean ± SEM of training (TR, black bars) or test session step-down latency; $n = 6–7$ per group; $p > 0.05$ in Student's *t* test. (D) Animals received bilateral intra-mPFC infusions of Veh or Ani and 15 min later were trained in IA using a strong footshock. LTM retention was evaluated 2 or 7 days later. Data are expressed as mean ± SEM of training (TR, black bars) or test session step-down latency; $n = 8$ per group; $^{**}p < 0.01$, $^{***}p < 0.001$ in Student's *t* test. TR = latency at the training session, in this and following figures. Each experimental condition had its own control group. Vehicle groups did not differ between them, therefore only one vehicle group was illustrated in (A), (C) and (D).

15 min before training, emetine did not affect IA short-term memory (STM), as evaluated 90 min post-training (Fig 3C, $p > 0.05$, $n = 7$ per group). Thus, pre-training administration of emetine does not affect IA memory acquisition. Intra-mPFC infusion of anisomycin, a protein synthesis inhibitor with a mechanism of action different from that of emetine, 15 min before training also impaired LTM tested 2 or 7 days after training (Fig 3D, $^{**}p < 0.01$, $^{***}p < 0.001$, $n = 8$ per group), but not STM tested 90 min post-training (Fig 3C, $p > 0.05$, $n = 6–7$ per group).

To further confirm the role of mPFC as a rapid learning system, we trained rats in IA and immediately after training we inactivated this region by means of muscimol microinfusion. Intra-mPFC muscimol hindered LTM retention when tested 2 or 7 days after training (Fig 4A, $^{*}p < 0.05$, $^{**}p < 0.01$, $n = 10$ per group), suggesting that mPFC neural activity around the moment of training is indeed needed for IA LTM formation. No change in STM retention was found when muscimol was infused immediately after training (Fig 3C, $p > 0.05$, $n = 6–7$ per group).

It has been proposed that mPFC is required for processing salient information (Lauzon, Ahmad, & Laviolette, 2012; Lauzon, Bishop, & Laviolette, 2009). Therefore, we next asked whether saliency of the information to be encoded somehow determines the involvement of mPFC in LTM consolidation. We induced a low-arousing IA memory using a 0.3 mA footshock as unconditioned stimulus. Previously, we demonstrated that such stimulus generates a short-lasting LTM enduring no more than 2 days (Bekinschtein et al., 2007). Temporary inactivation of mPFC after weak IA training also impaired retention, suggesting that both highly salient and low salient sensory experiences are processed by mPFC during LTM formation (Fig 4B, $^{*}p < 0.05$, $n = 8–10$ per group).

It has been consistently found that mPFC and neighboring cortical regions are required for retrieving remote but not recent memories (Frankland & Bontempi, 2005). However, this assumption has been recently challenged (Einarsson & Nader, 2012; Tse et al., 2011). Temporary inactivation of mPFC 15 min before a retention test session carried out either 2, 14, 28 or 42 days after training, impaired memory expression. Animals that received intra-mPFC muscimol showed significantly lower step-down latencies than their respective control group regardless the time spent from the moment of training (Fig 5A, Mus 2-days, $^{***}p < 0.001$; Mus 14-days, $^{**}p < 0.01$; Mus 28-days, $^{**}p < 0.01$; Mus 42-days, $^{*}p < 0.05$; $n = 10–12$ per group, in Student's *t* test). These findings suggest that mPFC temporal inactivation caused flat graded retrograde amnesia and demonstrate that this structure is critical for recalling fear-motivated LTMs, irrespective of their age (Fig 5A, Mus vs. Veh 2-days, $^{***}p < 0.001$, $q = 9{,}324$; Mus vs. Veh 14-days, $^{***}p < 0.001$, $q = 6{,}293$; Mus vs. Veh 28-days, $^{***}p < 0.001$, $q = 6{,}439$; Mus vs. Veh 42-days, $^{*}p < 0.05$, $q = 4{,}293$; $n = 10–12$ per group, in Newman–Keuls Multiple Comparison Test after ANOVA). Experiments utilizing pre-test intra-dorsal CA1 microinfusions of muscimol suggest that inactivation of the dorsal hippocampus impairs recent memories. Animals that received intra-CA1 Mus infusions 15 min before a retention test session carried out 2 or 14 days post-training showed significantly lower step-down latencies than their respective control groups. No differences were found between Mus and the respective control

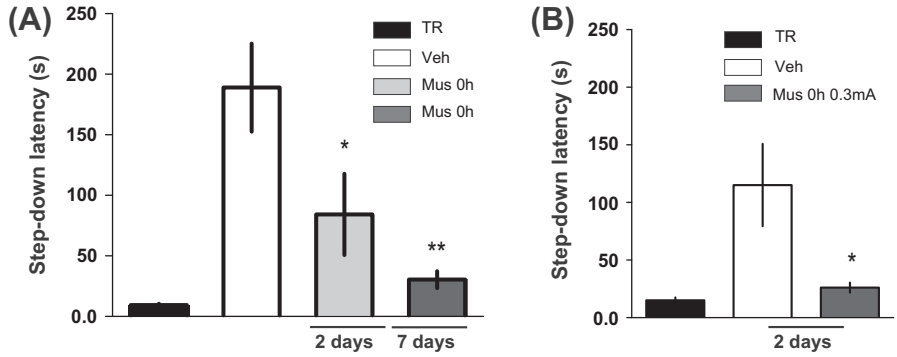

Fig. 4. Neural activity is required in mPFC around the moment of training for normal long-term memory consolidation. (A) Animals were trained in IA using a strong footshock and immediately after training received bilateral intra-mPFC infusions of vehicle (Veh) or muscimol (Mus; 0.1 μg/μl). LTM retention was evaluated 2 or 7 days later. Data are expressed as mean ± SEM of training (TR, black bars) or test session step-down latency; $n = 10$ per group; $^{*}p < 0.05$, $^{**}p < 0.01$ in Student's t test. Vehicle groups did not differ between them, so only one vehicle group was illustrated here. (B) Animals were trained in IA using a weak footshock and immediately thereafter received bilateral intra-mPFC infusions of Veh or Mus. LTM retention was evaluated 2 days later. Data are expressed as mean ± SEM of training (TR, black bars) or test session step-down latency; $n = 8$ per group; $^{*}p < 0.05$ in Student's t test.

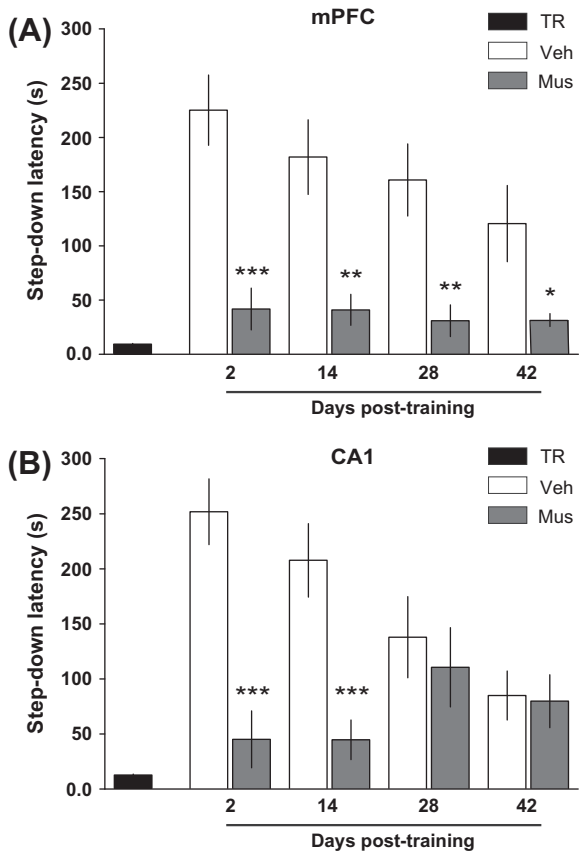

Fig. 5. Neural activity is required in mPFC for recalling both recent and remote IA memories. (A) Animals were trained in IA using a strong footshock. LTM retention was evaluated 2, 14, 28 or 42 days later. Animals received bilateral intra-mPFC infusions of vehicle (Veh) or muscimol (Mus; 0.1 μg/μl) 15 min before the test session. Data are expressed as mean ± SEM of training (TR, black bars) or test session step-down latency; $n = 10–12$ per group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, in Student's t test. (B) Animals were trained in IA using a strong footshock. LTM retention was evaluated 2, 14, 28 or 42 days later. Animals received bilateral intra-CA1 infusions of Veh or Mus 15 min before the test session. Data are expressed as mean ± SEM of training (TR, black bars) or test session step-down latency; $n = 10–12$ per group; $^{***}p < 0.001$, in Student's t test.

groups in test sessions carried out 28 or 42 days after training (Fig 5B, Mus 2-days, $^{***}p < 0.001$; Mus 14-days, $^{***}p < 0.001$; $n = 10–12$ per group, in Student's t test). When muscimol injected groups were analyzed, no differences were found between them (Fig 5B, $p > 0.05$ in all cases, $n = 10–12$ per group, in Newman–Keuls Multiple Comparison Test after ANOVA).

4. Discussion

Our results demonstrate that IA memory consolidation requires protein synthesis in the mPFC early after training and that retrieval of both recently acquired and well-consolidated IA LTM involves the functional participation of this cortical area. The amnesic effects of two protein synthesis inhibitors and a $GABA_A$ receptor agonist given into mPFC around the time of training are typical of brain regions playing key roles in memory formation, like the dorsal hippocampus or the amygdala (Alberini, 2009). In this context, and consistent with our findings, recent reports described an early involvement of mPFC in memory processing in an odor–reward task (Carballo-Márquez et al., 2007), in a cued fear conditioning using multiple US shocks (Choi et al., 2010; Gilmartin & Helmstetter, 2010) and in spatial memory (Leon et al., 2010). In addition, a recent study reported that anterior cingulate cortex is required for rapid memory formation and recall of recent and remote contextual fear memories (Einarsson & Nader, 2012). Most of these studies used different protein synthesis inhibitors to determine whether this brain area is involved in memory formation. Given that these compounds, in particular anisomycin, have several side effects, their utilization as tools for studying the role of protein synthesis in memory consolidation has been recently questioned (Gold, 2006). As it has long been discussed, most of the critiques can be overcome using two different protein synthesis inhibitors. In the present study we used emetine as a primary compound, which is devoid of most of the side effects observed with anisomycin (Alberini, 2008; Hernandez & Abel, 2008).mPFC has a massive connection with the ventral and dorsal hippocampus (Jay, Glowinski, & Thierry, 1989; Jay & Witter, 1991; Vertes, 2006) and amygdala (Vertes, 2004). Prelimbic cortex receives inputs from the ventral part of the hippocampus while both the prelimbic and the anterior cingulate cortex receive inputs mainly from the dorsal hippocampus via retrosplenial and related cortices (Cenquizca & Swanson, 2007; Vertes, 2006). There is no direct mPFC efferent connection to the hippocampus, but an indirect and strong pathway connects mPFC with dorsal CA1 through midline nuclei of the thalamus (Vertes, 2006).

An apparent dissimilarity in memory processing between hippocampus and mPFC is their differential role in retrieving remote memories (Fig 5). Pre-test inactivation of the dorsal hippocampus

impaired recent memories but it appears not to significantly affect remote memories. Our present findings using pre-test muscimol infusions are consistent with those previously reported showing that CNQX given in the dorsal hippocampus 15 min before a test session performed 24 h, but not 31 days after training, impairs IA memory expression. (Izquierdo et al., 1997). Nevertheless, given that muscimol-injected groups have no differences between them (Fig 5B), and that a prerequisite to compellingly endorse the hypothesis of systems consolidation is that memory retention in the muscimol group must be greater at the remote than at recent time points, our present findings failed to find evidence for supporting systems consolidation. Controversy still persists regarding whether or not the hippocampus has a temporary role in the storage and retrieval of memories (Frankland & Bontempi, 2005; Squire & Zola, 1996; Sutherland & Lehmann, 2011). In addition, one cannot rule out the possibility that dorsal and ventral parts of the hippocampus may exhibit different temporal gradients in IA memory retrieval. In contrast, we found that mPFC is required for recalling not only remote but also recently stored information (2-days old memory). Our data contrast with previous reports showing the involvement of the mPFC and neighboring regions only in remote memories (Frankland et al., 2004; Lesburguères et al., 2011; Maviel et al., 2004), but are consistent with findings showing that the anterior cingulate cortex is involved in recall of recent contextual fear and IA memories (Einarsson & Nader, 2012; Holloway & McIntyre, 2011).

The impairment of IA memory expression caused by mPFC inactivation could be due to the fact that prelimbic cortex can reduce fear expression, as shown in animals subjected to multiple shocks (Corcoran & Quirk, 2007; Stevenson, 2011). However, in our experimental conditions (one foot-shock) no signs of freezing or other fear-related measures, like rearing, jumping or defecation were observed during training or test session. Of course, it cannot be totally rule out the possibility that a mild undetected state of fear is actually present in control animals. Relevant to our findings are those works showing normal fear expression to unconditioned shocks during acquisition (Choi et al., 2010; Runyan, Moore, & Dash, 2004), which taken together with our findings of normal STM after emetine or anisomycin administration (Fig 3C), are consistent with the idea that mPFC is important for the formation of LTM about emotional experiences but not for processing fear expression itself. In agreement with our results, it has been reported that mPFC is important for storage of multi-trial trace fear conditioning memory (Runyan et al., 2004).

In conclusion, our results indicate that the mPFC behaves as a rapid learning node within a neural network, necessary to IA memory acquisition, consolidation and retrieval of both recent and remote memories.

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