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Differential Role of the Dorsal Hippocampus, Ventro-intermediate Hippocampus, and Medial Prefrontal Cortex in updating the Value of a Spatial Goal

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ABSTRACT: Encoding of a goal with a specific value while performing a place navigation task involves the medial prefrontal cortex (mPFC) and the dorsal hippocampus (dHPC), and depends on the coordination between mPFC and the ventro-intermediate hippocampus (vHPC).The present work investigates the contribution of mPFC, dHPC, and vHPC when the rat has to update the value of a goal. Rats were trained to navigate to an uncued goal in order to release a food pellet in a continuous place navigation task. When they had reached criterion performance level in the task, they were subjected to a single ''flash session'' in which they were exposed to an aversive strobe light during goal visits instead of receiving a food reward. Just before the flash session, the $GABA_A$ agonist muscimol was injected to temporarily inactivate mPFC, dHPC, or vHPC. The ability to recall the changed value of the goal was tested on the next day. We first demonstrate the aversive effect of the strobe light by showing that rats learn to avoid the goal much more rapidly in the flash session than during a simple extinction session in which goal visits are not rewarded. Furthermore, while dHPC inactivation had no effect on learning and recalling the new goal value, vHPC muscimol injections considerably delayed goal value updating during the flash session, which resulted in a slight deficit during recall. In contrast, mPFC muscimol injections induced faster goal value updating but the rats were markedly impaired on recalling the new goal value on the next day. These results suggest that, contrary to mPFC and dHPC, vHPC is required for updating the value of a goal. In contrast, mPFC is necessary for long-term retention of this updating. © **2013 Wiley Periodicals, Inc.**

KEY WORDS: muscimol; continuous place navigation task; temporary inactivation; rat

INTRODUCTION

The interplay between the hippocampus and frontal cortex is crucial for a variety of cognitive processes central to spatial navigation, such as consolidation of memories (Bontempi et al., 1999; Frankland and Bontempi, 2005), decision making (Rudebeck et al., 2008; Kennerley and Walton, 2011; Hillman and Bilkey, 2012), and spatial planning (Granon and Poucet, 1995; Poucet et al., 2004). Additional studies have shown that neurons in both hippocampus and frontal cortex encode the spatial goal of a rat engaged in spatial navigation (Hok et al., 2005; 2007). Such correlates were evidenced as rats had to perform a continuous place navigation task in which they had to enter an unmarked circular goal in a cylindrical environment to release a food pellet from an overhead dispenser (Lenck-Santini et al., 2002). In this task, place cells in the dorsal hippocampus (dHPC), in addition to firing in their main place field, also discharged selectively (though less robustly) while the rat was in the goal zone (Hok et al., 2007). In contrast, medial prefrontal cortical (mPFC) neurons had large place fields centered on the goal zone (Hok et al., 2005). Furthermore, mPFC goal activity was strongly altered by bilateral lesion of the ventro-intermediate region of the hippocampus (vHPC) (Burton et al., 2009).

This last finding strongly suggests that the mPFC and vHPC act in concert for goal encoding, a possibility which is supported by their anatomical and functional connections. Indeed, vHPC is the origin of a direct pathway to the infralimbic and prelimbic areas of mPFC (Swanson, 1981; Jay et al., 1989; Hoover and Vertes, 2007). High-frequency stimulation of this pathway results in the induction of NMDA-dependent receptor long-term potentiation in the mPFC (Jay et al., 1995). There is no direct mPFC output to the hippocampus, but indirect and moderate projections via the parahippocampal and entorhinal cortices (Beckstead, 1979; Takagishi and Chiba, 1991; Vertes, 2004), and possibly a more important connection to both dorsal and ventral hippocampus via the thalamic nucleus reuniens (Dolleman-Van der Weel et al., 1997; Vertes, 2004, 2006; Vertes et al., 2007).

In spite of the above-mentioned evidence for a functional dialogue between the mPFC and dHPC in goal encoding, the exact contribution of each structure to flexible goal processing is unclear. It is known that mPFC is well situated to integrate affective information for the production of flexible and adaptive behavior (Heidbreder and Groenewegen, 2003; Morgane et al., 2005) while dHPC underlies flexible navigation (Morris et al., 1982). In addition, vHPC is usually assumed to be involved in emotional or sensorimotor

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Abbreviation used: dHPC, dorsal hippocampus; MAPK, mitogen-activated protein kinase; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; PBS, phosphate buffered saline; vHPC, ventro-intermediate hippocampus.

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processes (Bast and Feldon, 2003; Bannerman et al., 2004), but how these different structures might cooperate in flexible goal processing is unknown.

To probe their specific contribution to flexible goal processing, we asked whether mPFC, dHPC, and vHPC are necessary for rapidly and flexibly updating the value of a goal and adapting behavior accordingly. More specifically, we trained rats in the continuous place navigation task in which they received a food reward on each visit to the goal. Once they were efficient in reaching the goal, we reversibly inactivated mPFC, dHPC, or vHPC with a bilateral intra-cerebral infusion of the $GABA_A$ agonist muscimol just before a test session in which the value of the goal was changed. In this test session, reaching the goal resulted in the rat being exposed to a short strobe aversive light instead of receiving a food reward. The results showed that vHPC is required for updating the value of the goal but not mPFC or dHPC. In contrast, mPFC is necessary for long-term retention of this updating.

MATERIALS AND METHODS

Subjects

Long–Evans black hooded male rats (R. Janvier, St.-Berthevin, France, $n = 45$) weighing 300–350 g were housed one per cage at 20 ± 2°C, under controlled lighting conditions (light on from 07:00 to 19:00). They had free access to water and were food deprived to 85% of ad libitum body weight. All procedures complied with both the regulations specified by the European directive (*2010/63/EC)* and French institutional guidelines (authorization n°13–76 to BP).

Surgery

Rats were deeply anesthetized by an intra-muscular (i.m.) injection of xylazine (15 mg/kg; Rompun, Bayer, France) and ketamine (100 mg/kg; Imalgène, Merial, France) and placed in a Kopf stereotaxic apparatus (Kopf instruments, Tujunga, CA). After a midline incision of the scalp was made, the skin and muscles were carefully retracted to expose the skull. Holes were drilled above the target regions. Bilateral implantation of guide cannulas was aimed at the following coordinates relative to bregma: mPFC, AP +3.5 mm, *L* ±0.5 mm, and DV −3 mm (below the dura); dHPC, AP −3 mm, *L* ±2.4 mm, and DV −3 mm; vHPC, AP −5.3 mm, *L* ±5 mm, and DV −5 mm (Paxinos and Watson, 2005; see Fig. 1). The guide cannulas were anchored to the skull with four small stainless screws and secured with dental cement. Stainless steel stylets, which extended 0.5 mm beyond the tips of the guide cannulas, were placed inside them to prevent occlusion. After surgery, the rats received an injection of antibiotic (Terramycine, 60 mg/kg, i.m.; Pfizer, Paris, France) and analgesic (Tolfédine, 0.06 mg/kg, subcutaneous; Vetoquinol, Lure, France) as post-operative treatment. They were placed back in their home cage for at least 1 week of recovery before the first training session.

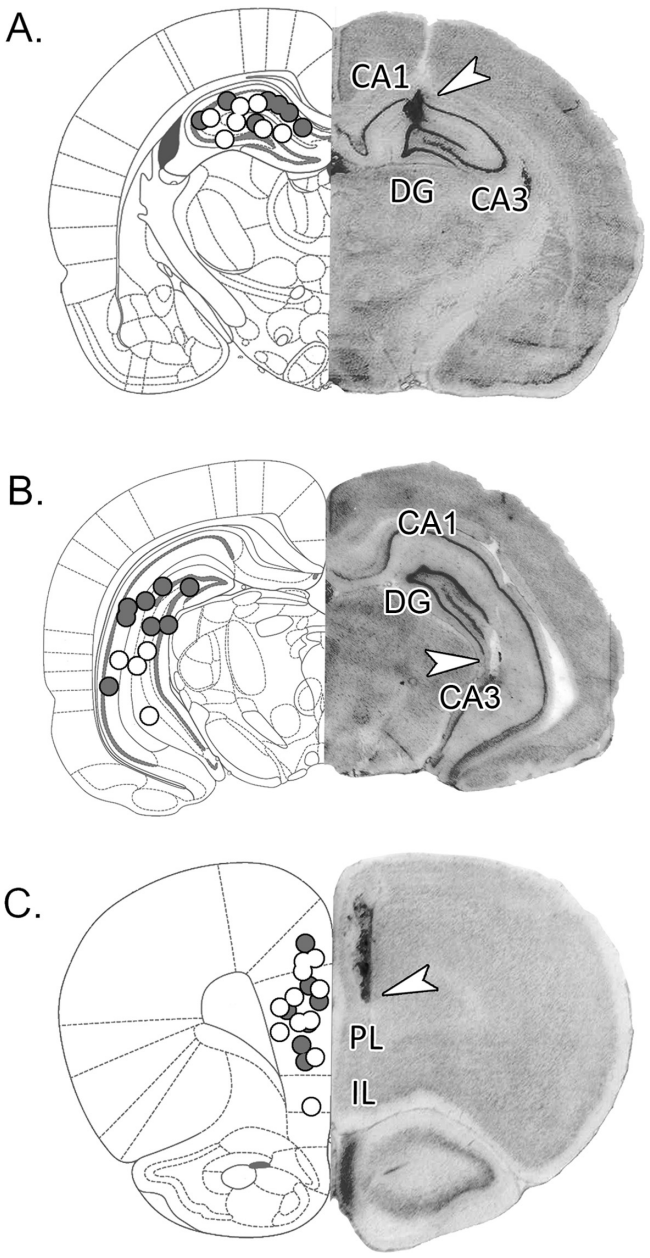

FIGURE 1. Schematic illustration of coronal sections of the rat brain showing the approximate location of bilateral medial prefrontal cortex (A), dorsal hippocampus (B), and (C) ventro-intermediate hippocampus infusion. Muscimol: gray dots. PBS: empty dots.

Apparatus

The apparatus was a white circular arena (76 cm diameter) with opaque walls 50 cm high and a plastic floor that was wiped with alcohol before each session and between animals to prevent accumulation of uncontrolled odors. The arena was at the center of an evenly lit area. A black cue-card attached to the wall of the cylinder covered 90° of internal arc. Several

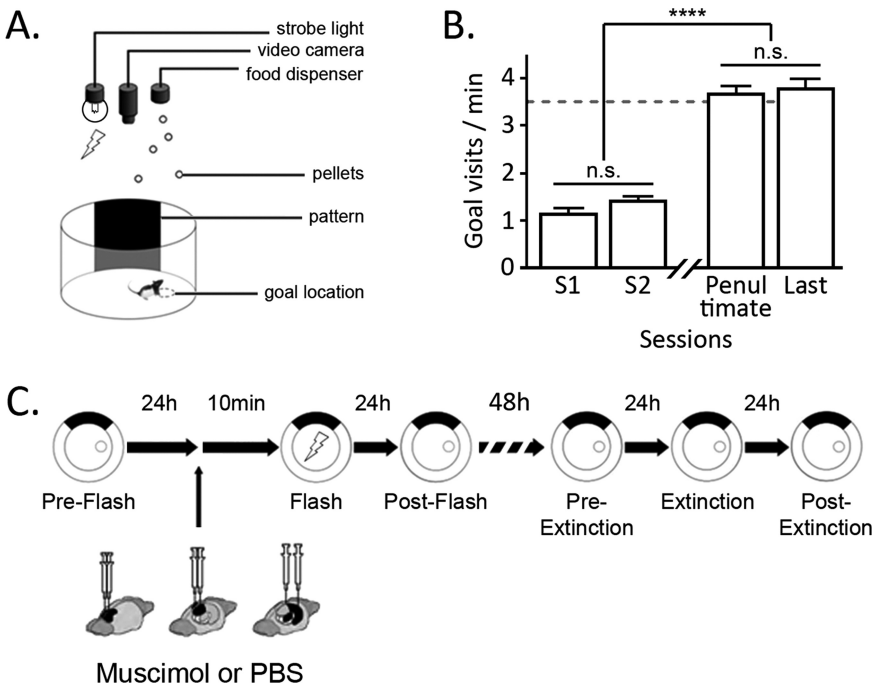

FIGURE 2. (A) Sketch of the place preference task. The rat must visit an unmarked goal to release a food pellet from an overhead feeder. To find and eat a food pellet, the rat has to forage around the cylinder. (B) Evolution of the learning performances. Rats significantly increased the number of goal visits until the learning criterion (dashed line, 3.5 ± 1 goal visits per minute; Bonferroni multiple comparison *t*-test: $t > 15.5$, $P < 0.0001$). (C) Twenty-four hour after the last training session (pre-flash session), rats were assigned to the PBS or muscimol group. Injection was made 10 min before the flash session (goal associated with stroboscopic flash). Recall was done the next day during the post-flash session (goal associated with pellet release). Following 2 days of re-acquisition, rats were tested during an extinction session (no pellet, no flash associated with the goal) and, the next day, tested for recall on a post-extinction session.

distal cues were attached to the walls of the room. When manually activated by the experimenter via a remote control, a food dispenser located 2 m above the arena dropped 20 mg food pellets which, after hitting the ground, could end up anywhere on the apparatus floor. Next to the food dispenser, a strobe light and a video camera were fixed to the ceiling above the cylinder. The strobe light was switched off for all testing except for a single "flash" session (see below). In contrast, the video camera was switched on during all testing to record the behavior of the animals. The video recording system and equipment for controlling the experiment were in the same room.

Behavioral Procedure

Rats were trained in a continuous place navigation task which is a modified version of the place preference task originally designed by Rossier et al. (2000) and Lenck-Santini et al. (2002) in which they were required to reach an unmarked circumscribed zone, the goal, to get a food reward (Fig. 2A). Three days before the first training session, animals were food deprived and daily handled before training started. Rats were daily exposed to a 10 min training session. During the first session, the goal was cued with a 20 cm diameter black metal disc put directly onto the arena floor. Rats had to visit the goal to trigger the overhead food dispenser. Because the pellet could go anywhere in the cylinder, the rat had to forage over the entire area to find and eat it before initiating another visit to the goal. In the second session, the goal was again directly signaled by a black metal disc, which, however, was reduced to 10 cm in diameter. Starting from the third session, the black metal disk cue was removed so that the rat were required to rely on the spatial cues provided by the room and the cue-card attached to the wall of the arena to locate the goal. Rats were then trained until they reached the learning criterion of 3.5 ± 1 visits to the goal per minute during two consecutive sessions (Fig. 2B), which took an average of 24 ± 1 sessions. The last training session was referred to as the pre-flash session. At this point, rats were randomly assigned to a phosphate buffered saline (PBS)-injected (control) or a muscimol-injected (experimental) group. Testing continued in two distinct stages (Fig. 2C).

Stage 1

On the day following the last training session, animals were intra-cerebrally injected with either PBS or muscimol 10 min before they were tested to the "flash session". During this session, each visit to the goal resulted in the activation of the strobe light (1.5 Hz, 45 W) instead of the food dispenser. The strobe light was activated as long as the rats stayed in the goal. Because the strobe light was supposed to be unpleasant and because no food pellet was delivered on each goal visit, it was

expected that rats would rapidly learn to avoid the initially appetitive goal. On the day following the flash session, testing was resumed in the usual way: rats were not injected, the strobe light was constantly switched off and visits to the goal were rewarded with the delivery of a food pellet. We assumed that the latency of the very first visit to the goal during this session would reflect the memory of the aversive nature of the goal. If the rat remembered that visits to the goal during the flash session had unpleasant consequences, they should delay their first visit to the goal during the session that followed. This session was named the "post-flash session" and measured the rats' ability to recall the changed value of the goal.

Stage 2

Following the post-flash session, rats were further trained in the standard procedure (goal visits were rewarded with food pellets) for two more days with one session per day. Their performance during the second retraining session was referred to as the pre-extinction score. On the day following the pre-extinction session, they were subjected to the "extinction session" in which visits to the goal were unreinforced and did not result in either a food reward being delivered or exposure to the strobe light. Testing was resumed in the usual way on the day following the extinction session with visits to the goal being rewarded with the delivery of a food pellet. This "post-extinction session" reflected the memory of the extinction session, as measured by the latency of the very first visit to the goal (Fig. 2C).

All sessions were recorded and digitized with a Viewpoint videotrack system (Champagne au Mont D'Or, France) for off-line analysis of goal visits and latency of first goal visits during each session. For statistical analyses, performances of the recall session (latency) were compared to the last training session in order to compare two sessions during which animals did not receive intra-cerebral injections.

Intra-Cerebral Drug Infusion

Rats were handled and habituated to the infusion procedure by mock treatment 2 days before the flash session. On infusion days, rats were gently restrained while the stylets were removed and replaced with sterile infusion needles (30G) that extended 1 mm below guide cannulas, and replaced in their home cage during injection. Ten minutes before the flash session, rats were given bilateral infusions of muscimol (Sigma-Aldrich, UK) at a concentration of 1.0 μg/μl in PBS or only PBS for PBS rats. Animals received 0.25 μl in both sides of the target structure at a rate of 0.20 μl/min. Needles were connected with PE-20 tubing to a 10 μl Hamilton syringe connected to an infusion pump (Harvard Apparatus). Needles were left in place for 1 min following the infusion to allow diffusion of the PBS or muscimol. Stylets were replaced after infusion. Muscimol is an agonist of $GABA_A$ receptors, whose activation causes profound inhibition of local neurons. This effect is observable within 5 minutes following injection and lasts several hours. Given the injected volume and duration of the test session, the radius of inactivated brain tissue is estimated to be 1.5–2.0 mm, that is, sufficiently extended to consider that the target structure is silenced (Edeline et al., 2002).

Histology

Rats were injected with a lethal dose of sodium pentobarbital (i.p.) and decapitated. The brain was removed and immediately frozen by dry ice. Brains were sectioned (40 μm sections) and stained with cresyl violet. The sections were examined under a light microscope to determine the location of cannula placement. Most of the mPFC cannula tips were in the prelimbic cortex and few were located in the anterior cingulate cortex (one muscimol-injected rat and two PBS-injected rats). Following histological checking of correct cannula placements, the final size of each group was mPFC: PBS $n = 10$, muscimol $n = 7$; dHPC: PBS $n = 8$, muscimol $n = 7$; vHPC: PBS $n = 5$, muscimol $n = 8$.

Statistical Analysis

Behavioral performance (i.e., overall activity, number of goal visits for all sessions and latency of first visit during pre- and post-flash, and pre- and post-extinction sessions) was analyzed using two-way repeated measure ANOVAs and Bonferonni post-hoc test for flash versus extinction sessions. One-way repeated measure ANOVAs were done for analysis of training sessions, and for independent analysis of PBS and muscimol groups during flash session. All other comparisons were done with paired or unpaired *t*-test when appropriate. A value of $P < 0.05$ was considered to indicate a statistically significant difference ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$). Statistical analyses were performed using GraphPad Prism 5.02 (GraphPad Software, San Diego, CA) and SPSS/PC statistics 11 software package (SPSS).

RESULTS

Behavior in Normal Animals

To validate the experimental design, the evolution of behavioral performances during training sessions which preceded exposure to the flash was assessed by measuring the number of goal visits per min. As it can be seen in Figure 2B, rats learned to reliably visit the goal to release a food pellet [$F(3,44) = 189.8$, $P < 0.0001$].

During the flash session, the rats were exposed to a strobe light supposed to be unpleasant on each goal visit. They were, therefore, expected to learn to avoid the goal more rapidly than during the extinction session in which goal visits were simply not rewarded with a food pellet. To compare the dynamics of behavior during flash and extinction sessions, we conducted an analysis of within-session goal visits using 2 min bins, which revealed that goal visits, though decreasing in both conditions [$F(4,176) = 11.6$, $P < 0.0001$], did so faster in the flash session than in the extinction session [$F(1,44) = 5.67$, $P < 0.05$; Fig. 3B]. Furthermore, compared to the pre-flash session, animals delayed their first visit to the goal during the post-flash

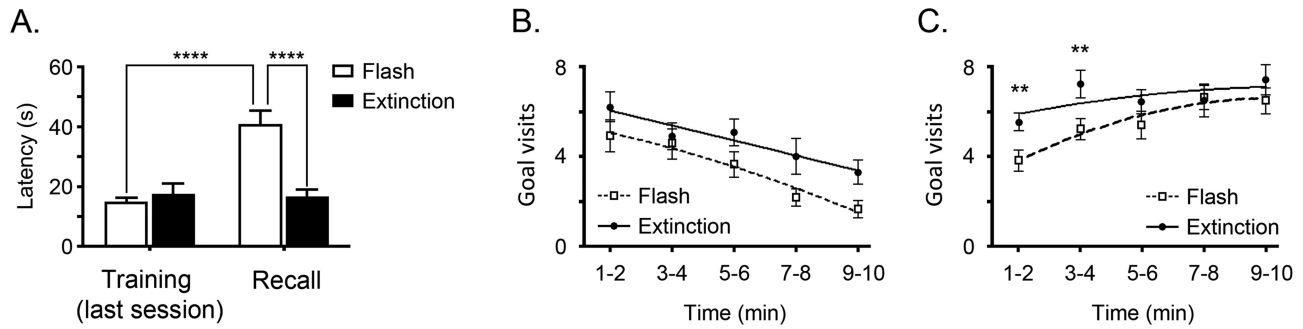

FIGURE 3. Comparison between flash and extinction conditioning in normal control animals. (A) Latency of the first goal entrance. Twenty-four hour after the flash session, the rats delayed the entrance in the goal but not following the extinction session. (B) Performance (i.e., goal visits) during the flash (open squares) versus the extinction (black dots) sessions (bin = 2 min). The flash session induced a faster decline of the goal visits through the 10 min of the session. (C) Performance (i.e., goal visits) during post-flash versus post-extinction sessions. Flash group had lower goal visits during the first two bins (bining = 2 min).

session (from ~15 s during the pre-flash to 40 s during post-flash; paired *t*-test, $P < 0.0001$). In contrast, there was no difference in latency of first visit to the goal for pre- and post-extinction sessions (~17 s, paired *t*-test: $t = 0.281$, ns). In addition, the first goal visits occurred significantly later during the post-flash than during the post-extinction session (unpaired *t*-test: $t = 4.385$, $P < 0.0001$; Fig. 3A) and the number of goal visits during the first 4 minutes was much reduced in the post-flash session compared to the post extinction session (unpaired *t*-test: $t \geq 2.705$, $P < 0.01$; Fig. 3C). In short, the strobe light had an aversive effect as rats rapidly learned to avoid the goal during the flash session. In addition, rats remembered this during the post-flash session as they delayed their first visit to the goal and avoided it for a few minutes before resuming pre-trained goal visit behavior.

Behavioral Effects of dHPC, vHPC, and mPFC Inactivations

Overall activity

Comparison of overall activity, measured by distance covered during each 10-min session, revealed a strong decrease during the flash session in control (PBS) rats compared to the pre-flash and post-flash sessions (Bonferonni post-test, $P < 0.05$; Table 1). This decrease was expected as a result of the procedure employed during the flash session. A similar decrease was found following inactivation of dHPC and mPFC, but not vHPC. In addition, no significant difference was found between the muscimol groups and their matched control groups during either flash or post-flash sessions (Table 1).

Updating of goal value during the flash session

The time-course of goal visits during the 10 min flash session was measured using 2 min bins. A three-way repeated-measure analysis of variance was run on this data using the following design: two between factors were "group" (dHPC, vHPC, mPFC), and "treatment" (muscimol, PBS) and the repeated measure was "time" (i.e., bins, $n = 5$). The analysis yielded a significant effect of "time", $F(4,195) = 10.142$, $P < 0.001$, and an interaction "group × treatment", $F(2,195) = 9.207$, $P < 0.001$, meaning that the the time course of goal visits was differentially affected by inactivation of the different brain areas. Following this overall analysis, a comparison of goal visits during the flash session in respective PBS and muscimol groups was done for each structure (Fig. 4A–C).

TABLE 1.

Locomotor Activity. Mean distance traveled by the dHPC, vHPC, mPFC, PBS, and muscimol groups during the 10 min of the pre-flash, the flash, and the post-flash sessions

	dHPC		vHPC		mPFC	
	PBS	Muscimol	PBS	Muscimol	PBS	Muscimol
Pre-flash	109.6 ± 6.7	110.3 ± 2.7	113.5 ± 6.3	122.6 ± 11.6	124 ± 2.3	117.4 ± 8.6
Flash	80.3 ± 4.6[a,b]	82.6 ± 4.2[c,d]	78.6 ± 7.7[a,b]	102.3 ± 9.4	90.1 ± 2.2[a,b]	74.2 ± 8.5[c,d]
Post-flash	102.4 ± 8.8	112.2 ± 4	112.1 ± 3	123.7 ± 12.6	112 ± 3.7	104.3 ± 8.9

Data are express in meters (averages ± S.E.M).
Statistically significant differences (Bonferroni multiple comparison *t*-test) for the dHPC, vHPC, and mPFC groups:
[a]PBS Flash vs Pre-Flash;
[b]PBS Flash vs Post-Flash;
[c]Muscimol Flash vs Pre-Flash;
[d]Muscimol Flash vs Post-Flash.

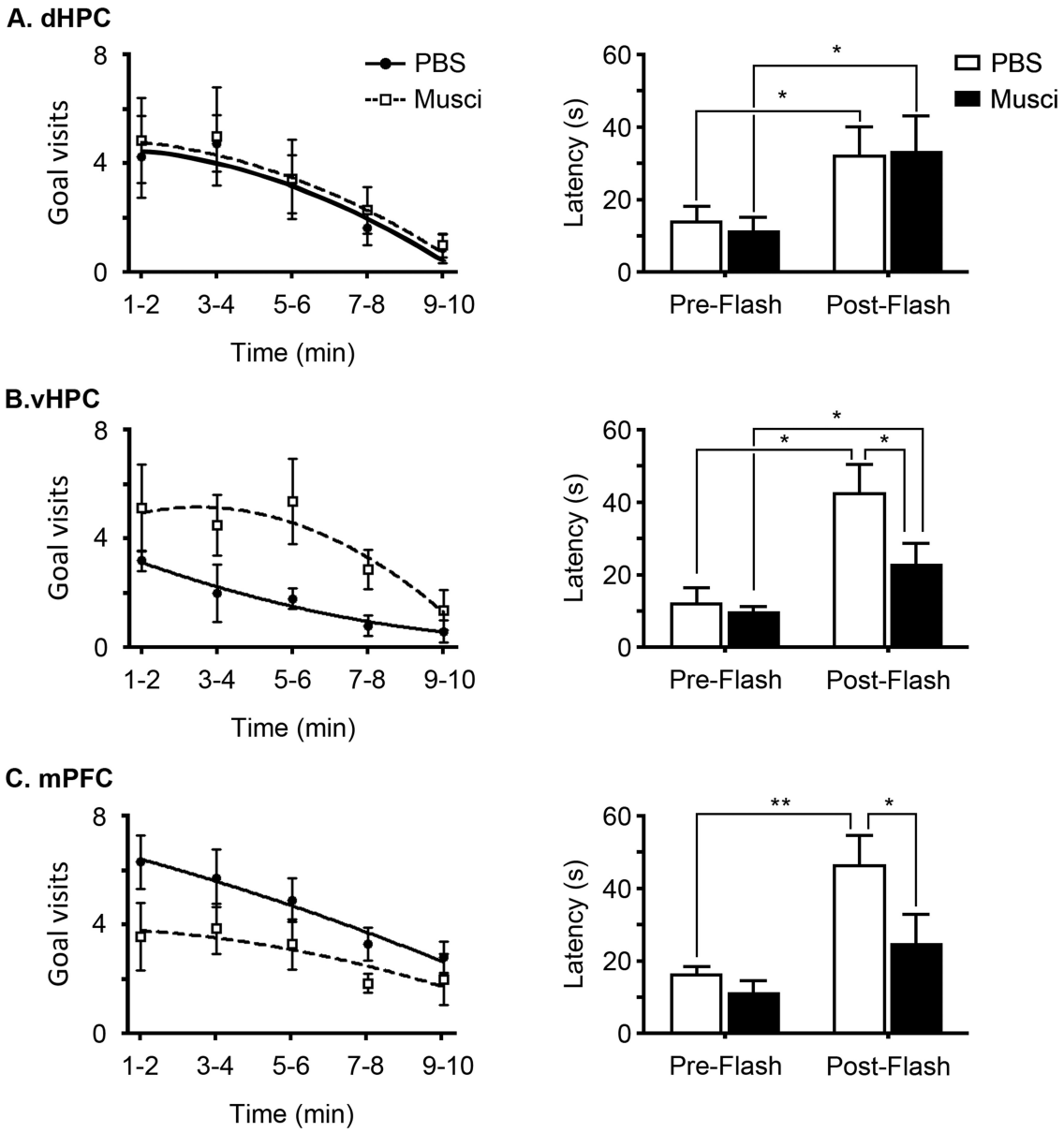

FIGURE 4. Effects of temporary inactivation during valence updating and the next day recall. (A,B,C: left column) Updating goal valence was unchanged during dorsal hippocampus inactivation (A, dHPC), delayed following ventro-intermediate hippocampal inactivation (B, vHPC) and faster with medial prefrontal cortex inactivation (C, mPFC). (A,B,C: right column) During the post-flash session, dHPC rats avoided the goal thus recalled its changed valence (A). vHPC rats delayed their first visit to the goal but did so less than PBS rats (B); mPFC rats did not recall the flash session as they crossed the goal earlier than PBS animals (C).

dHPC *(Fig. 4A, left).* There was no difference between PBS- and muscimol-injected rats. Goal visits decreased steadily during the flash session [$F(4,52) = 7.61$, $P < 0.0001$] in both groups. The dynamics of goal value updating were similar in the two groups (no significant effect of group and no significant group × time interaction).

vHPC *(Fig. 4B, left).* The analyses revealed a significant effect of time [$F(4,44) = 2.75$, $P < 0.05$] and group [$F(1,11) = 7.62$, $P < 0.05$]. Even though there was no significant interaction [$F(4,44) = 0.41$, ns], goal visits decreased rapidly in PBS-injected rats [$F(4,24) = 3.445$, $P < 0.05$] but not in muscimol-injected rats (ns, 1-way ANOVAs). As a consequence, muscimol-injected rats made more goal visits than PBS-injected rats, which might also explain why they did not significantly decrease locomotor activity during the flash session.

mPFC *(Fig. 4C, left).* Goal visits decreased rapidly in both PBS- and muscimol injected rats [main effect of time, [$F(4,60) = 4.14$, $P < 0.01$]. However, there was also an effect of group [$F(1,15) = 6.42$, $P < 0.05$] with muscimol-injected rats learning more rapidly the new value of the goal than PBS-injected

rats. As a result, muscimol-injected rats made fewer goal visits than PBS-injected rats.

In summary, only vHPC inactivation negatively interfered with correct updating of the goal value during the flash session.

Recalling changed goal value during the post-flash session

We assessed whether the change of goal value during the flash session was stored in long-term memory and was thus recalled during the post-flash session on the next day. To do so, we compared the latency of the very first visit to the goal during pre-flash and post-flash sessions, that is, two sessions with no injection and using the standard procedure with goal visits rewarded with food pellets (Fig. 4A–C).

dHPC *(Fig. 4A, right).* Both PBS- and muscimol-injected rats delayed their first goal visit during the post-flash session as shown by a significant increase of first visit latency compared to the pre-flash session [significant main effect of session, $F(1,13) = 12.76$, $P = 0.05$]. There was no significant difference between the two groups, and the increase in latency was significant for both groups (paired *t*-test: PBS, $P < 0.05$; muscimol, $P < 0.05$).

vHPC *(Fig. 4B, right).* The first goal visit was delayed during the post-flash session compared to the pre-flash session in both PBS- and muscimol-injected rats (significant main effect of session, $F(1,11) = 21.29$, $P < 0.001$). There was also a significant difference between the two groups with muscimol-injected rats visiting the goal much earlier than PBS-injected rats (Bonferonni post-test, $P < 0.05$). Moreover, the increase in latency was significant for both groups (paired *t*-test: PBS, $P < 0.01$; muscimol $P < 0.05$).

mPFC *(Fig. 4C, right).* In spite of a significant overall effect of session [$F(1,15) = 11.14$, $P < 0.01$], only PBS-injected rats delayed their first visit to the goal during the post-flash session (paired *t*-test, $P < 0.01$). There was a significant effect of group [$F(1,15) = 4.86$, $P < 0.05$], which was due to muscimol-injected rats that did not delay their first goal visit in the post-flash session. As a result, the first visit latencies of muscimol-injected rats were not significantly different in the pre- and post-flash sessions but were significantly lower than for PBS-injected rats in the post-flash session (Bonferonni post-test, $P < 0.05$).

In summary, recall of the changed value of the goal during the post-flash session was altered in rats that had the mPFC inactivated during the flash session. It was also altered, though to a smaller extent, in vHPC muscimol-injected rats and was unaffected in dHPC muscimol-injected rats.

DISCUSSION

The aim of these experiments was to investigate the specific contribution of mPFC, dHPC, and vHPC in learning a change in the value of a goal. Using a new behavioral procedure, we showed that (1) control rats rapidly learn to avoid a previously appetitive goal when its rewarding value is modified and becomes aversive and remember the new goal value for at least 1 day; (2) rats with an inactivated dHPC learn and they remember the new goal value as well as control rats; (3) rats with vHPC inactivation were impaired in learning the change in goal value, but could remember the change on the next day, though to a lesser extent than control rats; (4) mPFC-inactivated rats learned the new goal value during the flash session, but failed to remember it when tested 1 day later during the post-flash session. In the following, we briefly discuss the results of normal animals before turning to the effects of inactivating the different brain areas.

Main Features of the Behavioral Design

An important issue is whether, in our behavioral protocol, exposure to the flash induces learning of the new response–outcome relationship (Colwill and Triola, 2002). As a matter of fact, if we assume that the goal acts as a stimulus (S) associated with food reward as the outcome (O) and goal visit as the response (R), the flash session, in which food rewards are no longer delivered, might simply produce extinction of the previously learned behavior. Then extinction may superimpose an inhibitory S–R association upon those original outcome associations (Colwill, 1991; Rescola, 1993). Several features, however, argue against this interpretation. First, the flash session explicitly required rats to build a new association between the goal and a new US (the strobe light) so as to produce a new response (avoiding the goal rather than orienting to it), making the protocol very different from traditional extinction. Second, the decrease of goal visits proceeded faster in the flash session compared to the extinction session, which supports the notion that the aversive value of the goal was effective and was rapidly learned. Third, in the session that followed exposure to the flash, rats clearly delayed their first visit to the goal and were slow in relearning the task compared to the session that followed extinction session, which suggests that that the aversive nature of the goal was remembered 1 day later. Even though a latency of 40 s before the first visit to the goal may appear as a short delay for the post-flash session, it is 2.5 times more than for the pre-flash session. This delay might remain short during the post-flash session because the animals have to deal with the conflict between the memory of the aversive value of the goal and their food-deprived state which drives them to the goal. Together, these different points suggest that the decrease of goal visits during the flash session reflects the learning of the new (aversive) value of the goal and not just the learning that food is no longer available upon visiting the goal location (Rescola, 1993; Schiller et al., 2008; Archbold et al., 2010). This conclusion, however, must remain tentative because data from rats that did not experience the flash session before the extinction session are lacking, thus preventing us to precisely delineate both aspects of the task.

Effect of Inactivations on Goal Value Updating

Injection of muscimol 10 min prior to the flash session induced an inactivation of the target structure during the flash session, that is, when the rats had to update the goal value. In contrast, the post-flash session performed 24 h after the flash session was conducted using the standard training procedure and under normal brain conditions. Therefore, it permitted to evaluate the long-term consequences of temporary inactivations on the memory of goal updating. We found very different behavioral profiles for each studied brain area in both the flash and post-flash sessions. These effects were very unlikely to result from a motor deficit since no major difference in locomotor activity was found between PBS-injected and muscimol-injected rats when considering each session separately (i.e., no differences were seen between PBS-injected and muscimol-injected rats for pre-flash, flash or post-flash sessions). We, therefore, interpret the observed effects in terms of the cognitive processing required for goal value updating and its memory.

Dorsal Hippocampus

Visits to the goal during the first minutes of the flash session were unaffected by dHPC inactivation, thus demonstrating that dHPC-inactivated rats were able to localize the goal. Although this result seems not consistent with the prevailing view that the dHPC is implicated in spatial memory, it should be noted that the rats had been extensively trained in the task before the flash session. Therefore, this finding rather suggests an interpretation in terms of disengagement of the dHPC as a result of memory consolidation (Bontempi et al., 1999; Frankland and Bontempi, 2005). Due to the time-dependent reorganization of the neural circuitry for long-term memory, dHPC functional integrity may not be necessary for performing goal localization at this stage. Our results also show that dHPC inactivations had no effect on learning of the new goal value during the flash session, thus suggesting that this aspect does not depend on functional integrity of the dHPC. Finally, both groups exhibited a similar increase in their latency to visit the goal for the first time during the post-flash session, demonstrating their ability to recall the new value of the goal during this session. In summary, the results suggest that dHPC plays no essential role in rapid learning and long-term memory of the new value of the goal.

Ventro-Intermediate Hippocampus

In sharp contrast with the dHPC, ventro-intermediate hippocampal inactivations induced a clear impairment in goal value updating which, however, was not totally abolished as shown by the delayed build-up of avoidance behavior during the flash session. This result is consistent with recent data showing that the intermediate hippocampus is involved in the rapid encoding of new information from the external environment from the dHPC and internal, limbic-like cues from the ventral hippocampus (Bast et al., 2009). Nevertheless, another possible explanation relates to the putative role of the ventral hippocampus in stress, emotion, and affect (for reviews, Bannerman et al., 2004; McHugh et al., 2004; Fanselow and Dong, 2010). In our protocol, the new value of the goal was provided by exposing the rat to an aversive strobe light, which was supposed to be unpleasant but might also have created an anxiogenic context. If vHPC is important for processing an anxiogenic context, it is possible that its inactivation decreases the rat's sensitivity to the flash. In this view, vHPC-inactivated animals may not consider the strobe light as an aversive stimulus, leading them to avoid the goal later than control rats. This particular behavior may explain why they did not decrease locomotor activity during the flash session, as they kept visiting the goal. This learning deficit was also reflected in the post-flash session in which vHPC-inactivated increased their latency of the first visit to the goal (i.e., remembered the new value of the goal), though they were impaired compared to the PBS group. It is very likely that this deficit resulted from their primary impairment to update goal value during the flash session. Nevertheless, because flash sessions also involved extinction, it might be unclear whether the inactivation effects reflect impairments in learning that the location is not rewarded, learning the aversiveness of the location or some combination of the two. However, additional analyses suggest that inactivation of the ventral hippocampus may reflect an impairment in aversive processing per se: (1) goal visits decreased faster for the vHPC PBS(control) animals during flash session than during extinction session $F(1,26) = 13.22$, $p < 0.01$; (2) goal visits decreased similarly for the vHPC muscimol (inactivation) animals during flash session than for normal animals in extinction session ($F(1,29) = 1.35$, ns). Overall, these data suggest that during the flash session the vHPC inactivated rats behave as normal animals would do during an extinction session, that is, that the vHPC inactivated animals were impaired in aversive processing of the strobe light and not on the extinction component of the task.

Medial Prefrontal Cortex

Temporary inactivation of mPFC accelerated learning the new value of the goal during the flash session, suggesting faster goal value updating. Although surprising at first sight, this result could be caused by inactivation of a small part of mPFC, namely the infralimbic region, which is known to enhance the sensitivity of the rat to the goal value (Killcross and Coutureau, 2003). More specifically, it has been shown that rats with infralimbic cortical lesions exhibited increased sensitivity to reward devaluation, compared to sham or prelimbic lesioned rats. An alternative explanation for the faster learning of mPFC-inactivated animals could result of a dysfunctioning in the coordination between mPFC and orbitofrontal cortex (OFC) in the process of value updating. Sul et al. (2010) recorded neural activity in OFC and mPFC as rats had to choose between two arms with different reward probabilities in a continuous T-maze. The reward probability in each arm was changed across blocks of trials. The authors observed strong neural activity in

OFC and weaker activity in mPFC in relation to the animal's choice and its outcome. In addition, mPFC signals were more related to the animal's previous choice and its outcome. Their conclusion is that OFC is mainly involved in the updating of action value, whereas mPFC would mainly participate in working memory though it could also have a role in updating action value. One possibility, therefore, is that mPFC acts not only as a working memory store but also as longer-term memory store of the previously learned value which guides the rat's performance. In this hypothesis, OFC uses the learned mPFC-stored value to make an expectation of upcoming reward, and would continuously update goal value by comparing the reward actually obtained to the expected reward. Hence, if the learned value of the goal is degraded by mPFC inactivation, OFC could use the new goal value more rapidly as the reference value so that goal updating would be faster.

Given their excellent learning performance during the flash session, the poor memory scores of mPFC-inactivated rats in the post-flash session may seem puzzling at first sight. Contrary to the control group, mPFC rats did not avoid the goal when first introduced into the apparatus during the post-flash session, suggesting a deficit in long-term memory of the changed value of the goal. However, this finding is consistent with the conception that, while recent memories are hippocampus-dependent, remote memories may depend on the cortex, and in particular the medial prefrontal areas (Frankland and Bontempi, 2005). For example, Leon et al. (2010) demonstrated mPFC involvement in consolidation and recall of a recent memory by showing mitogen-activated protein kinase/extracellular signal regulated kinase (MAPK/ERK) pathway activation 2 h after the acquisition of single-day learning. In our study, mPFC was inactivated for a period of time extending at least 2 hours after learning the new value of the goal (Edeline et al., 2002). It is thus possible that mPFC inactivation resulted in impaired consolidation, by disrupting synaptic plasticity processes that allow transferring information from the hippocampus to the mPFC.

mPFC-vHPC Cooperation

Inactivation of both mPFC and vHPC had some effect on the updating (flash session) and recall (post-flash session) of the goal value. Even though these effects were different, they raise the question of the cooperation between the two structures. Based on previous work, we propose that vHPC could be involved in the processing of the anxiogenic aspect of the flash and the rapid behavioral adaptation (McHugh et al., 2004; Bast et al., 2009). Thus, vHPC inactivation would prevent rapid processing of the flash as an aversive outcome, thus delaying the correct adaptive response. In this view, the weak recall performance during the post-flash session would be a consequence of poor learning, and not an impaired long-term consolidation process. In contrast, inactivation of the mPFC would block memory retrieval of the old (appetitive) value of the goal. Since information about the aversive new value of the goal flows to both the mPFC and OFC, this would result in a facilitation of OFC processing of the new aversive value and, therefore, result in faster behavioral adaptation. However, mPFC inactivation would also result in impaired consolidation (Edeline et al., 2002; Leon et al., 2010), thus preventing the new goal value to be correctly remembered during the post-flash session.

CONCLUSION

In this study, we validated a new behavioral design that allows analyzing rapid learning in a spatial context. More specifically we showed that rats are able to rapidly adapt their behavior to a change in the value of a goal. Using this behavioral protocol, we found that functional integrity of the dHPC is not necessary for updating goal value, whereas, the ventral and intermediate parts of the hippocampus are required for such learning. The mPFC is probably involved in a loop concerned with updating goal value, but its main role concerns the long-term retention of this updating. Finally, although these brain structures appear to belong to a neural network in which each has its own role in learning the new value of a location, further experiments, with brain inactivations performed before the extinction session, should help clarifying the impact of the extinction procedure and the exact contribution of the different brain structures at recall stage.

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