HIPPOCAMPUS 24:1482–1492 (2014)

Retroactive Interference of Object-in-Context Long-Term Memory: Role of Dorsal Hippocampus and Medial Prefrontal Cortex

María Cecilia Martínez,[1] María Eugenia Villar,[1] Fabricio Ballarini,[1] and Haydée Viola[1,2]*

ABSTRACT: Retroactive interference (RI) is a type of amnesia in which a new learning experience can impair the expression of a previous one. It has been studied in several types of memories for over a century. Here, we aimed to study in the long-term memory (LTM) formation of an object-in-context task, defined as the recognition of a familiar object in a context different to that in which it was previously encountered. We trained rats with two sample trials, each taking place in a different context in association with different objects. Test sessions were performed 24 h later, to evaluate LTM for both object-context pairs using separate groups of trained rats. Furthermore, given the involvement of hippocampus (Hp) and medial prefrontal cortex (mPFC) in several recognition memories, we also analyzed the participation of these structures in the LTM formation of this task by the local infusion of muscimol. Our results show that object-in-context LTM formation is sensitive to RI by a different either familiar or novel object-context pair trial, experienced 1 h later. This interference occurs in a restricted temporal window and works on the LTM consolidation phase, leaving intact short-term memory expression. The second sample trial did not affect the object recognition part of the memory. Besides, muscimol treatment before the second sample trial blocks its object-in-context LTM and restores the first sample trial memory. We hypothesized that LTM-RI amnesia is probably caused by resources or cellular machinery competition in these brain regions when they are engaged in memory formation of the traces. In sum, when two different object-in-context memory traces are being processed, the second trace interferes with the consolidation of the first one requiring mPFC and CA1 dorsal Hp activation. © 2014 Wiley Periodicals, Inc.

KEY WORDS: rat; learning and memory; episodic-like memory; amnesia; muscimol

[1] **Laboratorio de Memoria, Instituto de Biología Celular y Neurociencia "Prof. E. De Robertis", Facultad de Medicina, Universidad de Buenos Aires-CONICET, Paraguay 2155 3° piso, Buenos Aires, Argentina;**
[2] **Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160, Ciudad Universitaria, Buenos Aires, Argentina.**
Grant sponsor: National Agency of Scientific and Technological Promotion of Argentina (ANPCyT); Grant number: PICT 2008-2189; Grant sponsor: University of Buenos Aires (UBACyT); Grant number: M685.
*Correspondence to: Haydée Viola, Laboratorio de Memoria, Instituto de Biología Celular y Neurociencia "Prof. E. De Robertis", Facultad de Medicina, Universidad de Buenos Aires-CONICET, Paraguay 2155 3° piso, C1121ABG Buenos Aires, Argentina. E-mail: hviola@fmed.uba.ar
María Cecilia Martínez is currently at Laboratorio de Fisiología de Circuitos Neuronales, Departamento de Fisiología, Facultad de Medicina, Instituto de Fisiología y Biofísica "Bernardo Houssay", Universidad de Buenos Aires-CONICET, Paraguay 2155 7° piso, C1121ABG, Buenos Aires, Argentina.
M.C.M. and M.E.V. contributed equally to this work.
Accepted for publication 3 July 2014.
DOI 10.1002/hipo.22328
Published online 8 July 2014 in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

Recognition memory refers to the ability to identify an object or a situation and judge if it was previously experienced or not (Warburton and Brown, 2010). This type of memory, though vital for the life of individuals, is quite common: for example, when we meet somebody on the street, we recall many facts we know about that person; likewise when we enter a familiar room, we can realize novel furniture arrangements. In particular, the recognition of an item in connection with a context (what-where) constitutes an important element of episodic memory, which also implies remembering about what-when (Clark and Martin, 2005). Episodic memories are routinely evoked when we retrieve events or occasions including spatial locations and the contextual features of the environment in which an event took place.

As it occurs with other memory types, the formation of recognition memory involves different stages (acquisition, consolidation, retrieval among them) and impairments happening at any of these could result in the absence of memory. Retroactive interference (RI) is a type of amnesia which has been widely studied for over a century and is characterized by the disruptive effect of a new learning experience over previously encoded material (Wixted, 2004). Here, we show that the acquisition or the retrieval of an object-context association pair can put in risk the long-term memory (LTM) formation of a previously learned association between a different object with another context. We also investigated the effective temporal window in which a second experience can induce interference over a previous one, the required features of that experience to be disruptive, as well as the brain regions involved in the phenomenon.

Several studies set to determine which brain regions were selectively involved in particular aspects of recognition memory tasks. The hippocampus (Hp) has been demonstrated to participate in LTM formation for object-in-context recognition memory because the post-training local infusion of a protein synthesis inhibitor impaired the consolidation of this task (Balderas et al., 2008). Another region notably involved in remembering an object in connection with the information about a certain place or time is the medial prefrontal cortex (mPFC) (Barker et al., 2007;

for review see Banks et al., 2012). It has been reported that local pre-training administration of NMDA receptor antagonist into the mPFC, abolished the acquisition of an object-in-place memory (Barker and Warburton, 2008). Also, the pharmacological lesion of this region impaired rodents to remember the order of events in a sequence (Devito and Eichenbaum, 2011) or to acquire and retrieve a list of associations between odors, contexts, and rewards (Peters et al., 2013). In addition, the reversible inactivation of the mPFC with lidocaine has demonstrated that this structure is also important for the encoding and the retrieval of spatial memory in the Hebb-Williams maze, a task specially designed to study navigation performance (Churchwell et al., 2010). Moreover, object-in-place memory has been shown to depend on the activity of the Hp and the prefrontal cortex (Barker et al., 2007; Barker and Warbuton, 2011; Kim et al., 2011) and temporary mPFC inactivation specifically reduced the rule-based object associations represented in hippocampal neurons (Navawongse and Eichenbaum, 2013). Together, these and other (Burton et al., 2009; Colgin, 2011) results suggest that both regions integrate a cooperative functional network involved in the processing of episodic events.

With the aim of analyzing if recognition LTM formation is susceptible to RI, in this work, we trained rats to learn two different associations between objects and their respective arena boxes. Separate groups of subjects were tested in these contexts in order to evaluate the LTM for each associate pair (object-context). Furthermore, given the involvement of the Hp and the mPFC in several recognition memories, we also analyzed the participation of these structures in the LTM formation of the object-in-context task. Besides, considering our previous work on memory competition (Martínez et al., 2012), we studied if the processing of an object-context association could interfere with a previously acquired pair association through a competitive mechanism taking place in the Hp and the mPFC.

Our results show that LTM formation for a novel object associated with a context can be impaired if subjects explore a different object in another context and this second experience can exert RI over the first one in a limited temporal window. Interestingly, RI was dependent on the presentation of objects in the contexts, being insufficient the presentation of only novel or familiar contexts. Finally, our results suggest that the mPFC and dorsal Hp are important brain regions involved in the processing of object-context pairs association. In particular, when both experiences are being consolidated in an overlapping time course, competition occurs between traces and only one of them can be effectively stored.

MATERIALS AND METHODS

Subjects

Male adult Wistar rats weighing 180–220 g were housed in groups of five to six per cage, maintained under a 12-h light/12-h dark cycle (21°C) with food and water ad libitum. They were handled for 3 min for three consecutive days to avoid emotional stress. All procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publications No. 80-23, revised 1996) and were approved by the Animal Care and Use Committee of the University of Buenos Aires.

Surgery and Drugs

Surgery

For cannulae implantation, rats were deeply anesthetized (70 mg/kg ketamine; 8 mg/kg Xylazine) and 22-G cannulae were stereotaxically aimed to either the CA1 region of the dorsal Hp at coordinates A −3.9 mm, L ±3.0 mm, V 3.0 mm, or to the mPFC at coordinates A +3.2 mm, L ±0.75 mm, V −3.2 mm (Paxinos and Watson, 2007, see Fig. 4). All coordinates are relative to the Bregma in a flat position with respect to the lambda. Cannulae were fixed to the skull with dental acrylic. Animals received a subdermal application of analgesics at the moment of the surgery (Meloxicam 0.2 mg/Kg) and were allowed to recover from surgery for 5-6 days. Drugs were infused using a 30-G needle with its tip protruding 1.0 mm beyond the guide. The entire bilateral infusion procedure took about 2 min, including 45 s for the infusions themselves, first on one side and then on the other. Cannulae were left in place for 1 additional min to minimize back-flow. Histological examination of cannulae placements was performed after the experiments by the infusion of 0.5 μL of 4% methylene blue in saline solution. Briefly, after the end of the behavioral procedures, methylene blue in saline was infused as indicated above. Animals were killed by decapitation 15 min after and their brains were sliced to check the infusion area (maximum spread of about 1.5 mm^3). Only data from animals with correct cannula implants (95% of the rats) were included in statistical analyses.

The $GABA_A$ agonist muscimol (Sigma, USA) was applied to temporarily inactivate the hippocampal subregion CA1 and the mPFC. The dose infused (0.1 μg of muscimol in 0.5 μL saline solution per side) was reported to be effective (Gonzalez et al., 2013).

Behavioral Training

Habituation

Initial habituation sessions were carried out to familiarize the rats with the apparatus in which training would take place. Habituation consisted of one daily session of 20 min in each of the arenas to be used throughout the experimental protocol. Unless indicated to the contrary, all subjects were habituated in two consecutive days to the arenas without objects.

Object-in-context

Object-in-context memory is defined as the recognition of a familiar object in a context different to that in which it was

previously encountered (Dix and Aggleton, 1999). Here, we trained rats with two sample trials, each taking place in a different context. In all the experiments, the sequential order of the contexts was balanced. The objects defined as Obj1 and Obj2 were also balanced along the experiments.

One of the contexts was a rectangular apparatus of dimensions 60 cm width × 40 cm depth × 50 cm height, made of white acrylic and with distinctive visual cues in each wall. The front wall was transparent and the back wall was hatched. The other apparatus had a circular shape and its dimensions were 50 cm diameter × 39 cm height, with a black plywood floor. Animals were exposed to both arenas (with the exception of the animals in control groups, which were only exposed to one of them). In the training session, the subject was introduced for 5 min in the context (CTX1) in the presence of a pair of identical objects (Obj1). Each context had a specific pair of objects associated to it. Objects were made of plastic, glass, or aluminum and had similar dimensions. Animals were left to explore the arena and exploration time for each of the objects was measured using a hand stopwatch. One hour after the first sample trial had concluded, the subject was introduced for 5 min in the second context (CTX2) in the presence of a new pair of identical objects (Obj 2). The exploratory activity of the subject was registered in the same way as in the first context.

Twenty-four hours after training, half of the subjects was tested by reintroducing them individually in CTX1 and the other half was reintroduced in CTX2 for 3 min in the presence of Obj1 and Obj2. In test session, animals expressed memory for object recognition associated to the context (object-in-context memory) if they spent more time exploring the incongruent object (i.e., the object which had been presented in other arena during training) than the congruent one. Exploration was defined as sniffing or touching the object with the nose or forepaws. The time of exploration for each object was recorded and expressed as a percentage of the total exploration time for both objects.

Object Recognition

A protocol for testing object recognition task was used only for data shown in Figure 3. In this case, the training session was identical to the object-in-context task training but in contrast to the usual protocol, in the test session, a completely novel object was presented with the object that had been presented during training in the context (familiar). Animals expressed memory for object recognition if they spent more time exploring the novel object instead of exploring the object that was associated to the context during the training.

Empty context trial

This was carried out by exposing the subject to an empty arena to which they had been previously habituated.

Familiar object in new context trial

For object habituation, animals were placed individually in a homecage for 20 min in the presence of a pair of identical objects. This procedure was repeated for two consecutive days previous to training. In the test trial, subjects explored a new context with these familiar objects included in it.

Familiar object in familiar context trial

Animals were habituated to this context in the presence of a pair of identical objects so that after two habituation sessions subjects were already familiarized with them.

New context: open field (OF, spatial exploration)

For the second learning trial taking place in a completely novel context, an open field arena was used (i.e., subjects were not habituated to this arena before training). The apparatus was a 50 × 50 × 39 cm arena with black plywood walls and wooden floor, divided in 9 squares by black lines. Exploratory activity was measured as the number of crossings between squares and the number of rearings, registered minute by minute. Trail training session consisted of a 5-min exploration session. A 5-min test session was performed at the following day. Habituation percentage for each subject was calculated with the formula: [(OF Tr − OF Ts)/OF Tr] × 100, where "OF Tr" is the total number of events (crossings or rearings) registered during training session and "OF Ts," the total number of events registered during test session. A higher habituation percentage (i.e., a larger decrease in exploratory activity) represents a stronger memory in this task.

Data Analysis

Statistical analysis of behavioral data was performed with Student's *t* test (paired samples were used for the object-in-place and object recognition experiments and independent samples for the OF experiments) using InfoStat software.

RESULTS

The Second Sample Trial Interferes With the Object-in-Context Memory Formation of the First One

Rats were submitted to an object-in-context experimental protocol consisting of two sample trials with novel objects in two different contexts (CTX1 and CTX2). Each context had a different pair of identical objects associated to it and both trials were separated by 1 h interval. Memory for the objects in each context was tested 24 h after training using separate groups of animals that had received the same training. For the test session, we used a pair of objects composed by one object associated to the context (congruent) and another that had been presented in the other arena (incongruent). Figure 1a left shows that the control group of rats trained only in the first context—without the second trial—showed exploratory preference for the object that had never been presented in CTX1

($t_{(7)} = 3.54$, $P = 0.009$). However, when animals received a second sample trial 1 h after the first had taken place, the LTM for the Obj1-in-CTX1 was impaired when it was tested in CTX1 24 h later (Exp group, $t_{(13)} = 0.19$, $P = 0.855$). In other words, when objects were tested in the context used for the first sample trial, the exploration times for each of them were similar (Obj1: 12.0 s ± 1.7 SEM vs. Obj2: 12.1 s ± 2.1 SEM; $t_{(13)} = 0.052$, $P = 0.959$) regardless whether they had been

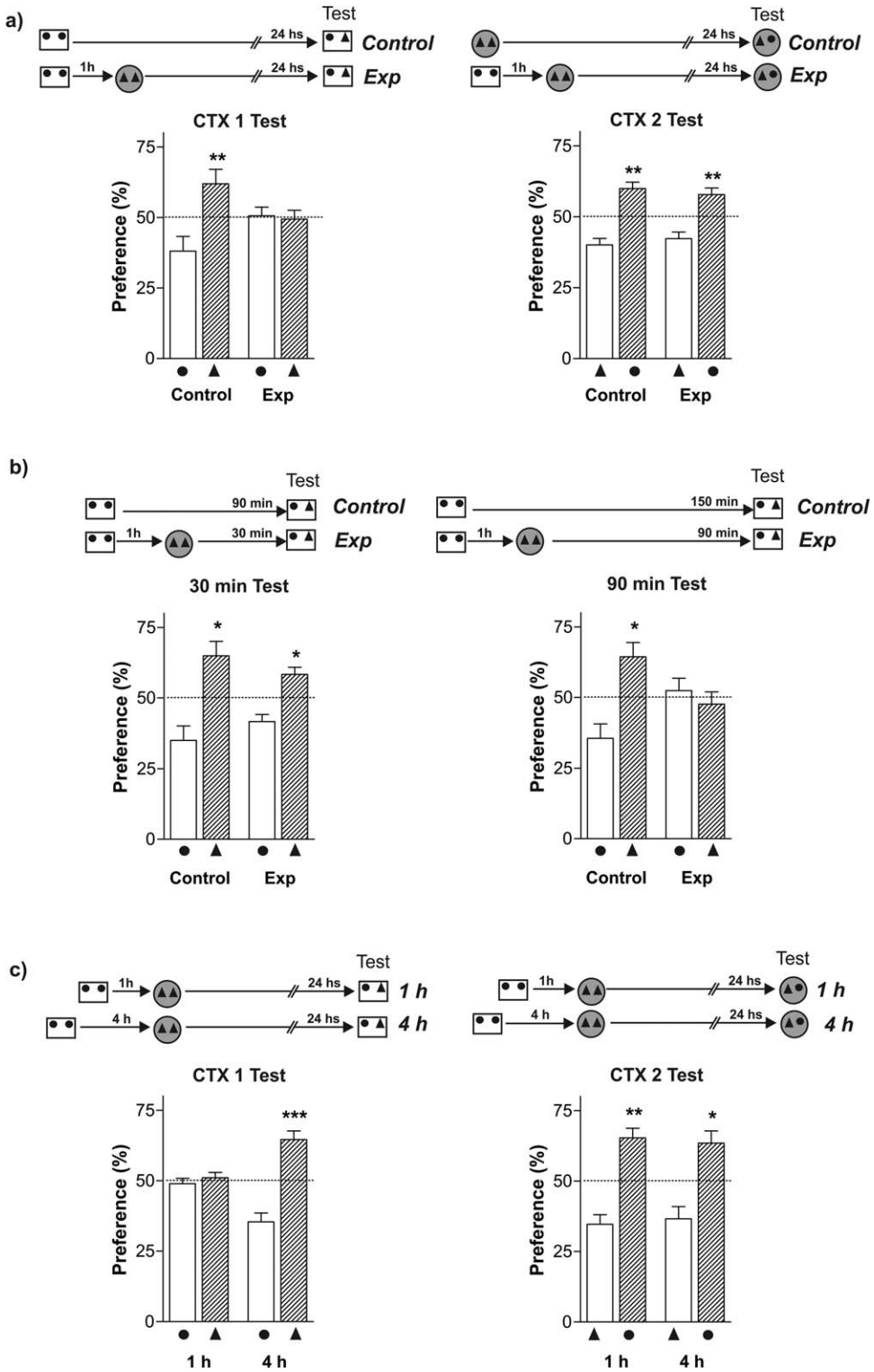


FIGURE 1.

associated or not to this context during training, evidencing RI of Obj1-in-CTX1-LTM. Interestingly, when a parallel group of rats was tested in CTX2, Obj2-in-CTX2-LTM remained unaffected by the previous sample trial in CTX1 because rats dispensed more time of the test session exploring the incongruent object (Fig. 1a right panel, Control group $t_{(7)} = 4.42$, $P = 0.003$; Exp group $t_{(12)} = 3.29$, $P = 0.006$). In this case, the subjects preferentially explored the Obj 1 (Obj1: 11.2 s ± 1.3 SEM vs. Obj2: 8.1 s ± 0.8 SEM; $t_{(12)} = 3.079$, $P = 0.0096$). Taken as a whole, these results show that the object associated to CTX1 in the training session results incongruent in both test contexts and it is explored more in both of them. In contrast, the object associated to CTX2 is explored less when tested in CTX2 than when it is tested in CTX1, evidencing the formation of Obj2-in-CTX2 LTM.

Next, we decided to study if this RI visible 24 h after training was related to impairments in the acquisition or the consolidation of the task. For that reason, we tested the short-term memory (STM) for Obj1-in-CTX1. Figure 1b shows that STM could be observed at 90 min when animals were trained in only one of the contexts as well as when they were trained with the two-trial protocol (Left panel, Control group $t_{(5)} = 2.91$, $P = 0.033$; Exp group, $t_{(5)} = 3.23$, $P = 0.023$). In contrast, when a test session was performed 150 min after training, memory was visible in control subjects but not in those submitted to the two-trial protocol (Fig. 1b Right panel, Control group $t_{(8)} = 2.82$, $P = 0.022$; Exp group $t_{(7)} = 0.56$, $P = 0.595$). Taken as a whole, these results suggest that information for Obj1-in-CTX1 can be effectively acquired but it is its stabilization what becomes impaired if another learning trial takes place in a close temporal lapse.

To test this possibility, we separated the two sample trials by a longer time interval to allow the completion of the consolidation of the memory for Obj1-in-CTX1 before the processing of the second trial took place. Figure 1c shows that there was RI when the temporal lapse between sessions was of 1 h but not when the intertrial interval was longer (Left panel, 1 h group, $t_{(9)} = 0.55$; $P = 0.593$, 4 h group, $t_{(10)} = 4.73$, $P < 0.001$; Right panel, 1 h group $t_{(6)} = 4.53$, $P = 0.004$; 4 h group $t_{(8)} = 3.08$, $P = 0.015$). Thus, LTM for both objects in their respective contexts can be consolidated when their acquisition trials are separated by a 4 h time lapse.

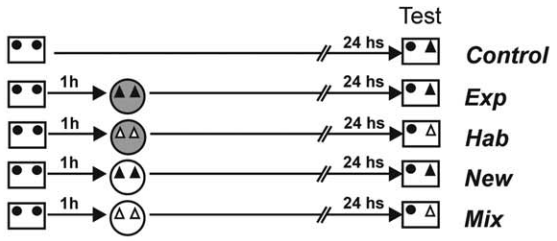
Interference Is Related to the Exposure to a Second Object-in-Context Sample Trial

In another series of experiments, we identified which features of the second trial are important for the RI in the LTM formation of the Obj1-in-CTX1. With that aim, we trained animals in the first context and 1 h after they were introduced in a second context (which was previously explored or not, depending on which group they belonged to) with objects (which were familiar or novel). All of these four possible combinations for the second sample trial induced RI (Fig. 2, Control group $t_{(5)} = 2.86$, $P = 0.036$; Exp group $t_{(5)} = 0.44$ $P = 0.676$; Hab group $t_{(6)} = 0.66$, $P = 0.533$; New group $t_{(7)} = 2.15$, $P = 0.075$; Mix group $t_{(8)} = 1.13$, $P = 0.290$). Therefore, either the acquisition of new information or the memory retrieval for the objects-in-context taking place in the second sample trial impaired the consolidation of the memory of Obj1-in-CTX1.

Object Recognition LTM Is Not Interfered by the Second Sample Trial

In order to confirm that the object-in-context LTM was specifically interfered—but not the recognition of the object itself—we studied if LTM for the object was preserved after a second learning experience occurring 1 h after the first sample trial. Thus, we performed the same training protocol as in Figure 1 but this time the test session included the object associated to the CTX and another object completely novel to the subjects. Figure 3a shows that rats submitted only to the first sample trial express LTM for object recognition (Control group $t_{(11)} = 5.96$, $P < 0.001$). When animals were submitted to a second sample trial consisting of a familiar CTX with novel objects (Exp) or the inverse situation (a novel CTX with familiar objects, Mix group), they also spent more time exploring the novel object (Exp group $t_{(8)} = 6.95$, $P < 0.001$; Mix group $t_{(9)} = 6.46$, $P < 0.001$). Therefore, these results demonstrate that RI is specifically observed for the object-in-context LTM, being intact the novel object recognition-LTM.

FIGURE 1. The second object-in-context sample trial interferes with the memory formation of the first one when it takes place 1 h after it. Schematic representation of the experimental protocol is presented on top of each panel. Preference index during test session ± SEM. The plain bar corresponds to the congruent object. The dashed line represents the chance level of performance (i.e. a 50% preference corresponds to no discrimination between congruent and incongruent objects). (a) Long-term memory for the first sample trial is interfered by the second sample trial. Separate groups of control subjects were trained in only one of the contexts and 24 h later were tested in the same arena. Subjects in the experimental groups performed two sample trials (one in CTX1 and the other in CTX2) 1 h apart during training and 24 h later were tested in only one of the arenas. **$P < 0.01$ vs. congruent object, paired *t* test. (b) Short-term memory for the first sample trial is not interfered by the second sample trial. Separate groups of control subjects were trained in only one of the contexts and were tested in the same arena 90 or 150 min after training. Subjects in the experimental groups performed two sample trials (one in CTX1 and the other in CTX2) during training and 30 or 90 min after training were tested in only one of the arenas as indicated in the graph. *$P < 0.05$ vs. congruent object, paired *t* test. (c) When both sample trials are separated by 4 h there is no retroactive interference. Subjects in the 1 h groups performed one sample trial in CTX1 and 1 h after they performed the second sample trial in CTX2. Subjects in the 4 h groups performed one sample trial in CTX1, and 4 h after, they performed the second sample trial in CTX2. All the groups were tested 24 h after training in only one of the arenas. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ vs. congruent object, paired *t* test.



Characteristics of the 2nd sample trial

	Control	Exp	Hab	New	Mix
CTX	—	Fam	Fam	Nov	Nov
OBJ	—	Nov	Fam	Nov	Fam

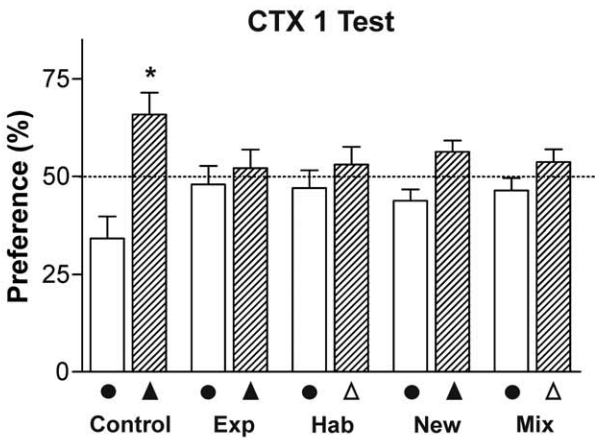


FIGURE 2. Interference on object-in-context LTM is related to the exposure to a second object-in-context sample trial. Preference index during test session ± SEM. The plain bar corresponds to the congruent object. The dashed line represents the chance level of performance (i.e. a 50% preference corresponds to no discrimination between congruent and incongruent objects). Subjects in the Control group performed only one sample trial in CTX1. Subjects in the other groups performed one sample trial in CTX1 and 1 h after they performed a second sample trial in a novel or familiar CTX2 with novel or familiar attributes as detailed in the table below. All the groups were tested 24 h after training in CTX1 *$P < 0.05$ vs. congruent object, paired t test.

Based on our previous findings (Martínez et al., 2012), we also studied if the exploration of a novel context (open-field, OF) could exert any effect on the object recognition LTM of the task. Therefore, we trained animals in the first context and 1 h after they were exposed to a novel OF arena for 5 min. Figure 3a shows that object recognition LTM was not impaired either by the exploration of a novel OF nor by the exposure to a familiar empty CTX (OF group $t_{(6)} = 4.87$, $P = 0.003$; Empty group $t_{(6)} = 4.14$, $P = 0.006$). To discard that the first learning trial could induce proactive interference on the second, we tested LTM in CTX2. Figure 3b shows that animals expressed LTM for the object explored in CTX2 (Control group $t_{(6)} = 3.94$, $P = 0.007$; Exp group $t_{(8)} = 3.77$, $P = 0.005$; Mix group $t_{(9)} = 4.65$, $P = 0.001$). Likewise, OF-LTM for the Exp group was consolidated, showing no significant differences with its respective control group (Fig. 3c, Crossings $t_{(10)} = 0.065$ $P = 0.949$; Rearings $t_{(10)} = 0.460$, $P = 0.655$). Thus, in contrast to object-in-context LTM consolidation, results in Figure 3 suggest that object recognition LTM was not sensitive to interference caused by a completely novel context (OF group), an empty but familiar context (Empty group) and neither to the mixture of a familiar context with novel objects (Exp group) nor familiar objects in a new context (Mix group).

Inactivation of the Hp and mPFC can Revert the Interference

Regarding the structures related with the processing of the LTM for the object-in-context task, we studied involvement of the Hp and the mPFC, which had been repeatedly associated to object recognition paradigms. To exert a reversible inhibition of these two areas, we applied local infusions of muscimol (Mus) 15 min before the second trial. Interestingly, muscimol treatment in CA1 dorsal Hp resulted in a loss of LTM for Obj2-in-CTX2 (Fig. 4a right panel, Veh group $t_{(9)} = 2.42$, $P = 0.039$; Mus group $t_{(11)} = 0.14$, $P = 0.889$) and a recovery of the memory trace of Obj1-in-CTX1 (Fig. 4a Left panel, Veh group $t_{(11)} = 0.72$, $P = 0.483$, Mus $t_{(9)} = 5.75$, $P < 0.001$). Likewise, muscimol administration into mPFC impaired the LTM formation for Obj2-in-CTX2 (Fig. 4b right panel, Veh group $t_{(13)} = 2.57$, $P = 0.023$; Mus group $t_{(12)} = 1.59$, $P = 0.138$) enabling the LTM consolidation for the Obj1-in-CTX1 (Fig. 4b Left panel, Veh group $t_{(9)} = 1.87$, $P = 0.094$; Mus group $t_{(11)} = 2.80$, $P = 0.017$). These results suggest that the Hp and the mPFC are both involved in the processing of the object-in-context task.

DISCUSSION

Our results show that object-in-context memory formation about the first sample trial is susceptible to interference by a different and subsequent object-context pair association experienced 1 h later (Fig. 1a). The results of the Exp group in Figure 1a left show that animals tested in CTX1 explore both objects equally (~12 s) so we consider that both objects are perceived as incongruent in that context. This clearly suggests that there is RI on the LTM of having explored Obj1 in CTX1. In contrast, when the test was done in the context where the second sample trial took place, subjects preferentially explored the Obj1 because they found it incongruent in CTX2. In this case, the less extensive exploration of Obj2 in CTX2 (~8 s) clearly shows that animals remember having explored this object-context pair association during the training performed the previous day.

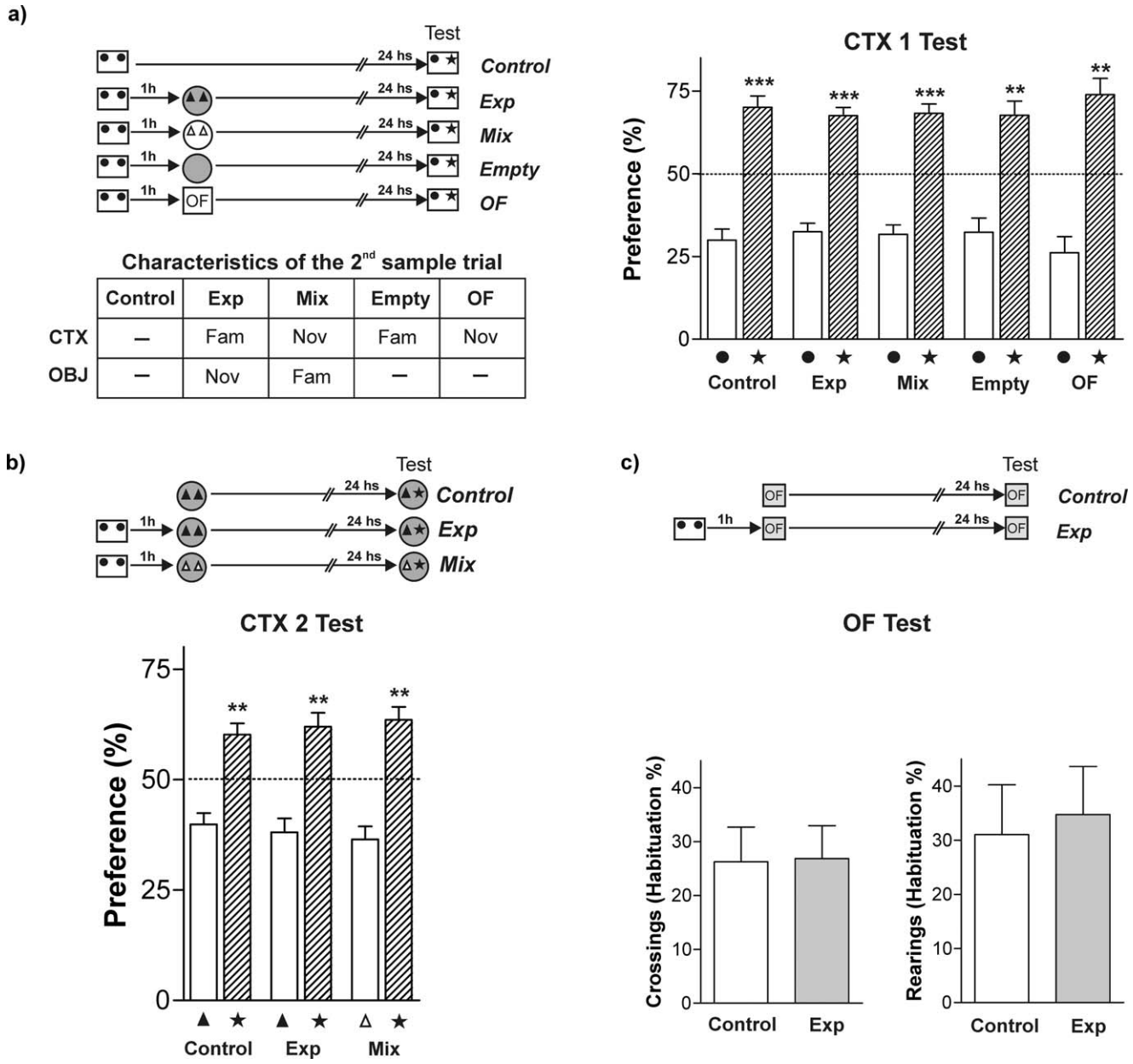


FIGURE 3. A second sample trial does not induce retroactive interference on the object recognition LTM. Schematic representation of the experimental protocol is presented on the top. The table below details training attributes. (a and b) Preference index during test session ± SEM. The plain bar corresponds to the congruent object. The dashed line represents the chance level of performance (i.e. 50% preference corresponds to no discrimination between congruent and novel objects). (c) Performance in the OF expressed as Habituation percentage ± SEM. (a) Retroactive interference is not observed for object recognition LTM. Subjects in the control group performed only one sample trial in CTX1. Subjects in the other groups performed one sample trial in CTX1, and 1 h after, they performed a second sample trial as described in the table. Twenty-four hours after training animals were tested in CTX1 in the presence of a congruent object and another completely unknown to them. $^{***}P < 0.001$, $^{**}P < 0.01$ vs. congruent object, paired *t* test. (b) There is no proactive interference for the second sample trial. Subjects in the control group performed one sample trial in CTX2 and subjects in the Exp and Mix groups performed one sample trial in CTX1, and 1 h after, they performed the second sample trial in CTX2. Twenty-four hours after training animals were tested in CTX2 in the presence of a congruent object and another completely unknown to them. $^{**}P < 0.01$ vs. congruent object, paired *t* test. (c) OF-LTM is unaffected by the first sample trial. Subjects in the control group were exposed to a novel OF and subjects in the Exp group performed one sample trial in CTX1, and 1 h after, they were exposed to a novel OF. They were tested in the OF 24 h after training. $P > 0.05$, Student's *t*-test.

Altogether these results suggest that there is no proactive interference of the first sample trial over the LTM of the second object-context pair (Obj2-in-CTX2) because Obj2 was explored less than Obj1 when the test was performed in CTX2. Instead, there is interference over first sample trial caused by the second sample trial. This RI works on the

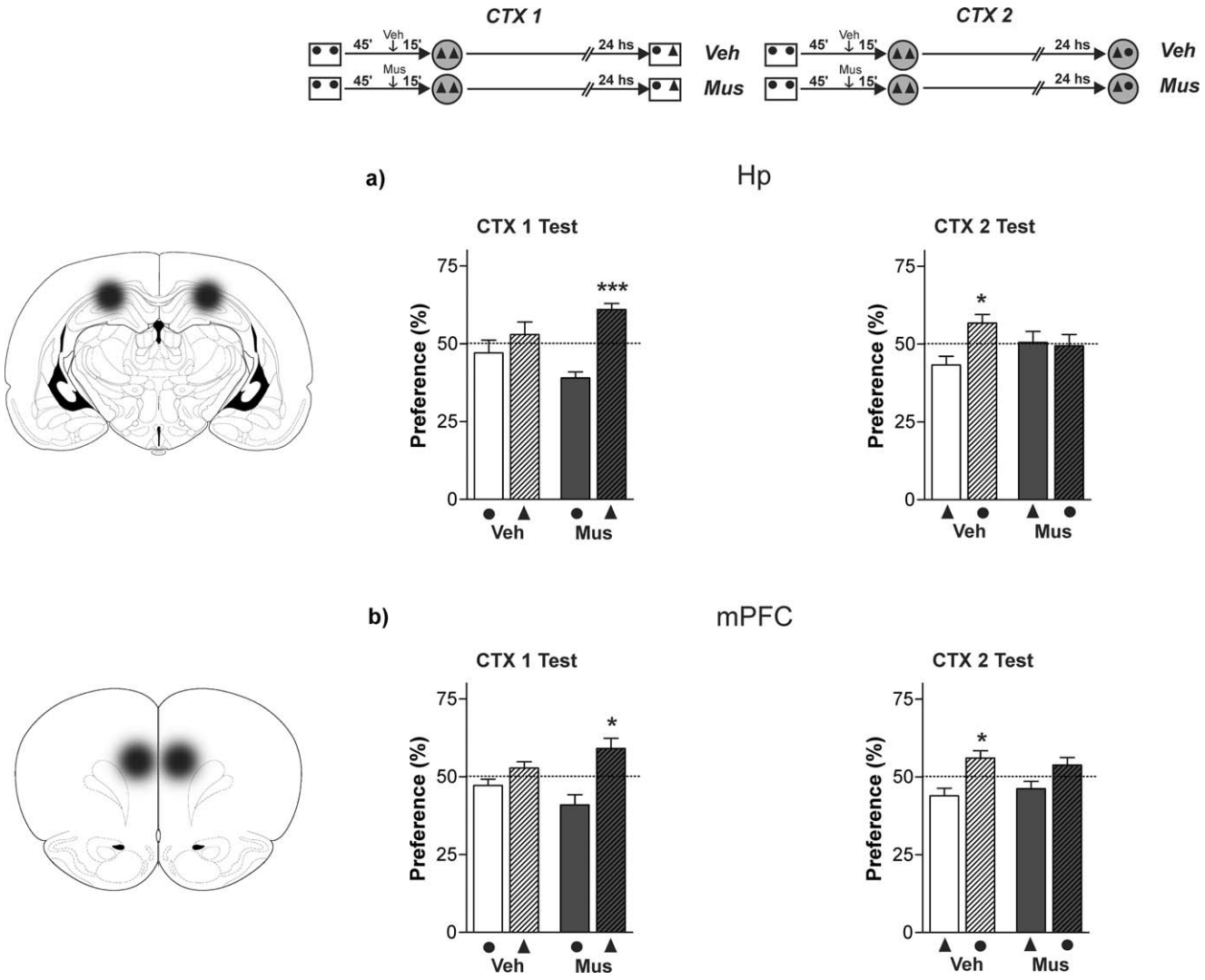


FIGURE 4. Local inactivation of the Hp and mPFC previous to the second sample trial can revert retroactive interference. Schematic representation of the experimental protocol is presented on top of the panel. Left: Schematic representation of the infusion area. Right: Preference index during test session ± SEM. The plain bar corresponds to the congruent object. The dashed line represents the chance level of performance (i.e. a 50% preference corresponds to no discrimination between congruent and incongruent objects). (a) Effects of the local infusion of Muscimol in the CA1 dorsal Hp. Subjects performed one sample trial in CTX1 and 1 h after they performed a second sample trial in CTX2. Fifteen minutes before the second sample trial, subjects received a local infusion of Veh or Mus. All groups were tested 24 h after training. $^{***}P < 0.001$, $^{*}P < 0.05$ vs. congruent object, paired *t* test. (b) Effects of the local infusion of Muscimol in the mPFC. Subjects performed one sample trial in CTX1, and 1 h after, they performed a second sample trial in CTX2. Fifteen minutes before the second sample trial, subjects received a local infusion of Veh or Mus. All groups were tested 24 h after training. $^{*}P < 0.05$ vs. congruent object, paired *t* test.

consolidation phase of the first pair learned because it only affects its LTM, leaving intact its STM (Fig. 1b). If the second trial was separated by 4 h from the first one, the RI was not observed (Fig. 1c). Such RI depends on the presentation of another object-in-context experience, regardless of whether they are familiar or novel (Fig. 2). Moreover, this interpolated trial specifically disrupted the consolidation of the Obj1-in-CTX1 memory and did not affect the Obj1 recognition memory formation (Fig. 3). In addition, we show that the inactivation of the CA1 dorsal hippocampal or mPFC regions before the second learning trial impaired the LTM formation for this pair association while restoring the first pair LTM expression (Fig. 4).

Around a century ago, it was postulated that the interference by the interpolation of certain materials or tasks could be one of the causes of everyday forgetting (Müller and Pilzecker, 1900). As memories are stabilized, they become less sensitive to interference. In that sense, the experiments shown here demonstrate that the RI observed in the LTM formation for an object-in-context task only occurs when the interpolation is

made 1 h after the first learning session and not when it is made 4 h later. However, the STM for that memory was intact 30 min after a training protocol that causes LTM interference. This temporal response strongly suggests that the second learning trial affected memory consolidation for the first one. To our knowledge, the present findings represent the first evidence of RI in the LTM formation of an object-in-context task. Several works focused on the STM-acquisition component of the recognition memory. Thus, they measured the performance of rats on a test carried out few min after a two-trial training protocol (two 5 min trials, 2 min apart from each other). Rats showed acquisition and STM for object recognition and temporal/spatial components of these tasks (Good et al., 2007). Such results are in agreement with ours and support the fact that the RI specifically acted on LTM formation without interfering on short-term aspects of memory. LTM-RI for an object recognition task could alternatively explain the results observed by Barbosa et al. (2012), who tested rats 24 h after a double trial session training. In this work, subjects' exploratory activity in the test session was directed to object present in the first trial, probably because its memory was not consolidated because of the presence of the second trial performed 1 h later.

Wixted (2004) suggests that the interference is the new learning itself which uses the resources available to consolidate the original trace. In accordance with this idea, experiments in our previous work suggested that competition for the consolidation of two Hp-dependent memory traces aroused when plasticity resources were in limited amounts (Martínez et al., 2012). Therefore, we hypothesized that in this object-in-context memory paradigm, the processing of the second trial could interfere in the offline processing of the first one by using common plasticity resources or by diverting the protein synthesis machinery that were aimed for the ongoing consolidation of the CTX1 trial. If that was the case then, which brain structures are involved in LTM formation of object-in-context task?

Because of the main role of the Hp in the processing of spatial and contextual information (Mumby et al., 2002; Balderas et al., 2008; Komorowski et al., 2009; Barker and Warburton, 2011), we reasoned that the LTM formation for this type of association between objects and contexts could be dependent on the activity of that region. As expected, the infusion of muscimol into the dorsal CA1 Hp impaired the expression of LTM for Obj2-in-CTX2 (Fig. 4a CTX2 test). Our data is also in agreement with a previous work in which the infusion of anisomycin into the Hp immediately after training blocked LTM but not STM of an object recognition task (Balderas et al., 2008). Moreover, the impairment in the consolidation of the second trial left intact the LTM expression for Obj1-in-CTX1, being Obj1 explored less than Obj2 because is not incongruent for the subject (Fig. 4a CTX1 test). This is why we consider that there could be a competition between the processes triggered by both sample trials within a critical time window. The role of the mPFC in the acquisition and STM formation of recognition memory is well characterized (Barker et al., 2007; Barker and Warburton, 2013). However, its role in the consolidation of this task has not been explored so far. Our experiments show that the muscimol inactivation of the mPFC before the acquisition of an object-in-context task impaired the expression of its LTM (Fig. 4b CTX2 test) leaving intact the LTM expression for the first sample pair (Fig. 4b CTX1 test). These results suggest that the object-in-context memory acquisition and consolidation require the activation of mPFC. Besides, the coincident processing of two different trials in mPFC could result in competition of the traces, leading to the expression of RI. In accordance with our work, it has recently found that the inactivation of mPFC before acquisition of a list association between odors and contexts, prevents the interference observed on the subsequent learning of conflicting information during the presentation of a second list (Peters et al., 2013). This pharmacological intervention blocked the formation of the memory of the first list, and reducing the proactive interference probably by preventing competition between the traces.

Remarkably, the blockade of the perirhinal cortex, insular cortex, or amygdala did not affect object-in-context memory (Balderas et al., 2008). In contrast, our results suggest that the dorsal Hp and the mPFC are critical for its LTM formation. Furthermore, recent findings from Bekinschtein et al. (2013) show that the blockade of R 5HT2A in the mPFC or the dorsal Hp impaired the recognition of the incongruent object in a short-term testing protocol. Those results prove that serotoninergic signaling in the mPFC is coupled to the hippocampal activity during retrieval of the object-in-context task. Even though in this work we did not explore the involvement of serotoninergic transmission in the formation of LTM for this task, the pharmacological inactivation of this region with muscimol lets us hypothesize that the interconnection between these regions is also required to consolidate the LTM for the object-in-context task.

There is increasing evidence for the involvement of overlapping networks of brain structures for different aspects of both spatial and recognition memory (Warburton and Brown, 2010; Banks et al., 2012; Barbosa et al., 2012; Barker and Warburton, 2013; Rossato et al., 2013). Our hypothesis is that the processes underlying acquisition, consolidation, and retrieval of object-in-context memories could compete inside these networks, resulting in a visible interference. In that sense, it was reported that neurons that responded to one event had elevated levels of CREB1 for a short period of time, making them more likely to be recruited by another event occurring in this time window (Zhou et al., 2009). Also by combining genetic and IEG techniques in rats exposed to two different environments, an activation of largely distinct cell populations in the dentate gyrus was observed, whereas there was a partial overlapping in CA1 (Deng et al., 2013). Thus, a plausible cellular competition mechanism in the CA1 dorsal Hp and in the mPFC could explain the molecular bases of the RI in the object-in-context LTM processing (Fig. 4). Nevertheless, the second trial did not interfere with the LTM consolidation for the object recognition memory (Fig. 3). A possible explanation for this is the link between hippocampal spatial representations

and extra-Hp memory components which could reinforce the identity of objects related to different contexts without resulting in RI (Hardt et al., 2013). Our results suggest that the activity of the CA1 dorsal Hp and mPFC is necessary to selectively assign and consolidate an explored object to the context. Similarly, it was observed that the mPFC blockade induced long-term generalization for contextual fear task (Xu and Südhof, 2013). It was observed that neurons in the anterior cingulate cortex actively responded when mice explored those places where objects were expected to be found (Weible et al., 2012).

Apart from the role of mPFC in memory recognition tasks, we cannot rule out the involvement of other cortices. It was demonstrated that rats with lesions of lateral entorhinal cortex were unable to recognize object-context associations yet showed normal object recognition and normal context recognition (Wilson et al., 2013a,b). If objects and contexts are considered as a part of an overall contextual environment, it could be thought that both the lateral entorhinal and the mPFC bind objects with their contexts to form a representation of a new contextualized environment. Besides, it has been recently shown that perirhinal cortex also contributes to the LTM formation of recognition memory (Seoane et al., 2012; Tinsley et al., 2012; Balderas et al., 2013) and its participation in object recognition is well documented (Winters et al., 2008, 2011; Albasser et al., 2011).

The main conclusion from the present experiments is that when two different object-in-context memory traces are being processed, the second trace interferes with the consolidation of the first one in a critical time window. We demonstrated that the mPFC and the CA1 dorsal Hp are involved in the consolidation of this behavioral task. The observed LTM-RI amnesia is probably caused by resources or cellular machinery competition in these brain regions when they are engaged in memory formation of the traces.

REFERENCES

Albasser MM, Amin E, Iordanova MD, Brown MW, Pearce JM, Aggleton JP. 2011. Perirhinal cortex lesions uncover subsidiary systems in the rat for the detection of novel and familiar objects. Eur J Neurosci 34:331–342.

Balderas I, Rodriguez-Ortiz CJ, Salgado-Tonda P, Chavez-Hurtado J, McGaugh JL, Bermudez-Rattoni F. 2008. The consolidation of object and context recognition memory involve different regions of the temporal lobe. Learn Mem 15:618–624.

Balderas I, Moreno-Castilla P, Bermudez-Rattoni F. 2013. Dopamine D1 receptor activity modulates object recognition memory consolidation in the perirhinal cortex but not in the hippocampus. Hippocampus 23:873–887.

Banks PJ, Bashir ZI, Brown MW. 2012. Recognition memory and synaptic plasticity in the perirhinal and prefrontal cortices. Hippocampus 22:2012–2031.

Barbosa FF, Pontes IMDO, Ribeiro S, Ribeiro AM, Silva RH. 2012. Differential roles of the dorsal hippocampal regions in the acquisition of spatial and temporal aspects of episodic-like memory. Behav Brain Res 232:269–277.

Barker GRI, Bird F, Alexander V, Warburton EC. 2007. Recognition memory for objects, place, and temporal order: A disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. J Neurosci 27:2948–2957.

Barker GRI, Warburton EC. 2008. NMDA receptor plasticity in the perirhinal and prefrontal cortices is crucial for the acquisition of long-term object-in-place associative memory. J Neurosci 28:2837–2844.

Barker GRI, Warburton EC. 2011. When is the hippocampus involved in recognition memory? J Neurosci 31:10721–10731.

Barker GRI, Warburton EC. 2013. Object-in-place associative recognition memory depends on glutamate receptor neurotransmission within two defined hippocampal-cortical circuits: A critical role for AMPA and NMDA receptors in the hippocampus, perirhinal, and prefrontal cortices. Cereb Cortex. [Epub ahead of print].

Bekinschtein P, Renner MC, Gonzalez MC, Weisstaub N. 2013. Role of medial prefrontal cortex serotonin 2A receptors in the control of retrieval of recognition memory in rats. J Neurosci 33:15716–15725.

Burton BG, Hok V, Save E, Poucet B. 2009. Lesion of the ventral and intermediate hippocampus abolishes anticipatory activity in the medial prefrontal cortex of the rat. Behav Brain Res 199:222–234.

Churchwell JC, Morris AM, Musso ND, Kesner RP. 2010. Prefrontal and hippocampal contributions to encoding and retrieval of spatial memory. Neurobiol Learn Mem 93:415–421.

Clark RE, Martin SJ. 2005. Interrogating rodents regarding their object and spatial memory. Curr Opin Neurobiol 15:593–598.

Colgin L. 2011. Oscillations and hippocampal–prefrontal synchrony. Curr Opin Neurobiol 21:467–474.

Deng W, Mayford M, Gage FH. 2013. Selection of distinct populations of dentate granule cells in response to inputs as a mechanism for pattern separation in mice. Elife 2:e00312.

Devito LM, Eichenbaum H. 2011. Memory for the order of events in specific sequences: Contributions of the hippocampus and medial prefrontal cortex. J Neurosci 31:3169–3175.

Dix SL, Aggleton JP. 1999. Extending the spontaneous preference test of recognition: Evidence of object-location and object-context recognition. Behav Brain Res 99:191–200.

Gonzalez C, Kramar C, Garagoli F, Rossato JI, Weisstaub N, Cammarota M, Medina JH. 2013. Medial prefrontal cortex is a crucial node of a rapid learning system that retrieves recent and remote memories. Neurobiol Learn Mem 103:19–25.

Good MA, Barnes P, Staal V, McGregor A, Honey RC. 2007. Context- but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats. Behav Neurosci 121:218–223.

Hardt O, Nader K, Nadel L. 2013. Decay happens: The role of active forgetting in memory. Trends Cogn Sci 17:111–120.

Kim J, Delcasso S, Lee I. 2011. Neural correlates of object-in-place learning in hippocampus and prefrontal cortex. J Neurosci 31: 16991–17006.

Komorowski RW, Manns JR, Eichenbaum H. 2009. Robust conjunctive item-place coding by hippocampal neurons parallels learning what happens where. J Neurosci 29:9918–9929.

Martínez MC, Alen N, Ballarini F, Moncada D, Viola H. 2012. Memory traces compete under regimes of limited Arc protein synthesis: Implications for memory interference. Neurobiol Learn Mem 98:165–173.

Müller GE, Pilzecker A. 1900. Experimentelle Beiträge zur Lehre vom Gedächtniss. Z Psychol 1:1–300.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H. 2002. Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. Learn Mem 9:49–57.

Navawongse R, Eichenbaum H. 2013. Distinct pathways for rule-based retrieval and spatial mapping of memory representations in hippocampal neurons. J Neurosci 33:1002–1013.

Paxinos G, Watson C. 2007. The Rat Brain in Stereotaxic Coordinates, 6th ed. Academic Press, San Diego.

Peters GJ, David CN, Marcus MD, Smith DM. 2013. The medial prefrontal cortex is critical for memory retrieval and resolving interference. Learn Mem 20:201–209.

Rossato JI, Radiske A, Kohler CA, Gonzalez C, Bevilaqua LR, Medina JH, Cammarota M. 2013. Consolidation of object recognition memory requires simultaneous activation of dopamine D1/D5 receptors in the amygdala and medial prefrontal cortex but not in the hippocampus. Neurobiol Learn Mem 106:66–70.

Seoane A, Tinsley CJ, Brown MW. 2012. Interfering with Fos expression in rat perirhinal cortex impairs recognition memory. Hippocampus 22:2101–2113.

Tinsley CJ, Narduzzo KE, Brown MW, Warburton EC. 2012. A role for the CAMKK pathway in visual object recognition memory. Hippocampus 22:466–476.

Warburton EC, Brown MW. 2010. Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. Neuropsychologia 48:2262–2272.

Weible AP, Rowland DC, Monaghan CK, Wolfgang NT, Kentros CG. 2012. Neural correlates of long-term object memory in the mouse anterior cingulate cortex. J Neurosci 32:5598–5608.

Wilson DIG, Langston RF, Schlesiger MI, Wagner M, Watanabe S, Ainge JA. 2013a. Lateral entorhinal cortex is critical for novel object-context recognition. Hippocampus 23:352–366.

Wilson DIG, Watanabe S, Milner H, Ainge JA. 2013b. Lateral entorhinal cortex is necessary for associative but not nonassociative recognition memory. Hippocampus 23:1280–1290.

Winters BD, Saksida LM, Bussey TJ. 2008. Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. Neurosci Biobehav Rev 32:1055–1070.

Winters BD, Tucci MC, Jacklin DL, Reid JM, Newsome J. 2011. On the dynamic nature of the engram: evidence for circuit-level reorganization of object memory traces following reactivation. J Neurosci 31:17719–17728.

Wixted JT. 2004. On common ground: Jost's (1897) law of forgetting and Ribot's (1881) law of retrograde amnesia. Psychol Rev 111: 864–879.

Xu W, Südhof TC. 2013. A neural circuit for memory specificity and generalization. Science 339:1290–1295.

Zhou Y, Won J, Karlsson MG, Zhou M, Rogerson T, Balaji J, Neve R, Poirazi P, Silva AJ. 2009. CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nat Neurosci 12:1438–1443.