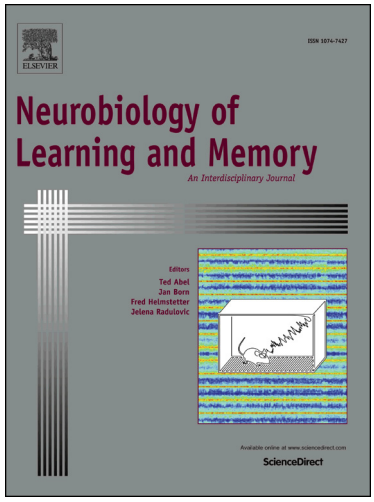

Memory consolidation and expression of object recognition are susceptible to retroactive interference

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Memory consolidation and expression of object recognition are susceptible to retroactive interference.

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Abstract:

With the aim of analyzing if object recognition long-term memory (OR-LTM) formation is susceptible to retroactive interference (RI), we submitted rats to sequential sample sessions using the same arena but changing the identity of a pair of objects placed in it. Separate groups of animals were tested in the arena in order to evaluate the LTM for these objects. Our results suggest that OR-LTM formation was retroactively interfered within a critical time window by the exploration of a new, but not familiar, object. This RI acted on the consolidation of the object explored in the first sample session because its OR-STM measured 3h after training was not affected, whereas the OR-LTM measured at 24h was impaired. This sample session also impaired the expression of OR memory when it took place before the test. Moreover, local inactivation of the dorsal Hippocampus (Hp) or the medial Prefrontal Cortex (mPFC) previous to the exploration of the second pair of objects impaired their consolidation restoring the LTM for the objects explored in the first session. This data suggests that both brain regions are involved in the processing of OR-memory and also that if those regions are engaged in another process before finishing the first consolidation process its LTM will be impaired by RI.

Keywords: Object recognition task; retroactive interference; long-term memory; dorsal hippocampus; medial prefrontal cortex; rat.

1. Introduction:

Recognition is the ability to distinguish the occurrence of a stimulus that was previously presented from one that was not (Squire, Wixted, & Clark, 2007). Animals can form recognition memories about the identity of individual objects and also about their location or recency (Barker, Bird, Alexander, & Warburton, 2007). In this work, we focus on the object recognition (OR) memory, which his based on the animal´s ability to discriminate a new object from an old one when they are presented in a familiar arena. This task is used to investigate the "what" aspect of episodic-like memories that also include the recall of information about "where and when" aspects of an event (Dere, Huston & De Souza Silva, 2005; Ergorul & Eichenbaum, 2004; Tulving, 2002).

A single exploration session episode in an OR task leaves a lasting complex memory trace. As a general mechanism of memories' formation, after the acquisition of information, the storage of a long-term memory (LTM) trace goes through a consolidation phase. This represents a labile period susceptible to disruption which probably accounts for an adaptive function, enabling those endogenous processes activated by an experience to modulate the strength of the memory (McGaugh, 2000). Quite recently this unstable period of consolidation was suggested to give new memories an opportunity to interact and communicate with others. In that sense, it was shown a correlation between the susceptibility to interference of a memory and learning transfer to the another memory task (Mosha & Robertson, 2015). Regarding this, there are many studies on the effect of Retroactive interference (RI) ,a type of amnesia characterized by the disruptive effect of a new learning experience over previously encoded material (Wixted, 2004). The objective of the present work is to investigate if OR -LTM formation is susceptible to RI within the consolidation

window. Besides we will determine what kind of events are able to interfere with the OR memory and which are the brain regions taking part in this process.

We have recently shown that object-in-context LTM formation is very sensitive to RI elicited by the exploration of a different context (novel or familiar) with different objects (novel or familiar) placed in it. This interference occurs in a restricted temporal window and works on the LTM consolidation phase, leaving intact the short-term memory (STM) expression (Martínez, Villar, Ballarini, & Viola, 2014). However, the memory for the object presented in the first trial is insensitive to the RI elicited by a different object when it is presented in a different context (Martínez et al., 2014). In other words, animals can remember the object but not the context in which it was explored during the training session. Thus, is it the OR memory immune to RI or is it necessary to increase the complexity of the task in order to observe interference on the rat's ability to remember the identity of the object? In order to resolve this issue, here we submitted rats to sequential object exploration sessions in an arena, changing the identity of the objects placed in it.

As it was previously mentioned, the formation of recognition memory includes several features to be encoded: a particular object or person ("what"), the context where the experience took place -which can be the arena itself or a location within the arena ("where")- and the particular time in which the event occurred ("when"). Moreover, recognition memory is widely viewed as consisting of two components: *recollection*, regarding to remembering specific details including the context and/or the particular time in which the experience took place, and *familiarity* which involves simply knowing that an item was presented (Squire et al., 2007; Yonelinas, 2002). There is an ongoing debate about the anatomical substrate of recognition memory. It was proposed that these components are relayed in different brain regions (Brown & Aggleton, 2001;

Warburton & Brown, 2015; Winters, 2004), being recollection dependent on the hippocampus and familiarity on the adjacent perirhinal cortex. However, an alternative perspective suggests that these structures work in a cooperative and complementary way and they are both involved in such components, what could be interpreted in terms of strong and weak memories (Clark, 2013; Cohen & Stackman, 2015; Squire et al., 2007). Related to this, the contribution of the perirhinal cortex in OR memory has been well demonstrated (Barker, Bashir, Brown, & Warburton, 2006; Ho et al., 2015; Mendez, Arias, Uceda, & Arias, 2015; Winters and Bussey, 2005); however, the involvement of the hippocampal (Hp) region remains controversial (Barker & Warburton, 2011; Broadbent, Squire, & Clark, 2004; Cohen et al., 2013; Kim, Kim, Lee, Park, & Ryu, 2014; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Rossato et al., 2007; Rossato et al., 2015; Vnek & Rothblat, 1996; Winters, 2004; Zalcman, Federman, de la Fuente, & Romano, 2015). Thus, we explored the participation of the dorsal hippocampus and the medial prefrontal cortex (mPFC), another region associated to recognition memory (Barbosa et al., 2013; Morici, Bekinschtein & Weisstaub, 2015; Pezze, Marshall, Fone, & Cassaday, 2015), in the formation of LTM for the "what" aspect of this memory.

In sum, our results suggest that OR-LTM formation was retroactively interfered only when a new (but not familiar) object was explored in the same arena within a critical time window related to the consolidation of this memory trace. This type of interfering session also impaired the expression of the OR memory when it occurred before the test session. Moreover, our data suggests that the dorsal Hp and the mPFC are both involved in the processing of OR memory formation, and that if these brain regions are committed in another process before finishing the consolidation of the former, this OR-LTM will be impaired.

2. Materials and methods:

2.1. Subjects: Male adult Wistar rats weighing 180-250 g were housed in groups of 5-6 per cage, maintained under a 12-h light/ 12-h dark cycle (21° C) with food and water *ad libitum*. They were handled for three min for three consecutive days to reduce 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.

2.2. 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 & Watson, 2007, see Fig. 5). 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 and antibiotics at the moment of the surgery (Meloxicam 0.2mg/Kg, gentamicin 3mg/Kg) and were allowed to recover from surgery for four 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 minutes 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 cannulae 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 (0.1µg of muscimol in 0.5µl saline solution per side) was reported to be effective (Gonzalez et al., 2013).

2.3. Behavioral training:

Habituation:

Initial habituation sessions were carried out to familiarize the rats with the apparatus in which training would take place (context). Habituation consisted of one daily session of 12 min in the arena to be used throughout the experimental protocol. Unless indicated to the contrary, all subjects were habituated in two consecutive days to the arena without objects.

Object Recognition (OR) task:

OR consists of a sample session in which a pair of objects is presented, delay and a test phase where rats explore this object and a novel one. In this paradigm, rats' spontaneous preference for novelty is used to calculate an index of the memory for the object explored in the sample session (Clark & Martin, 2005). As rodents present this innate preference by novel objects, OR task does not require explicit rule learning and also does not require extensive pre-training. They readily approach novel objects and explore them with their vibrissae, nose and forepaws. The percentage of time used to explore these novel objects serves as a measure of recognition memory for the familiar object.

In order to study if OR memory is susceptible to RI, we trained rats with two sample trials each of them taking place in the same context. The context was a rectangular apparatus of dimensions 60 cm width x 40 cm depth x 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. In the

training session, the subject was introduced for 5 min in the context in presence of a pair of identical objects (Obj 1). 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. The mean ± SEM of the total exploration time during the first sample session was 68.71±4.29 sec. One, three, four or seven hours after the first sample session had concluded the subject was reintroduced for 5 min in the context in presence of a novel pair of identical objects (Obj 2). The exploratory activity of the subject was registered in the same way as in the first sample trial. The objects defined as Obj 1 and Obj 2 was balanced along the experiments.

Twenty four hours (for LTM experiments) or three and a half hours (for STM experiments) after training half of the subjects were tested by reintroducing them individually in the context for 3 min in presence of Obj 1 and a novel object, and the other half in the presence of Obj 2 and a novel object. In the test session, animals expressed memory for object recognition if they spent more time exploring the novel object instead of the object that was presented during the training. The mean ± SEM of the total exploration time during the LTM test session after having experienced a single sample session training was 37.84±2.14 sec. Animals that in the test session had a total exploration time lower than 10 sec were exclude of the analysis. Exploration was defined as sniffing or touching the object with the nose or forepaws. The time of exploration for each object was recorded and it was expressed as a percentage of the total exploration time for both objects (Preference %).

OR-memory expression:

In order to study if OR memory expression was affected by another sample trial, the second sample session was given thirty minutes before the test (Fig 3). Test session was performed ninety minutes (for study OR-STM) or twenty-four (for OR-LTM) after training.

Different Context:

When the second sample session was carried out in different context a circular shape apparatus of 50 cm diameter x 39 cm height, with black plywood floor was used. The animals were habituated to it for different familiar context-experiments but not for the novel context-experiments of Fig 4.

Empty context trial:

Subjects were introduced in an empty arena to which they had been previously habituated or not.

Familiar object:

When it was required a previous familiarization to the objects, animals were individually placed in a home cage for 12 min in presence of a pair of identical objects. This procedure was repeated for two consecutive days previous to training.

2.4. Data analysis: Statistical analysis of behavioral data was performed with paired Student's t test by comparing the percentage of time exploration to novel versus to familiar object presented in the test session; using Graph Pad Prism® software.

3. Results:

The first objective of this work was to study if OR-LTM formation was susceptible to RI. Therefore, rats were trained with two sample trials occurring in the same context, separated by different inter-trial intervals (ITI). Each time, animals explored a different pair of identical objects. LTM for the objects present in the first (Obj 1) and in the second (Obj 2) trial was tested 24 h after training using separate groups of animals that underwent the same training. For the test session, also performed in the same arena used for training, animals explored one object that had been presented in the training and another that had never been presented before (Novel). **Fig 1A** shows that the control group of rats trained with a single trial, showed exploratory preference for the novel object ($t_{(7)}$= 3.37, P= 0.012). However, when animals received a second sample trial 1h or 3 h after the first one, both objects were explored equally reflecting that Obj 1-LTM was impaired (1h ($t_{(23)}$= 0.18, P= 0.856; 3h $t_{(11)}$= 1.63, P= 0.132). However, the RI effect of the second sample session over the first one was absent when the ITI was of 4h or 7h (4h $t_{(19)}$= 2.49, P= 0.022; 7h $t_{(17)}$= 5.82, P< 0.001), observing a higher exploration for the novel object than for Obj 1 which had been previously explored. **Fig 1B** shows that LTM for the Obj 2 was present at all the ITIs studied here (Ctrl $t_{(10)}$=4.17 , P=0.002 ; 1h $t_{(21)}$=2.30, P= 0.032; 3h $t_{(12)}$= 2.83, P= 0.015; 4h $t_{(20)}$= 3.05, P= 0.006; 7h $t_{(13)}$= 2.52, P= 0.025), suggesting that the first sample did not cause anterograde interference of the OR-LTM over the second sample trace.

Next, we decided to study if the RI, visible 24 h after training, was related to impairments in the short-term memory (STM) formation or to the consolidation of Obj1 memory. **Fig 2A** shows that STM for Obj 1 was observed at 270 min in the control group of rats submitted to a single trial protocol ($t_{(9)}$=2.29, p=0.047) as well as in the experimental group of animals (Exp) trained with the two-sample protocol ($t_{(8)}$=2.85, p=0.022). As expected, OR-STM for Obj 2 was also observed (**Fig 2B**; Ctrl $t_{(8)}$=3.10, p=0.015; Exp $t_{(8)}$=3.72, p=0.006).

Moreover, we wanted to know if another sample session could impair the expression of the memory for the objects. In these series of experiments the second trial was performed 30 min previous to the test. **Fig 3A** shows that both experimental groups of animals aimed to measure STM expression (Exp STM, $t_{(14)}$=0.07, p=0.945) and LTM expression (Exp LTM, $t_{(9)}$=0.43, p=0.680) failed to express the memory for Obj 1. Parallel control groups of rats trained without a second sample session expressed OR-STM (Ctrl STM, $t_{(9)}$=2.49, p=0.034) and OR-LTM (Ctrl LTM, $t_{(13)}$=2.26, p=0.041) when tested. These results suggest that the presence of a second sample trial close to the test session impairs the expression of both types of OR memory. Furthermore, **Fig 3B** shows that the first sample session had also a negative effect on the expression of the Obj2-STM when Obj 1 was explored close to the test (Exp -1h STM, $t_{(15)}$=1.17, p=0.258). However, if Obj1 was explored one day before the second sample session took place, the expression of Obj2-STM was not impaired (Exp -24h STM, $t_{(10)}$=4.55, p=0.001), resulting in an Obj 2-STM similar to that of the control group of rats (Ctrl STM, $t_{(12)}$= 3,23, p= 0.007). Taken as a whole these results suggest that the expression of OR-STM and OR-LTM is affected by the exposure to another object, provided that this occurs at least up to 90 min before the test.

Next, we decided to focus on the RI effect on the formation of OR-LTM described in Fig 1A, studying which features of the second sample session are important to exert the detrimental effect on the consolidation of Obj 1-LTM. With that aim, animals explored an Obj 1 pair in a given context and 1 h after, they were reintroduced in the same context, this time without objects (Empty) or containing novel objects (Novel) or a different pair of objects to which animals had been previously familiarized (Fam). The following day Obj 1-LTM was measured, testing the animals in the same context in the presence of Obj1 plus another object that had never seen before. **Fig 4A** shows that the exposure to the same empty context ($t_{(9)}$=3.15, p=0.012) or to the familiar objects ($t_{(15)}$=3.64, p=0.002) did not impair the consolidation of Obj1-LTM. As it was shown

in the Fig 1A, the presence of novel objects in the same context impaired the consolidation of the memory for Obj 1 identity ($t_{(12)}$=0.41, p=0.691). However, if the second sample took place in a novel context, irrespective of it being empty ($t_{(6)}$=4.87, p=0.003) or with novel ($t_{(11)}$=2.20, p=0.05) or familiar objects ($t_{(9)}$=6.46, p<0.001)in it, in all these cases Obj 1-LTM was preserved and RI was not observed (**Fig 4B**). Similar results were obtained if the second sample session occurred in a context different to the one used in the first trial but to which subjects had been previously familiarized (Empty: $t_{(6)}$=4.14, p=0.006; Novel: $t_{(8)}$=6.95, p<0.001; Fam: $t_{(17)}$=3.45, p=0.003) (**Fig 4C**) This results suggest that the OR-LTM is robust and is only interfered by the presence of novel objects in the same context.

Regarding the brain structures involved in the processing OR-LTM, we studied the role of the dorsal Hp and the mPFC, which had been found to be involved in different object recognition paradigms. Animals were infused with muscimol (Mus) 15 min before the second sample session, to exert a reversible inhibition of these areas, and OR-LTM was tested at the following day. **Fig 5A left panel** confirms the RI effect for Obj1-LTM in rats that were infused with vehicle solution (Veh) into CA1 dorsal Hp ($t_{(14)}$=0.86, p=0.405). Conversely, in the Mus group of animals the Obj1-LTM was recovered ($t_{(12)}$=4.06, p=0.002). In parallel, we evaluated Obj2-LTM and **Fig 5A right panel** shows an intact memory for the Veh group ($t_{(13)}$=6.81, p<0.001) in contrast with a loss of memory in the Mus group of animals ($t_{(13)}$=1.35, p=0.200). Likewise, Mus infusion into mPFC impaired the Obj2-LTM (**Fig 5B right panel**, Veh $t_{(5)}$=4.18, p=0.009; Mus $t_{(7)}$=0.390, p=0.708) enabling the consolidation for the Obj1-LTM (**Fig 5B left panel**, Veh $t_{(6)}$=0.03, p=0.974; Mus $t_{(7)}$=2.84, p=0.025). These results suggest that the Hp and the mPFC are both involved in the processing of the OR-LTM.

4. Discussion:

In the present work, we describe the temporal course of RI for OR-LTM (Fig 1A). Our results obtained after a comprehensive analysis suggest that only the presence of novel objects in the same training arena can induce RI for OR-LTM. When familiar objects were presented in the second sample session, they did not affect the process of consolidation of the object explored in the first session, probably because their OR-LTM must have been consolidated in the familiarization procedure.

We have recently published that RI for OR-LTM was not observed when different arenas were matched to different objects in the sample sessions (Martínez et al., 2014). Here, we add that if the interposed session occurs in a different arena, regardless whether it was novel or familiar to the rats and even if it did not contain objects or included novel or familiar ones, RI is not observed. We believe that the difference in the contexts between these two sessions contributes to the formation of the memory for the object's identity for each session. In agreement to this, Hardt et al. (2013) hypothesized that, when the neocortex is involved, one way to minimize confusion between similar memories is by linking the contents of a specific episodic memory to its unique spatio-temporal context. Then, it is possible that when rats explore different objects within the same context, memory formation for them gets in conflict. Why does the presence of a different arena prevent the development of RI for OR-LTM? It has been reported that two exploration sessions in different contexts induce the activation of diverging neuronal populations in the hippocampus, while repeating the exposure to the same context twice induces the activation of approximately the same neuronal population (Guzowski, McNaughton, Barnes, & Worley, 1999). So, when a new pair of objects is presented in the same context in the second sample trial, occurring up to 3 hours after the first session, a competition for the consolidation of both memory traces relative to the objects could emerge. We believe that this is because the

same hippocampal neuron population is required to form those memories, resulting in RI for Obj 1-LTM, as shown in Fig 1A.

Besides describing the process of RI for OR-LTM formation, we also show that both OR-STM and OR-LTM expression were impaired when a different sample session was experienced up to 90 min before the test (Fig 3A and B). We believe that the neural process associated to the interfering session involves the activation of different brain regions and the use of several resources also required for the expression of the memory related to the object. Thus, when these are not available at the moment of the retrieval, the memory trace expression is impaired. For instance, the hippocampus could be one of the brain structures involved in this phenomenon as has been shown to be involved in the consolidation and the retrieval of OR memory (Cohen et al., 2013; Rossato et al., 2007), matching our hypothesis.

Our results suggest that the dorsal Hp activation is required for the formation of the OR-LTM tested 24h after training (Fig 5A). In agreement, a muscimol or lidocaine infusion into the dorsal Hp of rodents at different stages of an OR task revealed a clear role of this brain structure in a non-spatial object memory formation (Cohen et al., 2013; Hammond, 2004; de Lima, Luft, Roesler, & Schröder, 2006). However, there are other reports showing no Hp participation or even contrasting results to those mentioned above (Kim et al., 2014; Oliveira, Hawk, Abel, & Havekes, 2010). Nevertheless, the specific knockdown of transcription factors or treatments with protein synthesis inhibitors into the dorsal Hp during a limited post-training time window resulted in impairments in the OR-LTM (Rossato et al, 2007; Zalcman et al., 2015). Also, extensive Hp lesions happening several days after the sample session impair the expression of the OR-LTM (Gaskin, Tremblay, & Mumby, 2003; Mumby, Piterkin, Lecluse, & Lehmann, 2005; O'Brien, Lehmann,

Lecluse, & Mumby, 2006). Taken as a whole, these findings illustrate the requirement of the dorsal Hp for the consolidation, expression and reactivation of OR-LTM.

Our results also suggest that the mPFC activity is required in order to form OR-LTM (Fig 5B). In agreement, it has been recently published that the pre-training local administration of a dopaminergic D1 receptor agonist into the prelimbic subregion impaired the OR-LTM in rats (Pezze et al., 2015). In addition, Rossato and colleagues (2013) demonstrated that the simultaneous activation of these receptors in the mPFC and the amygdala is required to consolidate the OR-LTM. Besides, the infusion of a protein synthesis inhibitor into the ventral prefrontal cortex disrupted the OR-LTM without altering the OR-STM (Akirav, Raizel, & Maroun, 2006). These results add up to others showing the role of the mPFC in object-in-context, object-in-place and temporal order object recognition memory (Morici et al., 2015; Warburton & Brown, 2015), highlighting the involvement of this brain region in object recognition tasks.

We have shown that the dorsal Hp and the mPFC are involved in the formation of the OR-LTM and also in the formation of the object in context- LTM (Martinez et al 2014). Moreover, we demonstrated that when the CA1 dorsal Hp or the mPFC were locally inactivated by using muscimol before the second training session, Obj 2-LTM was absent the following day whereas Obj 1-LTM was intact (Fig 5). These results are in accordance with the idea that both regions are engaged in OR-LTM formation and, if an ongoing processing is happening in these regions, a further activity requirement from them could impair the consolidation of the trace. However, are these structures interacting or acting independently? So far this question has not been solved, but considering the recent discovery of a monosynaptic projection from the prefrontal cortex to the hippocampus in mice, it is likely that these regions are engaged in the same circuit. Therefore, it is plausible to think that the mPFC could exert a top down control over the Hp in the processing of the OR memory as it occurs in the case of a contextual fear memory (Rajasethupathy et al., 2015).

In sum, our results suggest that the OR-LTM formation was retroactively interfered by a second training session only when a new (but not familiar) object was explored in the same arena within a critical time window. If the inter-session interval was extended to 4 or 7 h, Obj 1 and Obj 2-LTMs were both formed. We believe this probably occurred because Obj 1 consolidation was completed before the second sample trial and, in consequence, the RI was not developed. Interference was also observed on OR-memory expression, but not on OR-STM formation. Moreover, RI phenomenon on the OR-LTM formation was prevented by impairing the consolidation of the interfering sample session through the inactivation of the Hp or the mPFC. We suggest that these results could be explained by a competition mechanism occurring in the same neural circuits required for the formation and the expression of the OR memory of the objects. However, what mechanism could account for such effect? A wide series of reports strongly suggest that LTM formation depends on the synthesis of plasticity-related proteins (PRPs) which will be used at specific substrates (tagged sites induced by learning experiences) in order to establish the memory trace (for revision see Moncada, Ballarini, & Viola, 2015). Therefore, we believe that a plausible molecular event in the Hp and the mPFC underpinning the RI observed on the OR-LTM could be the competition for the PRPs induced by the LTM formation for Obj 1 and Obj 2. In that sense, we have previously reported that competition for activity-regulated cytoskeletal-associated protein (Arc), a protein required to consolidate two Hp-dependent memory tasks, caused interference between traces and resulted in RI (Martínez, Alen, Ballarini, Moncada, & Viola, 2012). The same rationale could be applied to the interference on OR memory expression shown here, taking into account recent results suggesting that the retrieval of memory also depends on protein synthesis (Lopez, Gamache, Schneider, & Nader, 2015). Besides, the fact that STM formation was not vulnerable to RI is in agreement to this postulated mechanism, as it has been widely postulated that the STM is independent of protein synthesis

(Davis & Squire, 1984; Gould et al., 2014; Igaz, Vianna, Medina, & Izquierdo, 2002; Motanis & Maroun, 2012), in consequence, it would not be affected by the competition for PRPs .

In conclusion, our results suggest that the OR memory consolidation and expression are both susceptible to be interfered by the exploration of a new object present in the same context and that the dorsal Hp and mPFC are two brain regions involved in this phenomenon.

Legends to figures:

Fig 1: OR- LTM formation is retroactively interfered by a novel object explored in the same arena within a critical time window.

Schematic representation of the experimental protocol is presented on top of each panel. Preference during test session is expressed as Mean ± SEM. The plain bar corresponds to the familiar object. The dashed line represents the chance level of performance (i.e., a 50% preference corresponds to no discrimination between familiar and novel objects).

Control subjects were trained once and tested 24 h after. Subjects in the experimental groups performed two sample sessions in the same context separated by inter-trial intervals of 1 h, 3 h, 4 h or 7 h, and 24 h later were tested for the object presented in only one of the sample session against a novel object. **(A)** Long-term memory for the object presented in the first sample session (Obj 1). **(B)** Long-term memory for the object presented in the second sample session (Obj 2). *** $p<0.001$, ** $p<0.01$ and * $p<0.05$ vs. familiar object, paired t test.

Fig 2: OR- STM formation is not affected by another exploration session of a novel object in the same arena.

Schematic representation of the experimental protocol is presented on top of each panel. Preference during test session is expressed as Mean ± SEM. The plain bar corresponds to the familiar object. The dashed line represents the chance level of performance (i.e., a 50% preference corresponds to no discrimination between familiar and novel objects).

Short-term memory for the object presented in the first sample trial is not interfered by the second sample session. Separate groups of Control subjects were trained with only one sample session and tested in the same arena 4h30 or 3h30 after training. Subjects in the experimental groups performed two sample sessions and 3h30 after training were tested for Obj 1 or Obj 2. **(A)** Short-term memory for the object presented in the first sample session (Obj 1). **(B)** Short-term memory for the object presented in the second session (Obj 2). ** $p<0.01$ and * $p<0.05$ vs. familiar object, paired t test.

Fig 3: OR-memory expression is impaired by a novel object explored in the same arena.

Schematic representation of the experimental protocol is presented on top of each panel. Preference during test session is expressed as Mean ± SEM. The plain bar corresponds to the familiar object. The dashed line represents the chance level of performance (i.e., a 50% preference corresponds to no discrimination between familiar and novel objects).

Control subjects were trained with only one sample session and were tested 30 min, 90 min or 24 h after it. Subjects in the experimental groups performed two sample sessions (Obj 1 first and Obj 2 second) being the second one 30 min before the test session. Short-term memory (30min post training) and Long-term memory (24hs post training) expression were measured. **(A)** Short-term and long term memory expression for the object presented in the first sample session (Obj 1). **(B)**

Short-term memory expression for the object presented in the second sample session (Obj 2). ** p<0.01 and * p<0.05 vs. familiar object, paired t test

Fig 4: Retroactive interference over OR- LTM formation requires exploring novel, but not familiar objects, in the same arena.

Schematic representation of the experimental protocol is presented on top of each panel. Preference during test session is expressed as Mean ± SEM. The plain bar corresponds to the familiar object. The dashed line represents the chance level of performance (i.e., a 50% preference corresponds to no discrimination between familiar and novel objects).

Experimental subjects performed one sample session and 1 h after they performed a second sample session, **(A)** in the same (=) Context that could be Empty or with Novel or Familiar pair of objects; **(B)** in a different (≠) Novel Context that could be Empty or with Novel or Familiar pair of objects or **(C)** in a different (≠) but Familiar Context that could be Empty or with Novel or Familiar pair of objects. All groups were tested for Obj1 24 h after training using the first sample context. *** p<0.001, ** p<0.01 and * p<0.05 vs. familiar object, paired t test.

Fig5: Local inactivation of the Hp or mPFC previous to the second sample session impaired Obj2-LTM and reverted retroactive interference on Obj1- LTM formation.

Schematic representation of the experimental protocol is presented on top of the panel. Left: schematic representation of the infusion area. Right: Preference during test session is expressed as Mean ± SEM. The plain bar corresponds to the familiar object. The dashed line represents the chance level of performance (i.e., a 50% preference corresponds to no discrimination between familiar and novel objects).

(A) Effects of local infusion of Muscimol in CA1 dorsal Hp. Subjects performed two sample sessions with an inter-trial interval of 1 h. Fifteen minutes before the second sample session subjects received a local infusion of Vehicle (Veh) or Muscimol (Mus). All groups were tested 24 h after training, *** $p<0.001$, ** $p<0.01$ vs. familiar object, paired t test.

(B) Effects of local infusion of Muscimol in mPFC. Subjects performed two sample sessions with an inter-trial interval of 1 h. Fifteen minutes before the second sample session subjects received a local infusion of Veh or Mus. All groups were tested 24 h after training. ** $p<0.01$, * $p<0.05$ vs. familiar object, paired t test.

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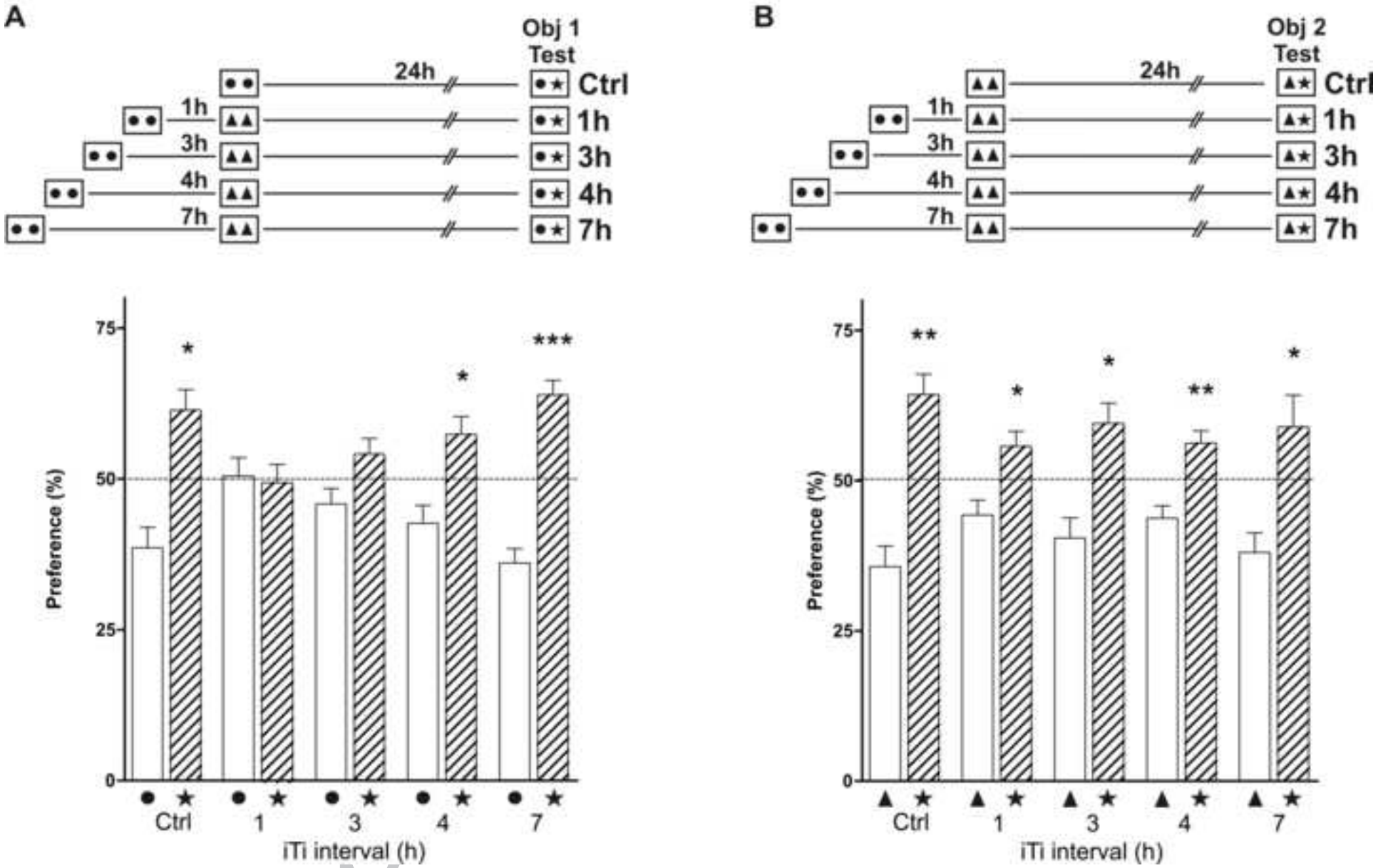

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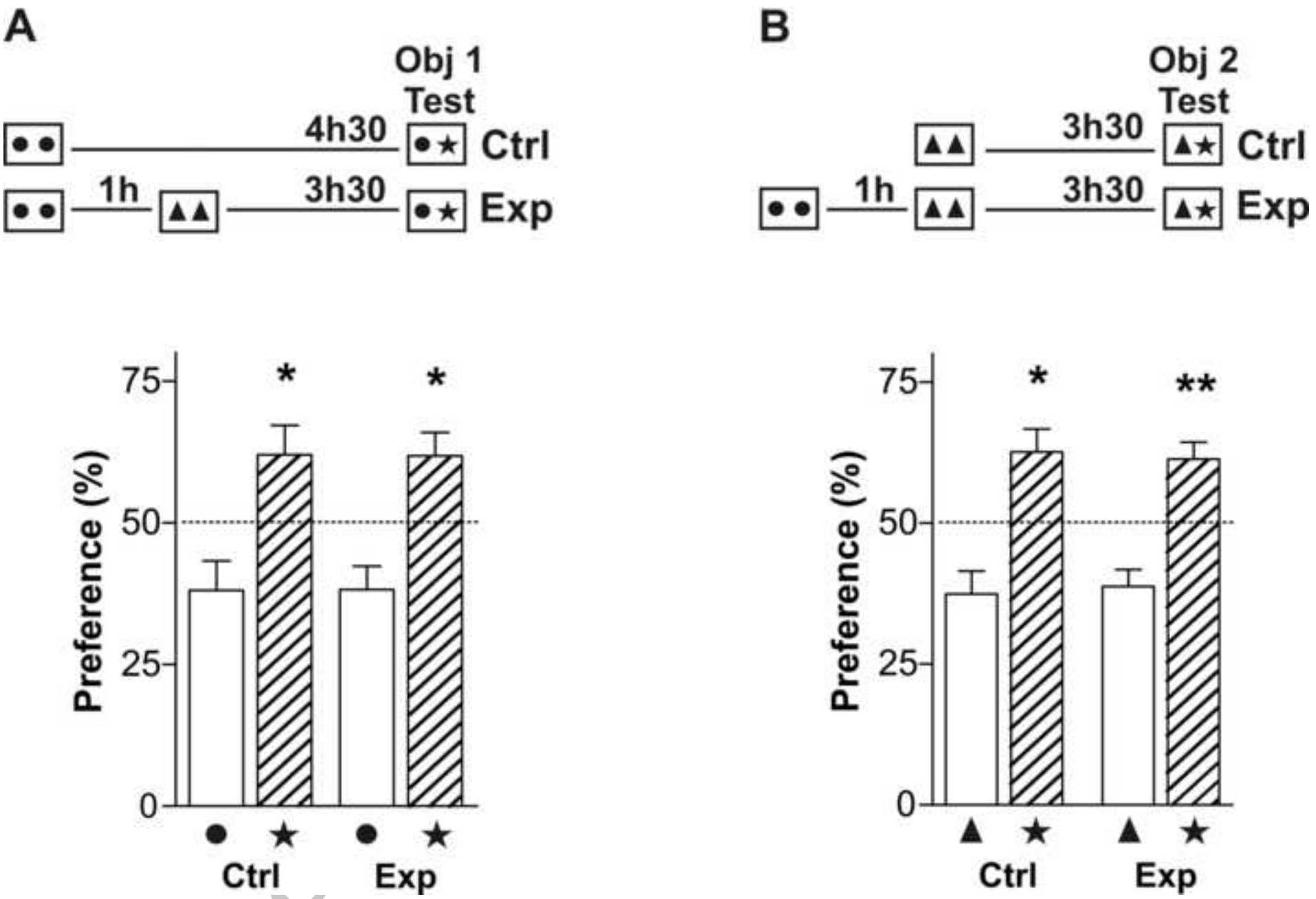

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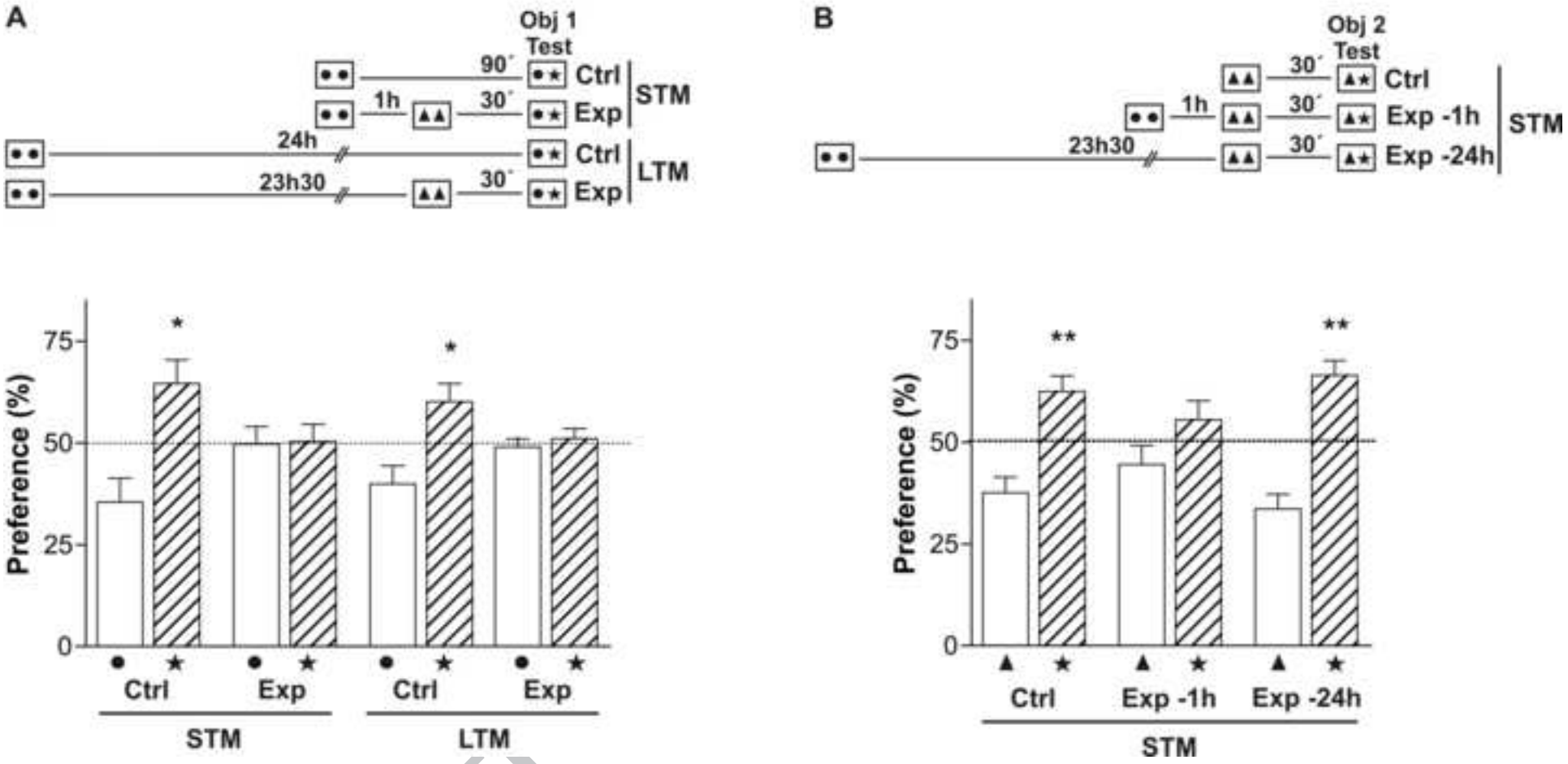

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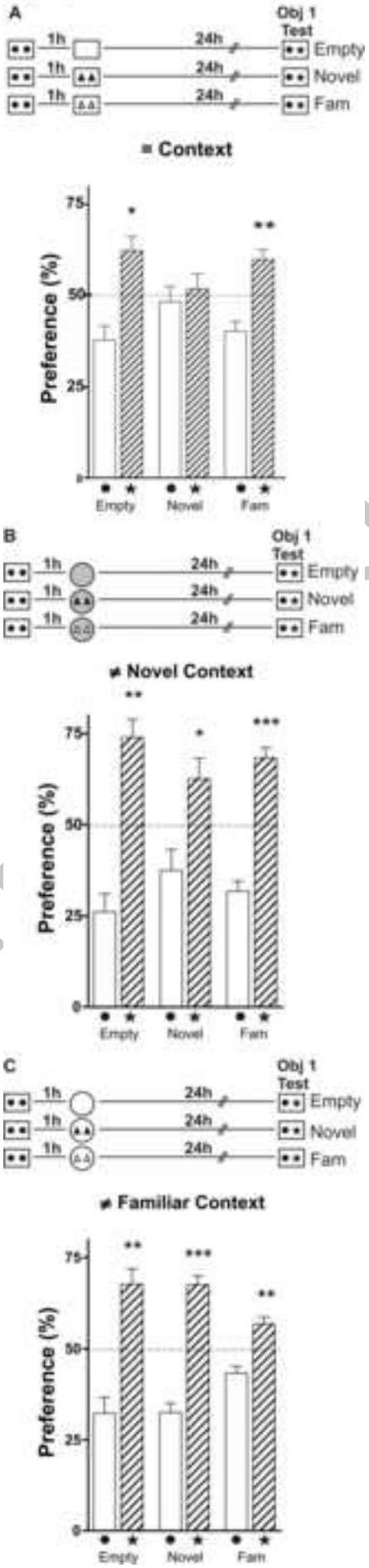

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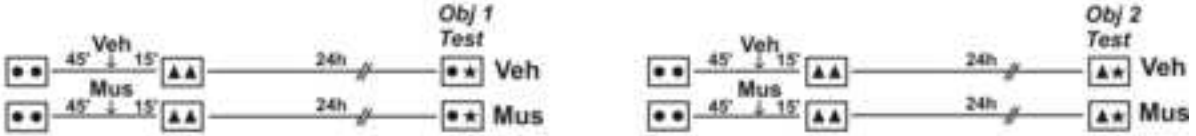

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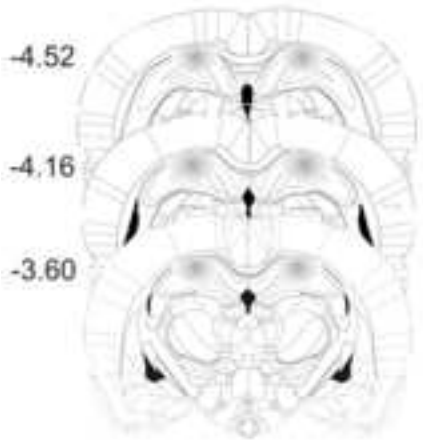

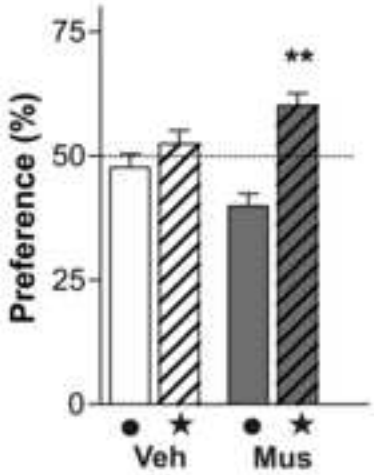

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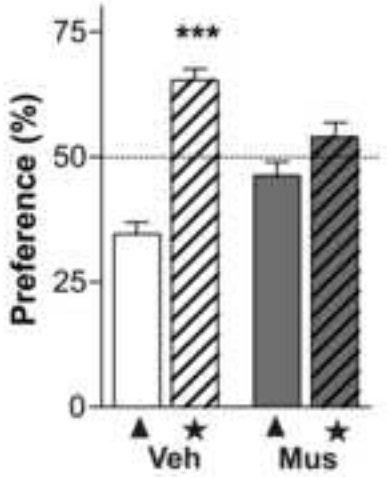

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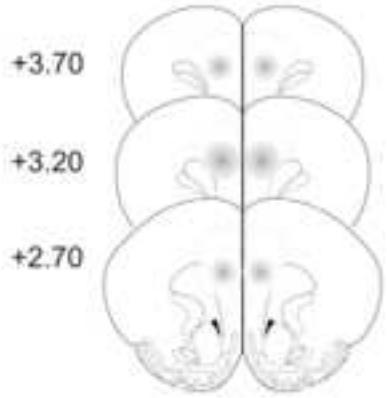

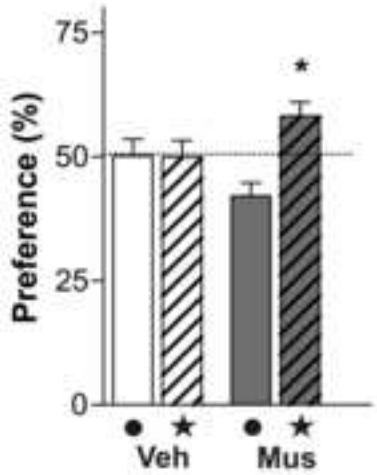

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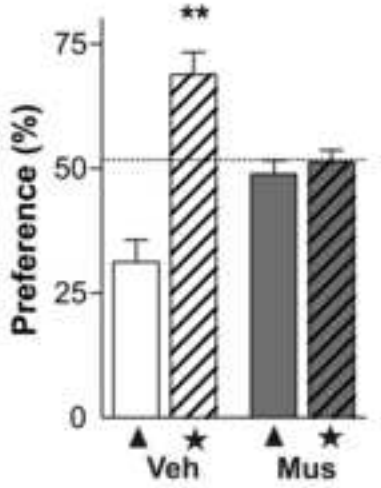

Highlights

1) Object recognition long-term memory is susceptible to retroactive interference
2) The exposure to a novel object in the same arena exerts an interfering effect
3) The interference acts on the consolidation and the expression of OR memory
4) Dorsal hippocampus and medial prefrontal cortex are involved in OR memory