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HIPPOCAMPAL ALPHA7 NICOTINIC RECEPTORS MODULATE MEMORY RECONSOLIDATION OF AN INHIBITORY AVOIDANCE TASK IN MICE

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Abstract—CF-1 male mice were trained in an inhibitory avoidance (IA) task using either a mild or a high footshock (0.8 or 1.2 mA, 50 Hz, 1 s). A retention test was given 48 h later. Immediately after the retention test, mice were given intradorsal hippocampus infusions of either choline (Ch, an α7 nicotinic acetylcholine receptor (α7nAChR) agonist, 0.08–1.30 μg/hippocampus), or methyllycaconitine (MLA, an α7nAChR antagonist, 1.0–30.0 μg/hippocampus). Memory retention was tested again 24 h later. Methyllycaconitine impaired retention performance regardless of footshock intensity and its effects were long lasting. Ch impaired retention performance only in those mice trained with a high footshock. On the contrary, Ch enhanced retention performance when mice were trained with a mild footshock. These effects were long lasting and dose- and time-dependent. Retention performance was not affected in drug-treated mice that were not subjected to memory reactivation, suggesting that the performance effects could not be attributable to non-specific effects of the drugs. Methyllycaconitine effects were dose-dependently reversed by choline, suggesting that MLA and Ch interact at the α7nAChR. Altogether, results suggest that hippocampal α7nAChRs play a critical role in reconsolidation of an IA response in mice, and may also have important implications for dynamic memory processes. This is the first presentation, to our knowledge, indicating that a specific receptor (α7nAChR) is able to modulate consolidated memories after retrieval.

Key words: inhibitory avoidance, acetylcholine, alpha7 nicotinic receptors, reconsolidation, memory, hippocampus.

Memory consolidation regards the underlying processes occurring after a learning situation where memory is stabilized and strengthened. Initially it is assumed that new memories are labile and sensitive to "disruption" before undergoing a series of processes that render the memory representation progressively stable (McGaugh, 1966, 2000). In 1968, Misanin and colleagues posited that retrieval renders the memory labile again. Along this line, several groups have recently found evidence to support the idea and coined the term "reconsolidation" (Przybyslawski et al., 1999; Nader et al., 2000). This process shares many features with memory consolidation but they are not identical and, moreover, reconsolidation is not considered to be a recapitulation of consolidation (Taubenfeld et al., 2001; Lee et al., 2004; Alberini et al., 2006; Tronson and Taylor, 2007). Some authors considered reconsolidation as a "post-activation state" where there is an increased sensitivity to amnesic agents, such as protein synthesis inhibitors, which might correlate with an enhanced plasticity of the neuronal circuitry that encodes the memory trace, or parts of it (Dudai, 2007). Accordingly, memory reconsolidation, from an adaptive point of view, allows new information to be integrated into the established memory to update it (Tronel et al., 2005). Reconsolidation may also serve to strengthen those memory traces that are most often used. According to this framework, memory is first consolidated, then retrieved (remembered either deliberately or spontaneously), and afterward, the engram is updated by the process of memory reconsolidation (Hardt et al., 2010).

Experimental and clinical evidence has given support to the hypothesis that cerebral acetylcholine (ACh) plays an essential role in mnemonic phenomena (Decker and McGaugh, 1991; Fibiger, 1991; Prado-Alcalá et al., 1993; Gallagher and Colombo, 1995; Baratti et al., 2009; but see, Blokland, 1996). Thus, it has been consistently found that central or systemic administration of anticholinergic drugs and lesions of the cholinergic system cause memory impairment while drugs that enhance cholinergic activity improve memory (Fibiger, 1991; Prado-Alcalá et al., 1993; Power et al., 2003). We recently reported that memory consolidation and reconsolidation are impaired when ACh synthesis is disrupted by i.c.v. administration of the reversible inhibitor of the sodium-dependent high-affinity choline uptake (HACU) hemicholinium (HC-3) (Boccia et al., 2004). Hemicholinium specifically affects cholinergic neurons by interfering with acetylcholine synthesis (Simon et al., 1976). The impairment inversely correlated with the time between training (learning) and the first retrieval session (Boccia et al., 2004, 2006). These results suggested, for the first time, that central cholinergic mechanisms are critically involved in memory reconsolidation of an inhibitory avoidance (IA) memory in mice. What we do not know yet is which cholinergic receptors are involved. Two different families of receptors are recognized by acetylcholine, muscarinic receptors (mAChRs, a G protein-coupled receptor), and nicotinic receptors (nAChRs, ligand-gated ion

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Abbreviations: Ach, acetylcholine; Ch, choline; IA, inhibitory avoidance; LTM, long-term memory; MLA, methyllycaconitine; nAchR, nicotinic receptor; SS, saline solution; STM, short-term memory.

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channels) (Albuquerque et al., 2009). Both are known to participate in encoding and retrieval but neither have been separately tested for their independent participation on post-retrieval memory processes (Buckingham et al., 2009).

Nicotinic receptors have homo or heteropentameric structure and are expressed in both the central and peripheral nervous system. They have been implicated in several physiological functions, such as learning and memory (Levin et al., 2006), and in several disorders (neurodegenerative and neuromuscular) (Taly et al., 2009). In vertebrates, 17 nAChR subunits have been identified ($\alpha1-\alpha10$, $\beta1-\beta4$, γ, δ and ϵ) which can co-assemble to generate a diverse family of AChR subtypes (Buckingham et al., 2009). The two main nAChR subtypes expressed in the CNS are $\alpha7$ and $\alpha4\beta2$ (Wevers and Schröder, 1999), which are highly expressed in different brain regions and have been implicated in several nervous system disorders such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder and tobacco addiction (Taly et al., 2009).

The key role of the hippocampus in the consolidation of many forms of memory, including inhibitory avoidance and maze tasks, has been amply documented (Izquierdo et al., 2002). Intra-hippocampal administration of nicotine enhances (Felix and Levin, 1997; Levin et al., 1996) and the nAChR antagonists, dihydro-β-erythroidine (DHβE; Felix and Levin, 1997; Levin et al., 2002) and mecamylamine (Ohno et al., 1993) impair working memory.

Martí Barros et al. (2004) reported that nAChRs in the CA1 region of the hippocampus participate in acquisition and consolidation of both short-term memory (STM) and long-term memory (LTM) of an inhibitory avoidance task, as well as the retrieval of LTM. These findings support a general enhancing or supportive modulatory role for nAChRs in a variety of memories (working memory, STM, LTM) and different phases of memory (LTM retrieval).

Prior studies, to our knowledge, have not investigated whether nAChRs are involved in memory reconsolidation. The present experiments investigated the memory-modulating effects of post-retrieval intra-dorsal-hippocampal infusions of either choline (Ch), an $\alpha7$ nicotinic acetylcholine receptor ($\alpha7$nAChR)-agonist or methyllycaconitine (MLA), an $\alpha7$nAChRs-antagonist (Levin et al., 2006), on an IA response in CF-1 mice.

EXPERIMENTAL PROCEDURES

Subjects

CF-1 male mice from our own breeding stock were used (age: 60–70 d; weight: 25–30 g). They were caged in groups of 10–12 and remained housed throughout the experimental procedures. The mice were kept in a climatized animal room (21–23 °C) maintained on a 12-h light/12-h dark cycle (lights on at 6:00 AM), with *ad libitum* access to dry food and tap water. Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication N 80-23/96) and local regulations. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Drugs

Choline bitartrate (Ch) and MLA citrate hydrate were from Sigma Life Science (Sigma, St. Louis, MO, USA). All drugs where dissolved in sterile saline solution immediately before use. All doses were calculated as the free base. All other agents were of analytical grade and obtained from local commercial sources.

Inhibitory avoidance task (IA)

Inhibitory avoidance (IA) behavior was studied in a one-trial learning, step-through type situation (Boccia et al., 2004; Blake et al., 2008), which utilizes the natural preference of mice for a dark environment. The apparatus consists of a dark compartment ($20\times20\times15$ cm^3) with a stainless-steel grid floor and a small (5×5 cm^2) illuminated, elevated platform attached to its front center. The mice were not exposed to the dark compartment before the learning trial. During training each mouse was placed on the platform and received a footshock as it stepped into the dark compartment. Two footshock training conditions were used: a low footshock (0.8 mA, 50 Hz, 1 s) which yielded median retention scores of approximately 100 s, and a high footshock (1.2 mA, 50 Hz, 1 s) which yielded median retention scores at the ceiling and was used to reduce the influence of extinction on retention performance (Boccia et al., 2004, 2006).

At the times indicated for each experimental group, the retention tests were performed. Each mouse was placed on the platform again and the step-through latency was recorded. The retention test was finished either when the mouse stepped into the dark compartment or failed to cross within 300 s (ceiling score). In the latter case the mouse was immediately removed from the platform and assigned a score of 300 s (ceiling score). In the retention test session the footshock was omitted.

Intra-dorsal-hippocampal injections

Mice were prepared (Boccia et al., 2004, 2006, 2007) for the intra-dorsal-hippocampal injections of vehicle or drug solutions 48 h before training, so that a minimum of time was necessary for injection, which was administered under light ether anesthesia in a stereotaxic instrument. The preliminary surgery was also performed under ether anesthesia and consisted of an incision of the scalp. Two holes were drilled in the skull without perforating the brain, at the following stereotaxic coordinates AP: -1.50 mm posterior to bregma, L/R$+1.20$ mm from the midsagital suture and DV: -1.75 mm from a flat skull surface (Franklin and Paxinos, 1997), to bilaterally infuse the drugs after recovery. The skull was covered with bone wax and the mouse was returned to its home cage. Injections lasted 90 s and were driven by hand through a 30-gauge blunt stainless steel needle attached to a 5 μl Hamilton syringe with PE-10 tubing. The volume of each intrahippocampal infusion was 0.5 μl. Control injections were applied to the primary somatosensory cortex, forelimb region (Boccia et al., 2007) at the following stereotaxic coordinates: AP (0.62 mm anterior to bregma), L/R (2.50 mm from the midsagital suture), and DV (-1.50 mm) (Franklin and Paxinos, 1997).

The accuracy of intra-dorsal-hippocampal injections was determined by histological determination of the needle position on an animal-by-animal basis. For this purpose, the brains of injected animals were dissected, fixed in 4% parafomaldehyde/ buffer phosphate saline, and stored in 30% sucrose. They were then cut into 25 μm coronal sections with a cryostat. The deepest position of the needle was superimposed on serial coronal maps (Franklin and Paxinos, 1997). Coronal sections containing the deepest reach of the needle were Nissl stained to estimate the damage produced during the procedure (Fig. 1). Animals were excluded from the statistical analysis if the infusions caused excessive damage to the targeted structure or if the needle tips extended outside the target structure.

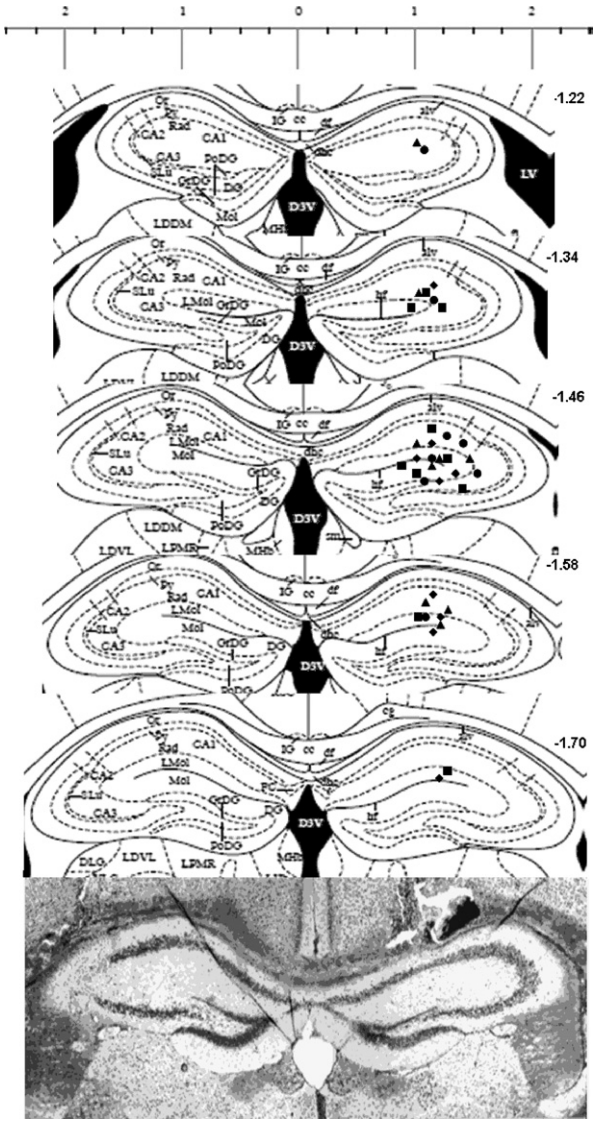

Fig. 1. Coronal brain image is adapted from the atlas of Franklin and Paxinos (1997). The symbols indicate the position of the injection in the hippocampus corresponding to the first experiment (● SS ■ Ch 0.30 ▲ Ch 0.80 ◆ Ch 1.30 μg/hippocampus) (see Fig. 2A). The last coronal section (Nissl stained) shows the trace of the needle (representative image).

Data analysis

Data are expressed as median latencies (sec) to step-through and interquartile ranges during the retention test and were analyzed, when appropriate, with the nonparametric analysis of variance of Kruskal–Wallis. The differences between groups were estimated by individual Mann–Whitney U-tests (two-tailed) (Siegel, 1956). In all cases, $P<0.05$ values were considered significant.

Experimental groups

In the first experiment, four groups of 10 mice each were trained with a low footshock; 2 days after training the mice were subjected to the first retention test. Immediately after the test, mice received a bilateral intra-dorsal-hippocampal infusion of saline (SS) or choline (0.30–1.30 μg/hippocampus). Mice were tested again one day and 21 days after training. The results of this experiment are shown in Fig. 2A.

A similar experimental protocol was performed using over-reinforced mice during the training trial (high footshock) (see IA task). The results of this experiment are shown in Fig. 2B.

In the second experiment, in order to test whether Ch effects on post reactivation memory processes are specific, four groups of 10 mice each were trained on the IA task. Half of them received the mild foot shock and 48 h later they received intra-dorsal hippocampal infusion of either SS or Ch (0.80 μg/hippocampus). The remaining groups were trained with the high footshock and received either SS or Ch (1.3 μg/hippocampus) 48 h later. Twenty-four hours later all groups were tested for retention. Note that in this experiment, memory was not reactivated before infusions (Fig. 3A).

The next experiment was performed in order to test whether the Ch effects on memory reactivation were time-dependent. For this purpose four different groups of mice were trained following the same training protocol as the one described above. Memory was reactivated 48 h after training and infusions were given 3 h after reactivation. All groups were tested again for retention 24 h later (Fig. 3B).

In the following experiment, we performed intra-dorsal hippocampal administration of MLA, an α7 receptor antagonist (Albuquerque et al., 2009), to further investigate the involvement of hippocampal α7-nicotinic receptors in post-reactivation memory processes. Again, we used two different footshock intensities during the training session, and replicated the experimental protocol described for choline. A dose-response curve was accomplished with 1, 10 and 30 μg MLA infused into the hippocampus. Mice not undergoing memory reactivation were infused with MLA (10 μg/hippocampus) 48 h after training; the same dose was used for mice receiving the infusion 3 h after the first retention test. The results of these experiments are shown in Fig. 3A, B and Fig. 4A, B.

To gain more insight about the possible participation of α7-nicotinic receptor subtype in post-reactivation memory processes, we also studied the interaction between Ch (agonist) and MLA (antagonist) (Albuquerque et al., 2009) when given immediately after memory reactivation. Thus, five groups of 10 mice each were trained with a low-intensity footshock. After the first retention test, performed 48 h after training, mice received intra-dorsal hippocampal injections of saline (0.5 μl), MLA (10 μg), or MLA (10 μg) plus Ch (0.30–1.30 μg) as a single injection, and were tested again 72 h after training. Note that the dose of MLA (10 μg) employed in this experiment impaired retention either when given to mice that were trained with a low or a high footshock. The results of this experiment are shown in Fig. 5.

Finally, three groups of 10 mice each were trained with a low footshock, and tested 48 h after training. Immediately after it they received an injection of saline (0.5 μl), Ch (0.80 or 1.30 μg/hippocampus), or MLA (10 μg/hippocampus) in the somatosensory cortex, forelimb region, and were tested again 72 h after training. The same experimental protocol was used for mice trained with a high footshock (Fig. 7A, B).

RESULTS

Step-through latencies among all of the groups during training were not significantly different (TSTL=12 (8–12); $H_{(37)}=9.38$; $P>0.05$).

Effects of choline on reactivated memory

The results of the first experiment are shown in Fig. 2. Intra-dorsal hippocampal injections of Ch administered immediately after the first retention test (T1) significantly influenced retention performance in a subsequent retention test (T2). The effect was strongly dependent on the

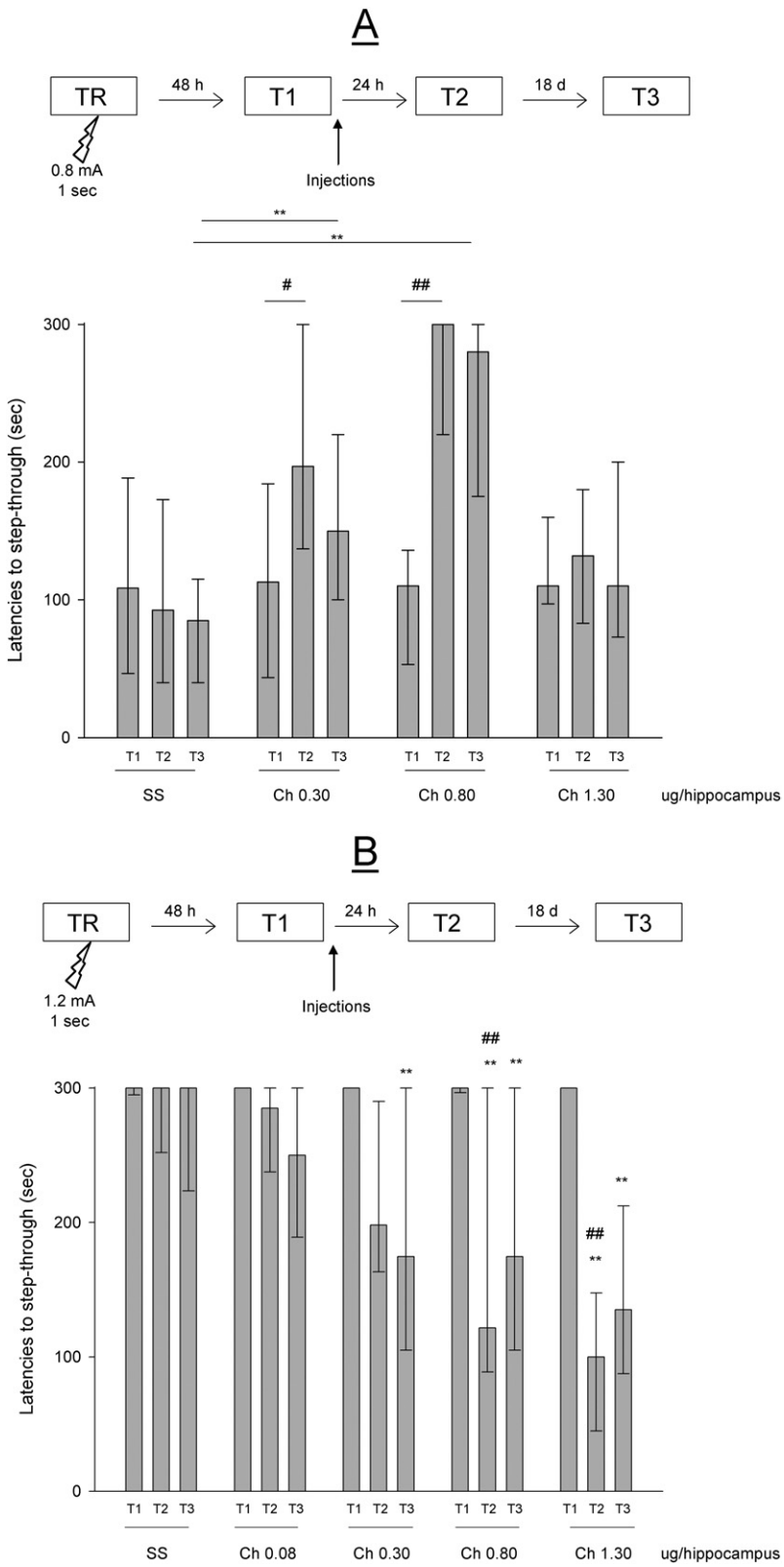

Fig. 2. Effects of intra dorsal hippocampal infusion of Choline (Ch 0.08–1.30 μg) on retention performance when given immediately after retrieval in mice trained either with a mild-footshock (A) or a high footshock (B). Each bar represents the median and interquartile range (n=8–10 mice/group). Test numbers represent successive tests. ** $P<0.01$, in all cases compared with its respective control test number (of SS control group); $^{\#}$ $P<0.05$ and $^{\#\#}$ $P<0.01$, in all cases compared with T1 (Mann–Whitney U-test, two tailed). Above each graph the behavioral experimental scheme is represented.

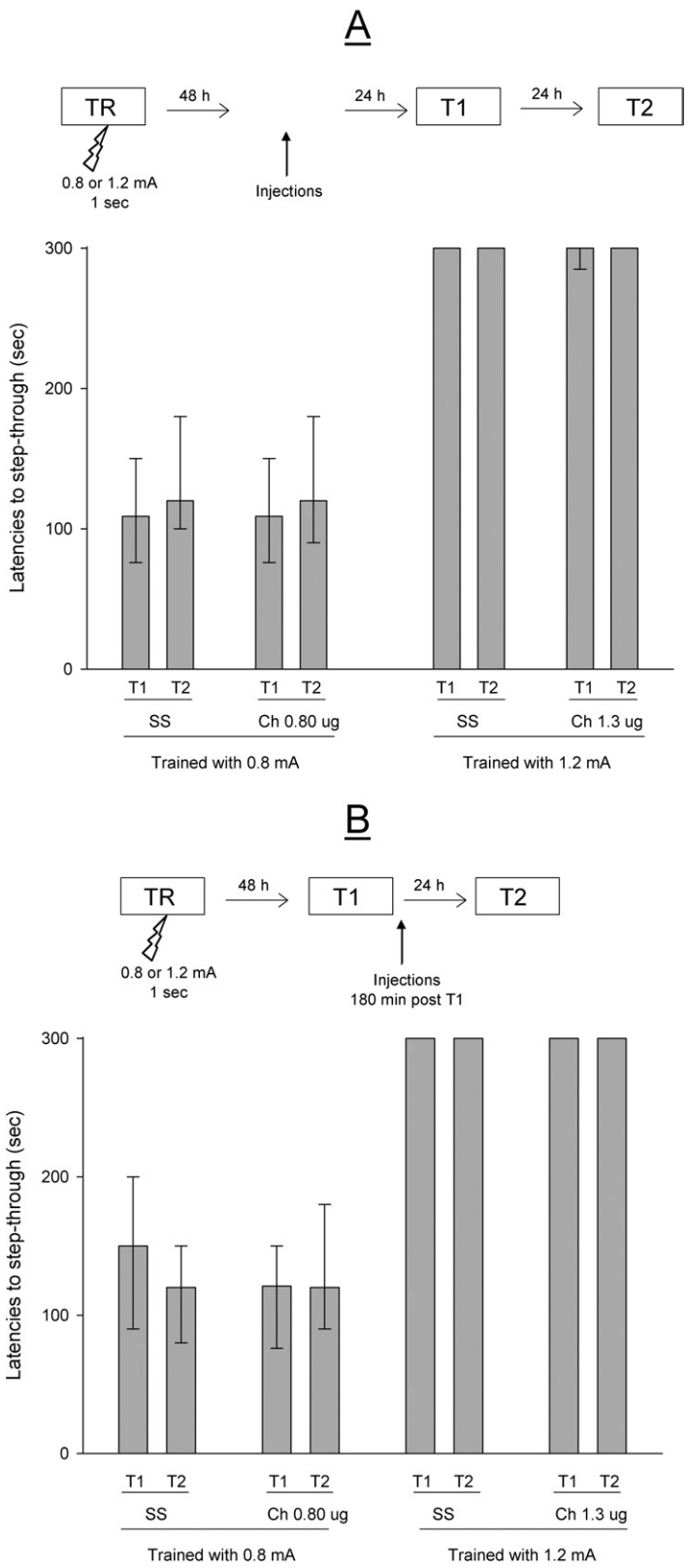

Fig. 3. Effects of intra dorsal hippocampal infusion of Choline (0.80 or 1.30 μg) on retention performance when given 48 h after training in absence of memory reactivation (A) or when delayed 180 min after memory reactivation (B). Each bar represents the median and interquartile range (n=8–10 mice/group). Test numbers represent successive tests. Above each graph the behavioral experimental scheme is represented.

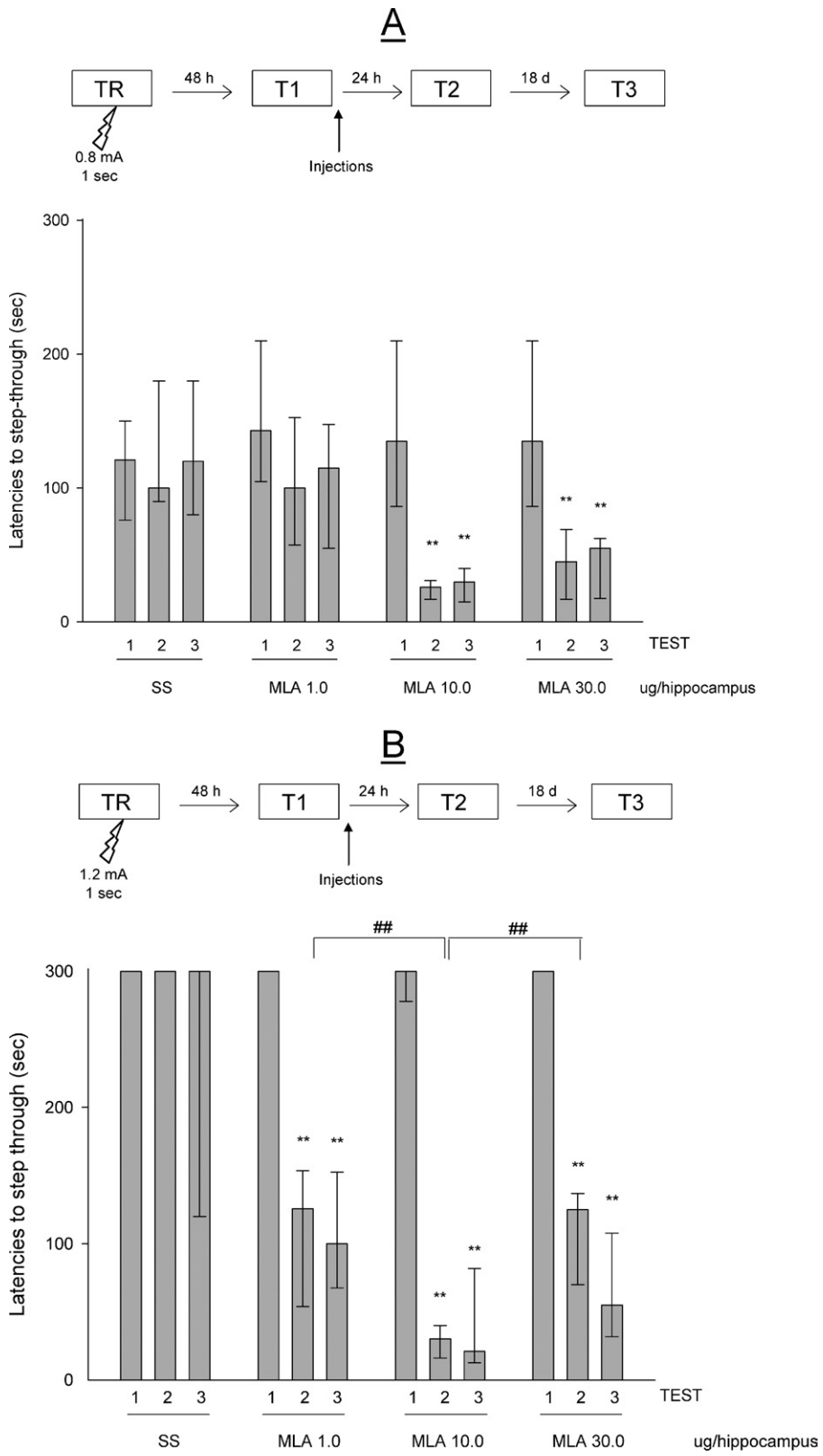

Fig. 4. Effects of intra dorsal hippocampal infusion of methyllycaconitine (MLA 1.0–30.0 μg) on retention performance when given immediately after retrieval in mice trained either with a mild-footshock (A) or a high footshock (B). Each bar represents the median and interquartile range (n=8–10 mice/group). Test numbers represent successive tests. ** $P<0.01$, in all cases compared with its respective control test number (of SS control group); ## $P<0.01$, when compared with T2 (of MLA 1.0 and MLA 30.0 μg) (Mann–Whitney U-test, two tailed). Above each graph the behavioral experimental scheme is represented.

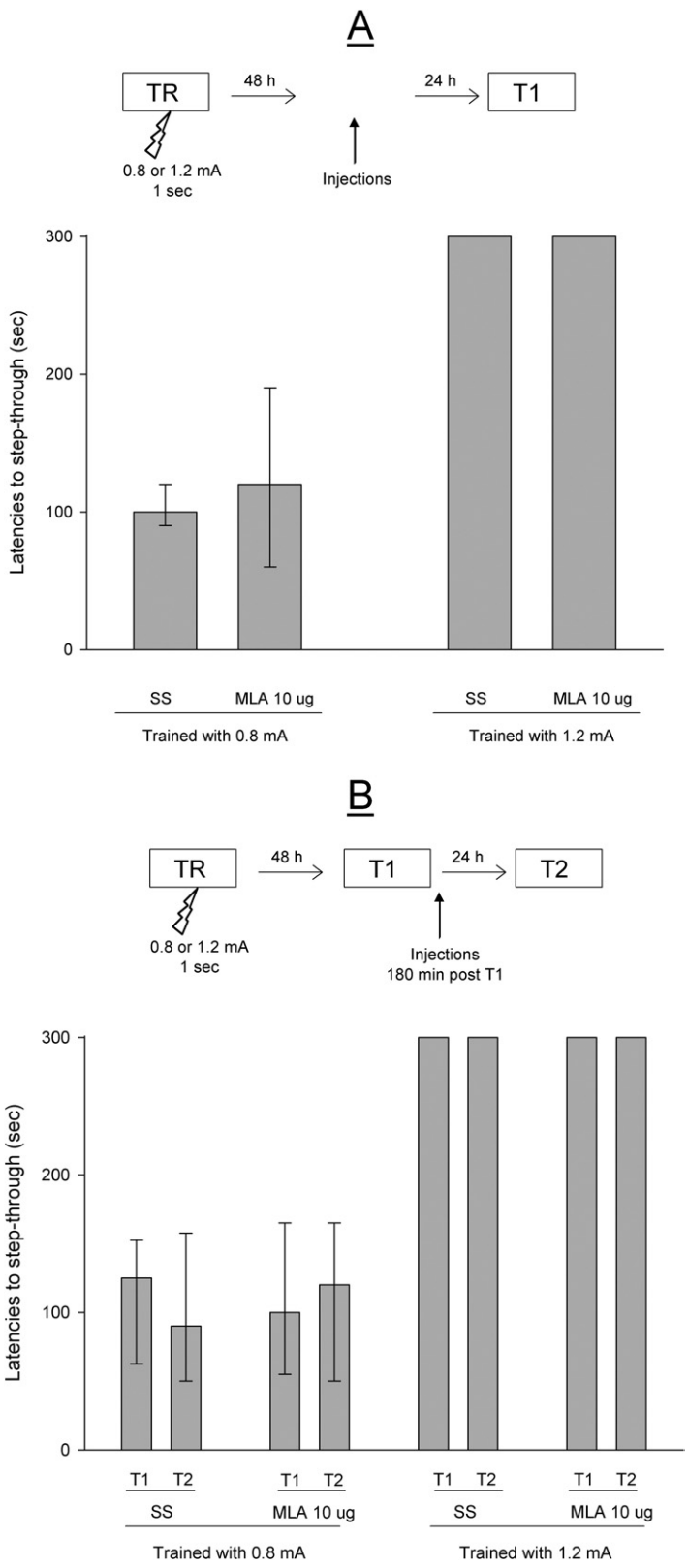

Fig. 5. Effects of intra dorsal hippocampal infusion of methyllycaconitine (MLA 10.0 μg) on retention performance when given 48 h after training in absence of memory reactivation (A) or when delayed 180 min after memory reactivation (B). Each bar represents the median and interquartile range (n=8–10 mice/group). Test numbers represent successive tests. Above each graph the behavioral experimental scheme is represented.

footshock intensity used in the training trial. Thus, there was an overall significant dose-response effect ($H_{(3)}=17.03$; $P<0.01$) of choline on retention performance of mice that received a low footshock during the training trial (Fig. 2A). Post-reactivation Ch injections enhanced retention performance in a dose-related manner, and statistical significance was reached at the doses of 0.30 and 0.80 μg/hippocampus ($P<0.05$, and $P<0.01$, respectively, in both cases as compared with T1). The higher dose of Ch (1.30 μg/hippocampus) was ineffective; thus, the dose-response curve was bell-shaped (Fig. 2A). The enhancing effect of Ch (0.80 μg/hippocampus) persisted up to 21 days after training ($P<0.01$ vs. saline-treated control group; Fig. 2A). In addition, mice that were injected with Ch (0.80 μg/hippocampus) 48 h after training, but did not experience the reactivation session, performed as well as the saline-treated group ($P>0.05$) on T2 (Fig. 3A). No effect of Ch was observed when the Ch (0.80 μg/hippocampus) injection was delayed 180 min after the end of the first retention test (Fig. 3B). In contrast, Ch administered immediately after the first retention test (T1) to mice trained with a high footshock, significantly reduced retention performance in the subsequent (T2) retention test (Fig. 2B). The effect of intrahippocampal Ch was dose-dependent. Thus, the doses of 0.80 and 1.30 μg/hippocampus significantly impaired retention performance ($P<0.01$, in both cases as compared with the first retention test), whereas the doses of 0.08 and 0.30 μg/hippocampus were without effect ($P>0.05$, in both cases compared the first retention test) (Fig. 2B). Choline-treated mice (0.30, 0.80 and 1.30 μg/hippocampus) trained with a high footshock exhibited a significant impairment of retention performance on 21-day test (T3) ($P<0.01$, in all cases compared with saline control group, Fig. 2B). When Ch (1.30 μg/hippocampus) was administered 48 h after training without memory reactivation, retention performance was not affected ($P>0.05$) (Fig. 3A). Retention latencies were not affected when Ch administration (1.30 μg/hippocampus) was delayed 180 min after the first retention test ($P>0.05$, Fig. 3B).

Effects of methyllycaconitine on reactivated memory

Methyllycaconitine impaired retention performance on T2 using either mild- or high-footshock during training and this impairment was dose-dependent (Fig. 4A, B). However when mice were trained with the mild footshock the lower dose (1.0 μg/hippocampus) did not reach statistical significance (Fig. 4A); on the contrary, MLA impaired retention performance at all doses employed in mice trained with the high footshock (Fig. 4B). Moreover, the impairment observed in those mice infused with MLA was evident even 21 days after training, using both training conditions (Fig. 4A, B; T3), suggesting a lack of spontaneous recovery.

Methyllycaconitine did not affect retention performance when infusions were given in the absence of memory reactivation or when infused 180 min after it (Fig. 5A, B). These results suggest that MLA effects could not be attributable to non-specific effects and, moreover, that MLA effects are not only dose-but also time-dependent, despite the footshock.

Methyllycaconitine—choline interaction

Methyllycaconitine impaired retention performance when infused immediately after memory reactivation (Fig. 6). MLA effects were dose-dependently reversed by choline (Fig. 6) suggesting that MLA and Ch interact at the α7nAChR and that this receptor seems to be critically involved in memory processes occurring immediately after retrieval.

Furthermore, we performed an anatomical control experiment in which animals were injected post-T1 with Ch or MLA in the primary somatosensory cortex, forelimb region. In this experiment, retention performance was not affected (Fig. 7A, B) using either a mild or a high footshock during training, supporting the site-specific effect of Ch/MLA on memory retention.

DISCUSSION

Acetylcholine signaling has been implicated in a number of learning and memory processes (Decker and McGaugh, 1991; Fibiger, 1991; Gallagher and Colombo, 1995). In this sense, it is well known that endogenous ACh is necessary for long-term memory consolidation (Boccia et al., 2004), memory retrieval (Boccia et al., 2003), memory extinction (Boccia et al., 2009), and memory reconsolidation (Boccia et al., 2004, 2006). In general, muscarinic and nicotinic receptor agonists enhance (Fibiger, 1991; Prado-Alcalá et al., 1993; Power et al., 2003), and muscarinic and nicotinic receptor antagonists impair learning and memory (Power et al., 2003), with particular effects on memory consolidation (Power et al., 2003).

In the last years there has been increased interest in the role of the nicotinic cholinergic neurotransmission in cognition processes, triggered, at least in part, by its significantly reduced activity in the brains of Alzheimer's disease patients (Oddo and LaFerla, 2006), and by some clinical studies demonstrating that (-)-nicotine might improve cognitive function in those patients (Levin et al., 2006).

The present study points to a key participation of the α7nAChR subtype on memory reactivation-induced processes (Nader et al., 2000; Boccia et al., 2004), probably memory reconsolidation, which is consistent with previous findings suggesting a role of ACh in those processes (Boccia et al., 2004, 2006).

Post-reactivation administration of the α7 receptor agonist Ch (Albuquerque et al., 2009), improves or impairs retention performance in a subsequent retention test depending on the intensity of the unconditioned stimulus used during the training trial. In mice trained with a low foot shock, post-reactivation bilateral intra-dorsal hippocampal injections of Ch enhanced retention performance. A necessary criterion to consider an effect on memory reconsolidation is that the procedure employed, Ch injections in our case, must be effective only following memory reactivation (Nader et al., 2000). It is also necessary to demonstrate a post-retrieval time-window of susceptibility of the original consolidated memory following its retrieval (Alberini et al., 2006). Both criteria were achieved under our experimental

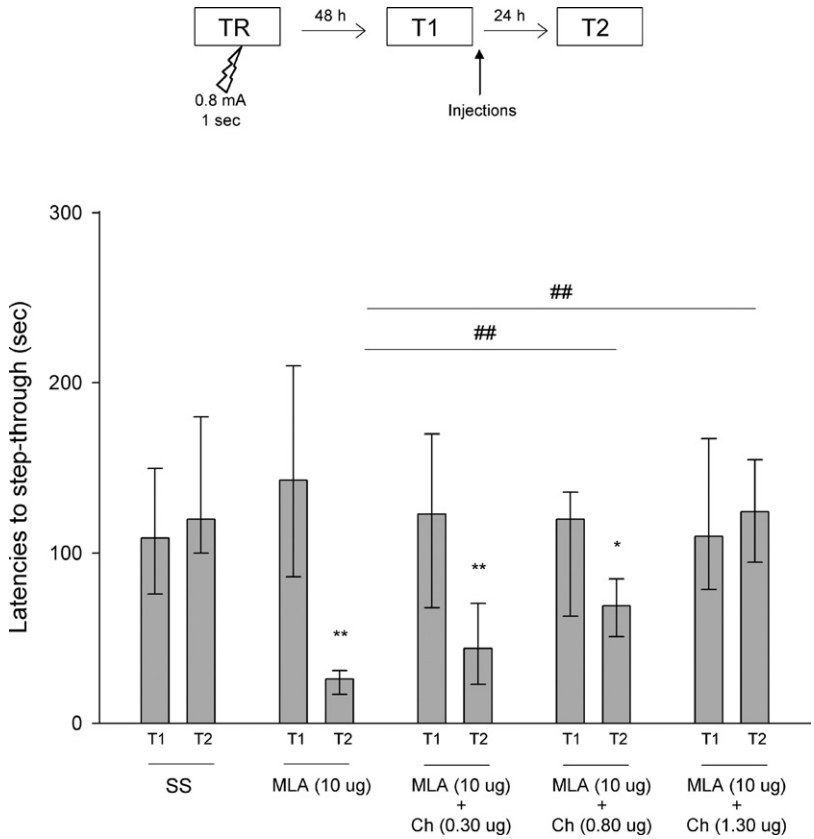

Fig. 6. Effects of intra dorsal hippocampal infusion of choline (Ch 0.30–1.30 μg) on methyllycaconitine (MLA 10.0 μg) memory reconsolidation impairment. MLA and Ch were given immediately after memory reactivation. MLA memory impairment was dose-dependently reversed with Ch. Each bar represents the median and interquartile range (n=8–10 mice/group). Test numbers represent successive tests. * $P<0.05$, ** $P<0.01$, in all cases compared with its respective control test number (of SS control group). ## $P<0.01$, in all cases compared with MLA (10 μg/hippocampus) treated mice. Above each graph the behavioral experimental scheme is represented.

conditions and, in addition, the effects of Ch on reactivated memories were dose-dependent. Retention performance enhancement did not vary in a monotonic way as a function of the Ch doses; rather, the dose-response curve was in the form of an inverted-U (Fig. 2A). This type of curve is typically observed for drug treatments given immediately after training (McGaugh and Roozendaal, 2009), and is most likely the result of the neuromodulatory influences exerted by such treatments on memory consolidation of newly acquired information (McGaugh, 2000; McGaugh and Roozendaal, 2009), and could indicate that similar modulatory effects occur during memory reconsolidation (Boccia et al., 2004, 2005). These post-reactivation effects of Ch on memory are, in general, in accordance with those previously reported by Gordon (1977); Horne et al. (1997), and Rodriguez et al. (1999), who employed different methodological approaches and post-reactivation treatments. In addition, the enhancement of retention induced by the optimal dose of Ch, that is the dose that produced the maximal effect, was long-lasting. This finding is similar to those reported for other drug treatments administered immediately after training to mice trained with a low foot shock in the IA task (Kopf and Baratti, 1994; Boccia and Baratti, 2000). On the contrary, it is worth pointing out that, in those mice trained with a high footshock, intra-dorsal-hippocampal infusions of the same dose of Ch impaired retention performance.

These apparently contradictory effects of Ch, when administered immediately after reactivation, on retention performance depending on the training conditions are very similar to those reported by Gold and van Buskir (1976). In that case, a dose of epinephrine that enhanced retention performance after low-footshock training produced amnesia if administered after high-footshock training. Accordingly our results are very similar, but in our case Ch was administered immediately after retrieval. We can speculate that post-retrieval treatments have important roles in modulating memory processes occurring after retrieval and seem to be very similar, though not identical (see Introduction), to that occurring after learning.

Methyllycaconitine, an α7nAChRs antagonist (Albuquerque et al., 2009), impaired retention performance in mice trained either with a mild or a high footshock when infused immediately after retrieval. In both cases, retention performance impairment varied as a function of the dose infused, although a tendency toward a U-shaped curve could be observed when mice were trained with a high footshock (Fig. 4B). Lack of spontaneous recovery suggests that MLA effects were long lasting (Bouton, 1993). Additionally, MLA effects were not observed in the ab-

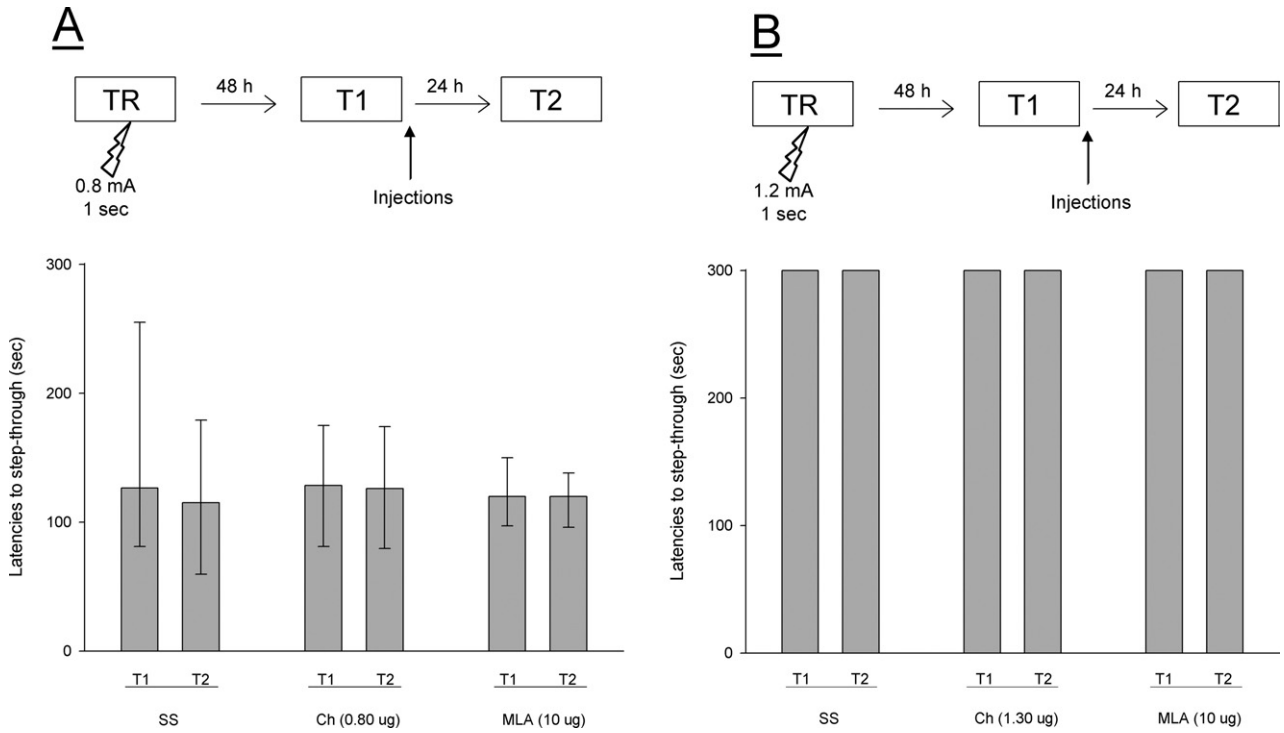

Fig. 7. Effects of choline (Ch 0.80 or 1.30 μg) and methyllycaconitine (MLA 10.0 μg) infusion in the forelimb primary somatosensory cortex after re-exposure in mice trained either with a mild- or a high-footshock (A, B respectively). Neither Ch nor MLA affected retention performance in a subsequent test performed 24 h after infusion independently of the training conditions. Each bar represents the median and interquartile range (n=8–10 mice/group). Test numbers represent successive tests.

sence of memory reactivation or when infusions were delayed 3 h after reactivation. Altogether, these results suggest that either impairment or enhancement of retention induced by post-retrieval administration of the drugs respectively, could not be attributed to non-specific influences on performance (Nader et al., 2000; Milekic and Alberini, 2002; Tronson et al., 2006).

Methyllycaconitine effects on memory reconsolidation were dose-dependently reversed by Ch when co-infused bilaterally immediately after memory reactivation, suggesting a potential pharmacological interaction at α7nAChR level and, moreover, involving this receptor critically on post retrieval memory processes.

No impairment of retention performance was observed in control experiments where either Ch or MLA were infused in the somatosensory cortex, forelimb regions in mice trained in both conditions (mild and high footshock). It is already known that α7nAChRs are present in the somatosensory cortex (Metherate, 2004), although these somatosensory nicotinic receptors do not seem to be involved in reconsolidation of inhibitory avoidance memory. Moreover, this lack of effect indicates some specificity for the locus of the drug-reconsolidation effect (hippocampus), although other brain areas should also be taken into consideration.

Methyllycaconitine effects on memory reconsolidation are in accordance with previous results obtained with post-retrieval i.c.v. injections of hemicholinium-3, a central inhibitor of ACh synthesis (Boccia et al., 2004), with the exposure of the experimental subjects to a new learning situation immediately after retrieval (Boccia et al., 2005), and with post-retrieval intrahippocampal injections of an inhibitor of the transcription factor NF-κB (Boccia et al., 2007). They are also in accordance with those reported by other authors using different pharmacological treatments (Bustos et al., 2006; Milekic and Alberini, 2002).

The amnesia found here should be a consequence of one of two possibilities: a storage deficit similar to the type of deficit assumed to occur after consolidation blockade, or a retrieval or performance deficit similar to that observed following extinction of conditioned fear (Dudai and Eisenberg, 2004). Reversal of amnesia could be studied and characterized by four experimental behavioral protocols: spontaneous recovery, saving, reinstatement and renewal (Holland and Bouton, 1999). If amnesia can be reversed, then it is likely that the original memory was still present, but was not behaviorally expressed, due to retrieval impairment.

The present results, at first glance, support a storage deficit interpretation, and are consistent with the memory reconsolidation hypothesis (Misanin et al., 1968). Alternative explanations to our results should consider that re-exposure to a previously learned situation could trigger extinction, the progressive decrease in the conditioned response resulting from repeated conditioned stimulus presentation without reinforcement (Myers and Davis, 2002, 2007). Further, extinction clearly implies a new

learning and not unlearning of the original memory (Myers and Davis, 2007).

This appears to be not exactly the case in MLA treated mice, since there was an acute drop in retention performance from T1 to T2, enduring until the last retrieval session. Since it has been consistently found that MLA impaired either learning, memory or attention in different species and several learning task (Martí Barros et al., 2004; Levin et al., 2006), it seems unlikely that MLA could be enhancing consolidation of the extinction memory.

As we did not find recovery of retention performance at least 21 days after training in mice treated with MLA, our results suggest that there could be a disruption of the memory trace rather than a retrieval deficit. This evidence is consistent with other studies showing absence of spontaneous recovery when different disrupting manipulations after memory retrieval were performed (Boccia et al., 2004, 2005; Bustos et al., 2006; Debiec and Ledoux, 2004; Duvarci and Nader, 2004), but it is in conflict with other reports, in which the use of multiple retention tests or increasingly long retention test delays after memory reactivation, leading to an initial retention deficit followed by memory recovery (Lattal et al., 2004; Power et al., 2006; Prado-Alcalá et al., 2006).

The effects observed after the administration of the α7nAChR agonist were opposite for both training conditions: post-reactivation Ch infusion led to performance enhancement in mice trained with a low footshock, but caused performance impairment in mice trained with the high footshock. The improvement observed for the low footshock training condition would be difficult to explain in terms of impairment of memory extinction. The development of an extinction phenomenon should lead to a progressive decrease in retention performance, and its impairment should block this decrease. In other words, retention performance in T2 should remain similar to or less than performance in T1, but never enhanced. Hence, this result could be explained through a facilitation of memory reconsolidation. The acute drop in retention performance observed from T1 to T2 after administration of the α7nAChR antagonist MLA is also in accordance with this interpretation, because if this drop had been caused by memory extinction improvement, then the reduction should have been progressive, not at once.

On the contrary, the high footshock training condition led to performance impairment, both after post-reactivation administration of the α7nAChR agonist and the antagonist. As it was previously mentioned for the low footshock training condition, the acute drop observed from T1 to T2 after the administration of MLA could be interpreted as memory reconsolidation impairment. However, post-reactivation administration of Ch led to a slight decrease of retention performance. Although we cannot provide a comprehensive explanation for this fact, it might be because of the facilitation of memory extinction rather than to impairment of memory reconsolidation. Hence, we cannot definitively discard a possible retrieval deficit yet, because having simply a poor performance does not mean absence of memory (Cahill et al., 2001).

At this point, it is worth pointing out that during the first retention test, 90–95% of mice trained with the high footshock did not enter the dark compartment; the opposite happened with mice trained with the mild footshock. Accordingly, mice not entering the dark compartment did not experience the CS—no US association (extinction training). However, it is important to mention that the mere exposure to the CS could be an extinction trial. Indeed, we have published that mice trained with the high footshock extinguish the avoidance response and, in order to do that, they need between four and five extinction training and/or reactivation sessions (Baratti et al., 2008).

The fast desensitization of α7nAChRs after its activation (Fenster et al., 1999; Mike et al., 2000; Quick and Lester, 2002; Gay et al., 2008) makes it difficult to distinguish between truly agonistic and antagonistic effects of an α7nAChR targeted drug. In fact, agonist efficacy *in vivo* is hard to reconcile with rapid α7nAChR desensitization *in vitro* and it is not clear whether the cognitive effects are the result of receptor activation per se or activation-induced receptor desensitization (Banerjee et al., 2005). Then, the possibility that agonists of this receptor actually function *in vivo* as inhibitors via desensitization has not been definitively resolved (but see Briggs et al., 2009). Nevertheless, it is likely that MLA and Ch are acting at the same site, since MLA effects were reversed with increasing doses of Ch and, furthermore, Ch effects were attenuated when co-infused with MLA (see Figs. 2A, B and 6).

The α7nAChR is highly expressed in the hippocampus and cerebral cortex (Albuquerque et al., 2009). The hippocampal α7nAChR acts as a presynaptic modulator of release of GABA and glutamate independently of its excitatory post-synaptic effects (Gray et al., 1996; Radcliffe et al., 1999). Methyllycaconitine or Ch effects on memory reconsolidation could be mediated through pre-synaptic effects on release of other neurotransmitters and/or post-synaptic effects; however, a resolution to this question is beyond the scope of the present paper. Studies are in progress in order to elucidate whether pre and/or post-synaptic effects of α7nAChR contribute to modulate post-retrieval memory processes.

Another approach to study the potential participation of α7nAChR on memory processes might be the use of α7nAChR knockout mice. However, the employment of these mice to study memory reconsolidation processes is limited since there are several studies reporting that these mice have impaired acquisition on different learning tasks (Young et al., 2004; Keller et al., 2005).

As was stated before, hippocampal α7nAChRs seem to be critically involved in working memory and also in acquisition, consolidation of STM and LTM, as well as retrieval of LTM of an inhibitory avoidance response (Ohno et al., 1993; Felix and Levin, 1997; Levin et al., 1996; Martí Barros et al., 2004).

Our results suggest that α7nAChRs in the hippocampus also participate in memory reconsolidation of an inhibitory avoidance response in mice. These findings may have important implications for understanding dynamic memory processes since this is the first report of evidence

that a specific receptor (α7nAChR) is able to modulate (enhance or impair) consolidated memories after retrieval.

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