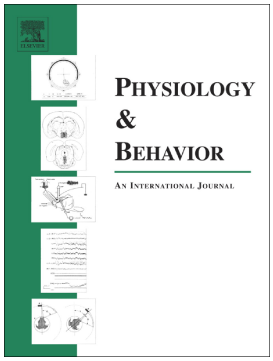

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Extinction and recovery of an avoidance memory impaired by scopolamine

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Keywords: amnesia; memory extinction; memory recovery; scopolamine; cholinergic dysfunction.

Highlights

- Pre-training administration of scopolamine causes memory impairment
- Despite cholinergic dysfunction, memory could be stored
- Extinction could be observed even in poorly expressed memories
- Memories that seem absent or lost can be recovered by a reminder

Abstract

Pre-training administration of scopolamine (SCP) resembles situations of cholinergic dysfunction, leading to memory impairment of mice trained in an inhibitory avoidance task. We suggest here that SCP does not impair memory formation, but acquisition is affected in a way that reduces the strength of the stored memory, thus making this memory less able to control behavior when tested. Hence, a memory trace is stored, but is poorly expressed during the test. Although weakly expressed, this memory shows extinction during successive tests, and can be strengthened by using a reminder. Our results indicate that memories stored under cholinergic dysfunction conditions seem absent or lost, but are in fact present and experience common memory processes, such as extinction, and could be even recovered by using appropriate protocols.

1. Introduction

Subjects exposed to new experiences acquire novel information and may store it, eventually causing behavioral changes. Once acquired, the new information is progressively stabilized over time through a consolidation process [28,29]. This consolidation process depends on a variety of conditions, meaning that it could be modulated through changes in the individual's internal state or by changing environmental conditions that modify the internal state. Among others, an important neurotransmitter involved in defining this particular state is acetylcholine. Thus, central cholinergic system has been implicated in learning and memory processes, either in invertebrates [6,13,44,46] and vertebrates [3,8-11,34,38,41], including human subjects. It also appears to be involved in modulation of acquisition, consolidation, reconsolidation, extinction, and retrieval of information.

High acetylcholine levels are necessary for acquisition of new information [23]. It has been consistently found that cholinergic blockade through administration of the muscarinic cholinergic antagonist scopolamine (SCP) before the training trial, results in acquisition impairments of several learning tasks, causing anterograde amnesia [6,8,9,13,16,17,33,42]. This type of amnesia, named scopolamine-induced amnesia, was also found in human subjects [19-21,40]. Traditional views considered the amnesic effect of SCP on human memory as due to acquisition impairment, and that other memory processes were not involved [19,20].

Several studies have evidenced recovery from amnesia, suggesting that under appropriate conditions a memory that would otherwise remain unexpressed may emerge [9,10,13,22,25,31,35,36,39]. These recoveries are the core of the still open debate about the nature of experimental amnesia. In the case of reversal of amnesia it could be speculated that despite the treatment inducing amnesia, the new information was actually stored, but the memory failed in controlling behavior when retrieved. A memory is behaviorally expressed when it effectively gains control of behavior. Hence, it is possible that during the retention test a memory can be retrieved but other processes (decision-making, for example) determine whether this memory will control behavior or will remain unexpressed.

Some studies suggested that SCP does not impair either memory acquisition or consolidation, but a long-term memory trace is stored, that could be recovered by appropriate treatments [9,13]. Hence, as SCP does not completely block memory formation, one could ask what is actually happening with this memory during the test sessions. The present work is aimed to evaluate how this stored but poorly expressed memory behaves, particularly whether this memory experience extinction, and to explore a physiological way to enhance its strength.

2. Materials and methods

2.1. Experimental animals

CF-1 male mice from our own breeding stock were used (age 45-60 days; weight 25-30 g). They were individually identified and housed in stainless-steel cages, 7 to 10 per cage. The mice were kept in a climatized animal room (21–23 °C) maintained on a 12 h light/dark cycle (lights on 06:00 h) 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 No. 80-23/96), and local regulations. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Behavioral procedures: Inhibitory avoidance (IA) task

The avoidance behavior was studied in one-trial learning, step-through type, which utilizes the natural preference of mice for a dark environment. The apparatus consisted of a dark compartment (20cm×20cm×15cm) with a stainless-steel grid floor and a sliding door opened in its front centre communicating with a small illuminated platform (5cm×5cm) attached to it and elevated 100 cm from the floor (conditioning context) [7].

The mice were not exposed to the apparatus before the learning trial. During training each mouse was placed on the illuminated platform. When the mouse stepped into the dark compartment with its four paws, it received a foot-shock of 0.4 mA (rms amplitude) - 50 Hz - 3 sec, that yields median retention scores at the ceiling, or a mild foot-shock of 0.2 mA (rms amplitude) - 50 Hz - 3 sec, used for repeated training (experiment 4) and as a reminder (T3 of experiments 1, 2, 3, and 5). During the retention tests, performed at the times indicated for each experimental group, 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 sec. In the latter case, the mouse was immediately removed from the platform and assigned a score of 300 sec. In the retention test sessions the foot-shock was omitted (except in repeated training conditions and when a reminder is delivered).

2.3. Drug administrations

Scopolamine hydrochloride (SCP) and scopolamine methylbromide (mSCP) were purchased from Sigma (St. Louis, MO). The drugs were dissolved in sterile saline solution immediately before their use, and were injected intraperitoneally at a volume of 0.1 ml/kg. All other agents were of analytical grade and obtained from local commercial sources. The doses of the drugs were calculated as the free bases, and were determined from previous experiments of our laboratory [8,9]. Experiments were carried out in a blinded fashion with regard to drug treatments.

2.4. Data analysis

Latencies to step-through either during the training or the retention test were expressed as medians and interquartile ranges. Comparisons among groups were performed with the non-parametric analysis of variance of Kruskal-Wallis or the Mann-Withney U test (depending on the number of groups included in each experiment), or with the non-parametric Friedman test (for comparison of repeated measures performed to the same group). The differences between groups were estimated, when appropriate, by the non-parametric post-hoc Dunn's test. The value of the statistic parameter (H for Kruskal-Wallis, Q for Friedman, U for Mann-Withney, and z for Dunn) is provided for each comparison, when appropriate. In all cases *P* values less than 0.05 were considered significant.

3. Results

3.1. A mild reminder can save the avoidance memory impaired by scopolamine

The first experiment was aimed to determine whether the pre-training administration of SCP allowed acquisition and consolidation of the avoidance memory. The behavioral protocol is represented in fig 1. Four groups of 10 mice each received an injection either of vehicle or SCP (0.5, 1.0 or 5.0 mg/kg), and 20 min later they were trained in the IA task. Retention tests were performed 24 (T1), 48 (T2) and 72 (T3) hours after training. During T3, once mice entered into the dark compartment received a mild foot-shock (0.2 mA, 50 Hz, 3 sec) as a reminder, and were tested again 24 hours later (at day 4 after training, T4).

The administration of SCP 20 min before the training session impaired retention performance in T1 of the IA task ($H_{(3,36)}$ = 21.80, $p<0.0001$; for Veh vs. SCP 0.5: $z=16.50$, $p<0.01$; for Veh vs. SCP 1.0: $z=18.55$, $p<0.01$; for Veh vs. SCP 5.0: $z=22.55$, $p<0.001$). Over consecutive tests performed 24 hours apart (T1-T3), retention performance progressively decayed (comparing performance in T1 vs T3; for Veh: $z=4.00$, $p<0.05$; for SCP 0.5: $z=11.50$, $p<0.05$; for SCP 1.0: $z=10.50$, $p<0.05$; for SCP 5.0: $z=13.00$, $p<0.05$), suggesting memory extinction development. The application of a mild foot-shock as a reminder during T3 was followed by significant improvement of retention performance in T4 (comparing performance in T3 vs T4 for each group; for Veh: $z=–20.50$, $p<0.05$; for SCP 0.5: $z=–33.00$, $p<0.001$; for SCP 1.0: $z=–31.50$, $p<0.001$; for SCP 5.0: $z=–31.00$, $p<0.001$) (Fig 1).

No significant differences were found during T4 among groups ($H_{(3,36)}$ = 7.481; $p>0.05$).

Results of this experiment confirm that pre-training administration of SCP does not blocked memory acquisition or formation. Instead, memory consolidation was possible, but the stored memory is poorly expressed in subsequent retention tests, unless a reminder is provided, after which memory becomes evident.

3.2. The mild foot-shock delivered in T3 induces development of a low level of avoidance memory in unshocked animals

This experiment was aimed to determine whether the mild foot-shock used as a reminder in T3 of the previous experiment was able to induce a similar level of avoidance memory in mice not receiving the foot-shock during training (unshocked mice). The behavioral protocol is represented in Fig 2. Four groups of 7-9 mice received an injection either of vehicle or SCP (0.5, 1.0 or 5.0 mg/kg), and 20 min later they were trained in the IA task, but without receiving the foot-shock (N_{Veh} = 9; $N_{SCP\ 0.5}$ = 9; $N_{SCP\ 1.0}$ = 8; $N_{SCP\ 5.0}$ = 7). Retention tests were performed 24 (T1), 48 (T2) and 72

(T3) hours after training. During T3, when mice entered into the dark compartment received a mild foot-shock (0.2 mA, 50 Hz, 3 sec), and were tested again 24 hours later (at day 4 after training, T4).

No differences were found in the performance among groups during the training session and during the tests (in all cases, $p>0.05$; for TR: $H_{(3,36)} = 6,741$; for T1: $H_{(3,36)} = 4.181$; for T2: $H_{(3,36)} = 2.845$; for T3: $H_{(3,36)} = 1.697$; for T4: $H_{(3,36)} = 4.069$), despite receiving or not vehicle or SCP 20 min before the training session.

Latencies to step-through of all groups increased after receiving the mild foot-shock on T3 (comparing performances on T4 vs T3 for each group; for Veh: $z=–20.00$, $p<0.05$; for SCP 0.5: $z=–21.50$, $p<0.05$; for SCP 1.0: $z=–25.50$, $p<0.001$; for SCP 5.0: $z=–26.50$, $p<0.001$). This mild foot-shock induced an avoidance level of about 30 to 50 sec, much lower than the elicited in mice trained with foot-shock (Figures 1 and 2).

3.3. The effects of SCP on memory are centrally mediated

This experiment was aimed to discard the possible involvement of peripheral effects of SCP on the observed behavior. With this in mind, methyl-scopolamine (mSCP), a quaternary analog of SCP with limited passage to the central nervous system, was used. The behavioral protocol and results are represented in Fig 3. Two groups of 10 mice each received an injection either of vehicle or mSCP (5.0 mg/kg), and 20 min later they were trained in the IA task. Retention tests were performed 24 (T1), 48 (T2) and 72 (T3) hours after training. During T3, when mice entered into the dark compartment received a mild foot-shock (0.2 mA, 50 Hz, 3 sec), and were tested again 24 hours later (at day 4 after training, T4).

No differences were found between groups at any test ($p>0.05$, comparing the same test number of Veh and mSCP, figure 3).

Results obtained for both groups were very similar to those obtained for vehicle-injected group of experiment 1. From T1 to T3, retention performance progressively decayed in both groups (T1 vs T3 for Veh: $Q=9.314$, $z=12.50$, $p<0.05$; T1 vs T3 for SCP 5.0: $Q=12.81$, $z=15.00$, $p<0.01$), suggesting memory extinction development. The application of a mild foot-shock as a reminder during T3 was followed by significant improvement of retention performance in T4 (comparing performance in T3 vs T4 for Veh: $z=–20.50$, $p<0.05$; and for SCP 5.0: $z=–23.00$, $p<0.05$) (Fig 1).

Taken together, these results suggest that no peripheral effect of mSCP is able to affect the measured behavior; therefore, the effects of SCP on performance are centrally mediated.

3.4. Level of avoidance behavior obtained after repeated training with the mild footshock

One group of 9 mice was repeatedly trained over 4 consecutive days in the inhibitory avoidance task, using the mild footshock (0.2 mA, 50 Hz, 3 sec). Their behavior was evaluated 24 hours apart, and in each test mice received the mild footshock as they entered into the dark compartment. The behavioral protocol and the results can be seen in figure 4.

A statistically significant progressive increase in the latencies to step-through can be observed from training to T2 ($Q=16.22$, $p<0.0001$; for TR vs T1: $z=–10.00$, $p>0.05$; for TR vs T2: $z=–26.00$, $p<0.01$; for TR vs T3: $z=–25.00$, $p<0.01$; for TR vs T4: $z=–24.00$, $p<0.01$). No further significant increase in latencies were observed after T2, reaching a plateau (for T2 vs T3: $z=1.00$, $p>0.05$; for T2 vs T4: $z=2.00$, $p>0.05$).

4. Discussion

The main finding of the present work is that despite pre-training administration of SCP memory acquisition and formation are produced, and that the stored memory shows extinction during successive retention tests.

‘‘Amnesia’’ is the clinical or experimental condition which makes a subject unable to demonstrate a memory [43]. It refers to a specific, acquired difficulty in learning new information and/or remembering information from the past [12]. Of course, if the individual did not store information, no memory was formed, and, therefore, amnesia occurs. However, it may happen that memory was in fact stored, but for some reason the individual fails to express it; that is, this memory cannot take control of behavior [43]. The process by which a memory drive behavior was named ‘‘memory expression’’ [26]. The capability of memories to guide behavior is a way to describe the strength of a memory, and this strength can be modified by modulation, depending on the history of each memory.

In experimental approaches, memory cannot be directly measured, but is inferred from a specific change in behavior [14]. This specific response is what will be actually measured and finally taken as a signal of the presence of memory. Therefore, amnesia is suspected when experimental subjects do not change their behavior in the expected way.

Many clinical conditions include varying degrees of amnesia [27]. In such cases it is not easy to decide whether this amnesia is due to a disappearance of memories, or the memories are still stored but the individual fails to express them. Because during

normal aging reduced cholinergic activity occurs in the central nervous system and as the degree of cholinergic impairment correlates fairly accurate with the magnitude of the progressive cognitive decline, it was early proposed a cause-effect relation by which the cognitive decline would be due to reduced number, or their activity, of central cholinergic neurons [4]. The progressive decline in cognitive abilities during normal aging includes increasing difficulty to access memories [32,37]. Geriatric mild cognitive impairment is characterized by episodic memory impairment such as forgetting details of a recently viewed movie or conversations, which correlates with a slight reduction in cholinergic activity. If the cholinergic deficit worsens, the cognitive manifestations become more pronounced. A mild cholinergic failure is also observed during the early stages of Alzheimer's disease [32,37]. From this perspective, a decrease in central cholinergic activity, thus preventing the action of acetylcholine, could reproduce some of the aspects that characterize aging. This can be achieved, for example, by blocking cholinergic receptors with SCP. Then, this drug was used as a model, though of limited application, of aging [4].

Scopolamine (SCP) has been used for years, and is still used, for the study of learning and memory processes. This drug exerts its pharmacological action by blocking all subtypes of muscarinic acetylcholine receptors (mAChRs). Blocking mAChRs by administering SCP before learning causes anterograde amnesia that has been repeatedly observed in animal models [6,8,9,13,16,17,33,42] and also in humans [19-21,40]. If the SCP is given after training, amnesia is also observed (but retrograde in this case), suggesting that central cholinergic system is also required for consolidation and storage of new information [6,8-10,41].

Although blocking cholinergic activity produce amnesia when administered either before or after the learning session, the effects are quite different. Minimum doses of SCP (0.1 mg/kg) are sufficient to produce anterograde amnesia, whereas much higher doses (above 4 mg/kg) are needed to produce retrograde amnesia, suggesting a different role for cholinergic neurons in acquisition and consolidation of new information.

The cholinergic system is required for directed attention and for detecting stimuli [23,24], so blocking the cholinergic system produces changes in attentional processes and information processing. For this reason, when an individual is presented with a stimulus while its cholinergic system has a decreased activity, processing of new information could be altered and amnesia might be observed. Therefore, the traditional view holds that the SCP prevents the acquisition, while it would have a much smaller effect on the consolidation / storage of the memory trace [8,20,42]. Along the same lines, the difference between the effects caused by the administration of the drug

before and after training led to the interpretation that acquisition is much more dependent on cholinergic system than consolidation, and proposed that muscarinic receptors exert different functions in both memory processes [8,42].

The results presented in this work confirm that the administration of SCP before the learning trial leads to memory impairment. However, they also show that memory is formed and stored, and that this memory is partially able to control behavior, that is, this memory is somehow expressed.

In fact, throughout the work, animals receiving SCP before the learning trial show differences in the latency to enter the dark compartment between training and T1, allowing to suspect that mice in fact processed and stored some new information.

In the first experiment, a poorly expressed memory progressively decays over three retention tests (T1-T3), suggesting the development of memory extinction. The fact that a very weak foot-shock delivered during T3 induced a full memory expression in T4 supports the idea that memory was successfully formed and stored, although weakly expressed.

Memory extinction develops through the repeated non-contingent presentation of the conditioned stimulus (CS) and the unconditioned stimulus (US) [30]. Then, memory extinction is the consequence of the process of new information storage, characterized by a decrease in the amplitude and/or frequency of a conditioned response (CR) when the contingent relationship between the CS and US is lost. The original memory and the new stored one provide opposite information about the predictive value of the CS in relation to the US: the original memory determines that CS predicts the later arrival of US, while the extinction memory predicts that CS is not followed by US. The original memory compete with the extinction memory for controlling behavior, and performance in the successive retention tests will be, among other factors, a consequence of the relative strength of both memories. If CS is repeatedly presented in absence of US, the extinction memory will become progressively stronger and the conditioned response (CR) will be less manifested. As the other memory processes, extinction can be modulated. In this sense, if extinction is enhanced, the new memory trace will be more efficient to be expressed and the CR will decay faster. But if extinction is blocked, this new memory could not be formed or, if formed, will fail in controlling behavior and the CR will remain with the amplitude of the original memory (but never enhanced).

In this work, animals receiving SCP extinguished their weak avoidance memory from T1 to T3. As extinction development involves a new learning process but not unlearning nor forgetting, the original memory trace should be conserved, and should be evidenced through an appropriate treatment. Four main protocols are commonly used in order to demonstrate the presence of a memory trace that remained silent. These

four protocols are spontaneous recovery, saving, reinstatement and renewal [18]. Throughout this work, a weak foot-shock was employed as a reminder to demonstrate that despite the very low latencies of step-through exhibited by animals during T3, memory was still stored, but remained silent, unexpressed. In this sense, a very low foot-shock applied at the end of T3, allowed memory to be expressed in T4.

Although recovery from amnesia were observed in all the experimental groups, it should be considered that the use of a ceiling score of 300 sec could be masking greater avoidance scores for the vehicle-injected group that would probably appear if no ceiling was used. Thus, a ceiling effect could be hiding an incomplete reversal of amnesia in SCP-injected mice.

The reversal of memory impairment observed from T3 to T4 in experiment 1, could be alternatively interpreted as the result of a second training training, as the shock delivered in T3 could be “adding” avoidance behavior from a higher starting point than during training. The mentioned possibility, mainly supported by the behavioral tagging hypothesis, seems very unlikely in the present case. The behavioral tagging hypothesis proposes that a weak event that induces transient changes in the brain can establish long-lasting phenomena through a tagging and capture process [45]. If this were the case, then the weak training performed repeatedly in experiment 4 should cause a progressive increase in avoidance latencies that should quickly reach the level of avoidance of the rest of the experiments. Opposite to this, latencies to step-through increases from TR to T2, but no differences were found after this second test session, suggesting that the maximal level of avoidance that could be attainable with this weak shock is much lower than the ceiling scores reached by the mice trained under the effects of SCP, even with the highest dose of SCP. Thus, the weak foot-shock delivered only once was enough to induce a ceiling avoidance level in experiment 1, but despite being delivered for 4 consecutive days induced a level of avoidance much lower in the repeated training experiment. For these reasons, behavioral tagging may not provide a suitable explanation for our findings.

Other alternative interpretations for the reversal of amnesia observed in experiment 1 could emerge from the possibility that the administration of SCP before the training session put animals in a state that makes them more prone to learn in T3, for example, by proactively modulating different memory processes. If this were the case, the weak foot-shock administered in T3 might have an intense effect on learning, thus explaining the great latencies observed in T4. The results obtained in experiment 2 suggest that this is not the case. Animals receiving the SCP on day 0 (the day of the training session) and receiving a foot-shock for the first time on day 3 (the weak foot-shock on

T3) did not display such hypothesized enhancement of learning. Therefore, the SCP does not exert this kind of proactive effects.

Animals receiving mSCP did not show differences with control mice, indicating that the assessed effects due to the administration of SCP are centrally mediated.

These results could be evidencing that a neural representation of a CS-US is formed despite the administration of SCP before the learning trial, and that this representation is reactivated by the reminder, thus entering in a labile phase. All these facts support the notion that scopolamine-induced amnesia could not be explained as a result of acquisition impairment.

In terms of its pharmacodynamics, SCP is relatively nonselective with respect to muscarinic receptor subtypes, and the drug does not discriminate very much regarding the brain regions. The septohippocampal pathways are the probably the site where scopolamine exerts its effects by blocking muscarinic receptors in the hippocampus. The hippocampal cholinergic system is necessary for stimuli processing entering by the direct and indirect septohippocampal pathways, that are likely relevant for cholinergic control of encoding versus retrieval modes in the hippocampus [15]. It was demonstrated in rats that cholinergic system in the amygdala is involved in extinction processes, as the administration of oxotremorine enhance extinction [11]. Acquisition and consolidation of the context-dependent extinction involves different neurotransmitter systems than context-independent extinction. Context-dependent extinction process, as the used in our work, is modulated by D1/D5 dopamine receptors [1] and by beta adrenoceptors [2]. It was recently suggested that the different ways to gain recovery of memory in operant procedures seem to be supported by shared mechanisms [5].

Neural circuits underlying memory extinction involve several interacting regions such as the amygdala, prefrontal cortex, midbrain, and hippocampus. It was proposed that extinction training implies the formation of neural networks that inhibit the networks formed during the acquisition of the avoidance memory [47]. It is not clear, however, whether memory recovery after extinction is caused by reactivation of the original neuronal ensembles activated during avoidance memory acquisition. If this were the case, then the hippocampus and the amygdala should be determinant for this recovery.

From these results, it could be expected that mild cholinergic dysfunction still allows memory formation, but the stored memory traces fail to be behaviorally expressed. However, these memories are in fact retrieved and can experience extinction, and may be restored by an appropriate reminder, allowing them to take control of behavior in subsequent evaluations. Thus, memory impairment observed in AD patients, in part caused by cholinergic dysfunction, might actually be a symptom of memories remaining

unexpressed rather than a real memory loss, at least in the initial phases of the disease. While the loss of cortical neurons is not very extensive, memories could still be there but remain unexpressed. If these unexpressed memories are labilized and then enhanced through appropriate protocols, it might be possible to get behavioral expression of the stored information, allowing a different approach to reduce memory impairment in cholinergic failure conditions.

Taken together, our results demonstrate that despite the cholinergic blockade by scopolamine, mice were able to process the novel information during training; that the avoidance memory trace was indeed stored; that the stored information remained even after extinction; and that the residual plasticity might be reflecting the persistent memory, evidenced by recovery from the experimental amnesia.

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Figure legends

Fig. 1 – Effect of the pre-training administration of SCP on retention performance. The pre-training administration of SCP causes memory impairment on T1. From T1 to T3 this weak memory extinguishes, and performance is recovered by delivering a reminder shock on T3. The behavioral protocol is represented above the graph. SCP (0.5 – 1.0 and 5.0 mg/kg, i.p.) was administered 20 min before training. Each bar represents the median and interquartile range (N=10 mice/group). TR: training session, T1-T4: retention tests.
is used to compare with the same test of Veh-injected group;
* is used to compare with performance on T1 of the same group;
& is used to compare with performance on T3 of the same group.

Fig. 2 - Effect of the pre-training administration of SCP on performance of mice trained without receiving the foot-shock (unshocked mice). The weak foot-shock delivered on T3 elicits retention performances of about 30 to 60 seconds. The behavioral protocol is represented above the graph. SCP (0.5 – 1.0 and 5.0 mg/kg, i.p.) was administered 20 min before training. Each bar represents the median and interquartile range (N_{Veh} = 9; $N_{SCP\ 0.5}$ = 9; $N_{SCP\ 1.0}$ = 8; $N_{SCP\ 5.0}$ = 7). TR: training session, T1-T4: retention tests.
& is used to compare with performance on T3 of the same group.

Fig. 3 - Effect of the pre-training administration of mSCP on performance of mice trained in the inhibitory avoidance task. Pre-training administration of mSCP is not followed by memory impairment. The behavioral protocol is represented above the graph. mSCP (5.0 mg/kg, i.p.) was administered 20 min before training. Each bar represents the median and interquartile range (N = 10 mice/group). TR: training session, T1-T4: retention tests.

* is used to compare with performance on T1 of the same group;

& is used to compare with performance on T3 of the same group.

Fig. 4 - Effect of repeated training using a weak foot-shock. The latencies to step-through increase from TR to T2, and no further increases were detected after T2. The behavioral protocol is represented above the graph. Each bar represents the median and interquartile range (N = 9 mice).

* is used to compare with performance on TR (the first training session).

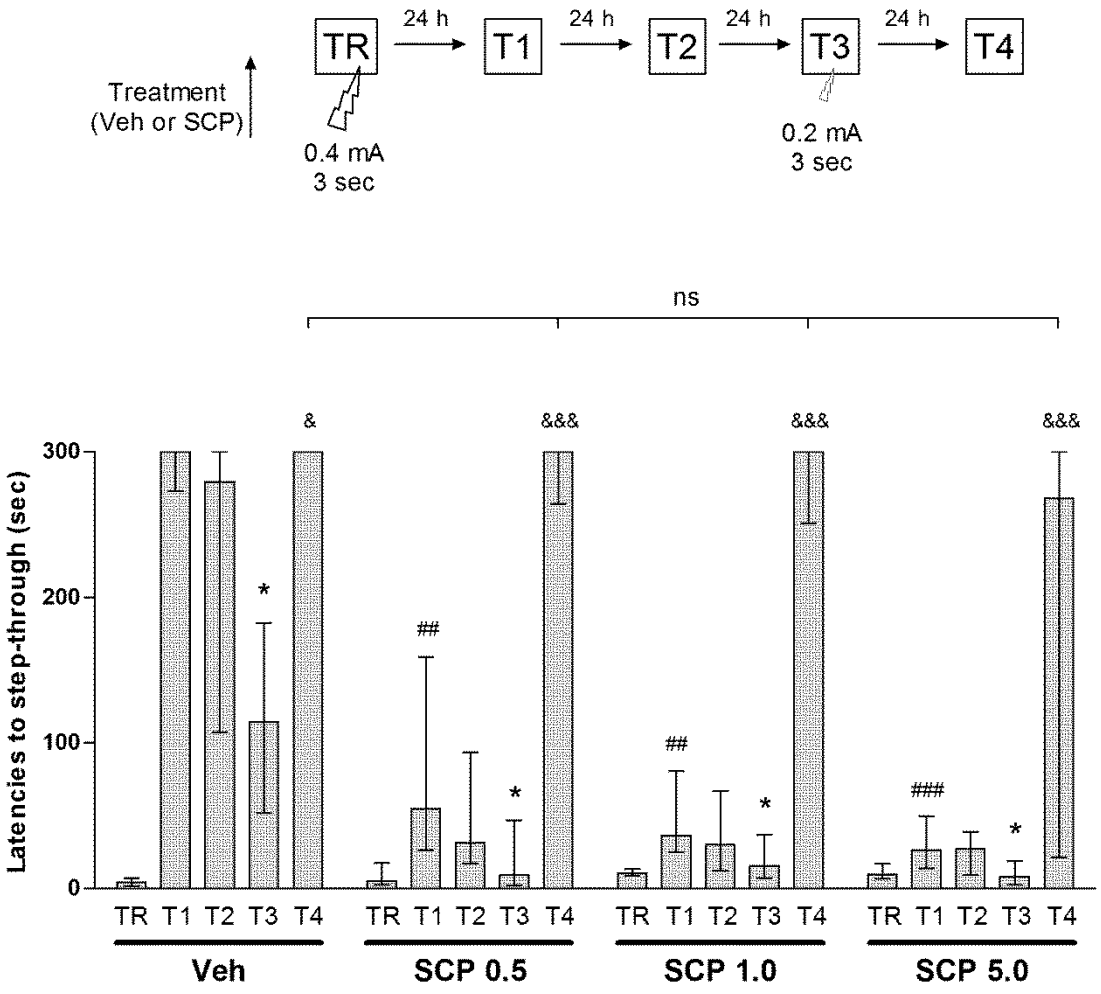

Figure 1

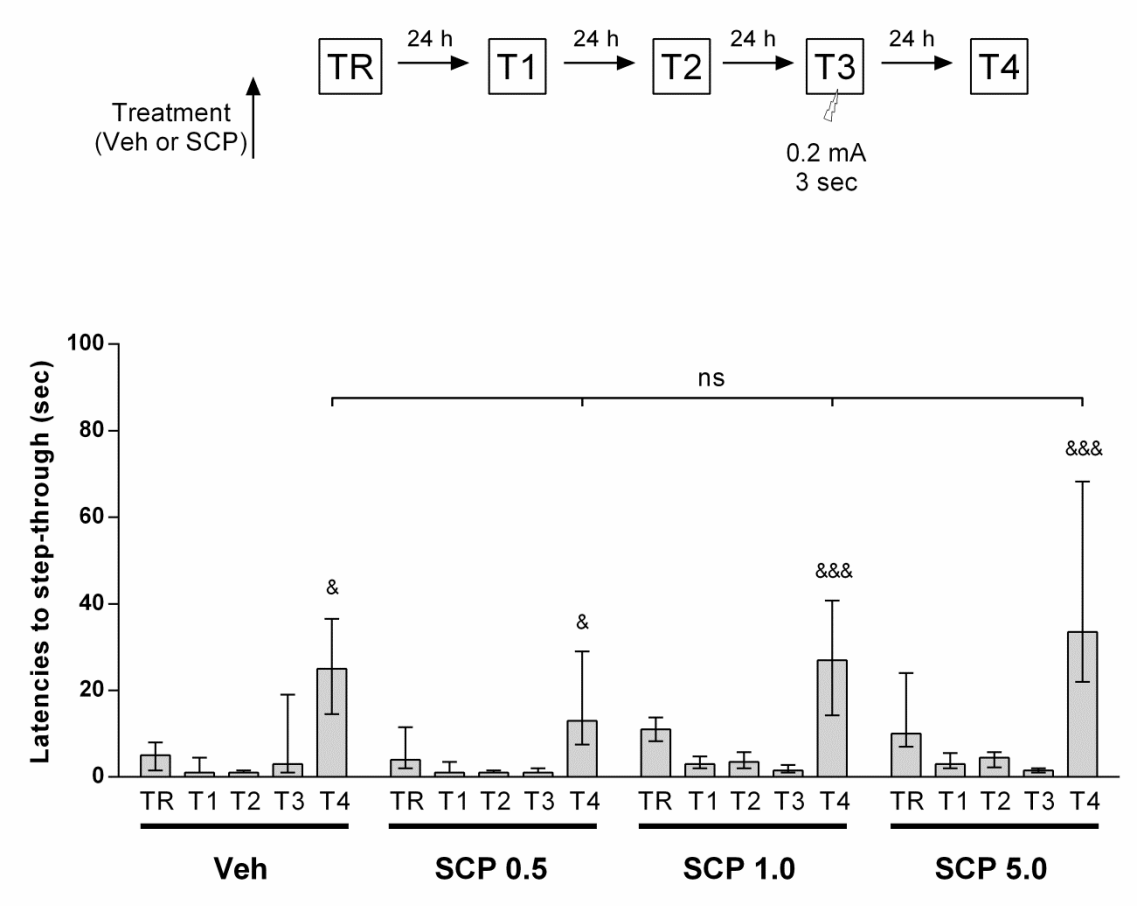

Figure 2

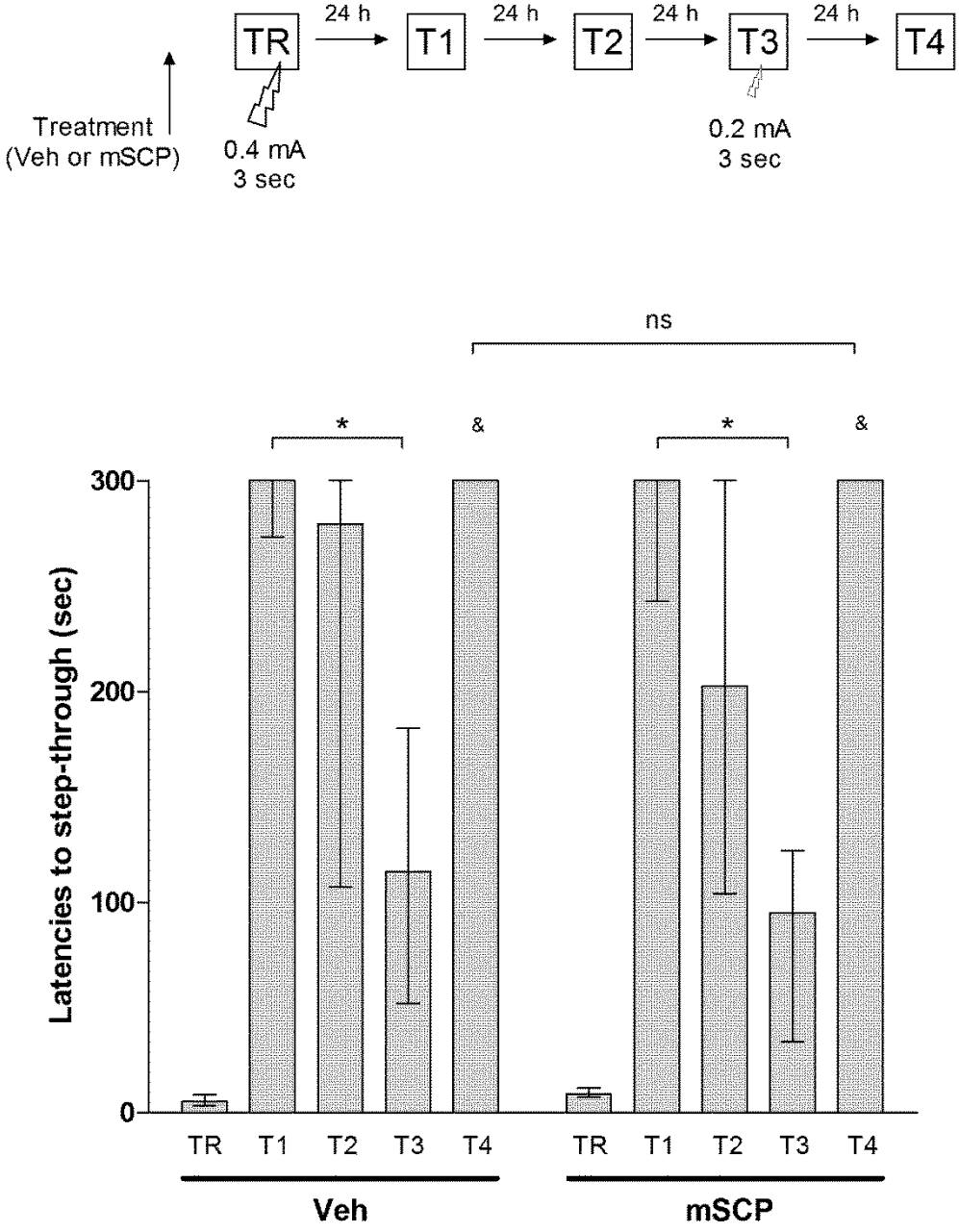

Figure 3

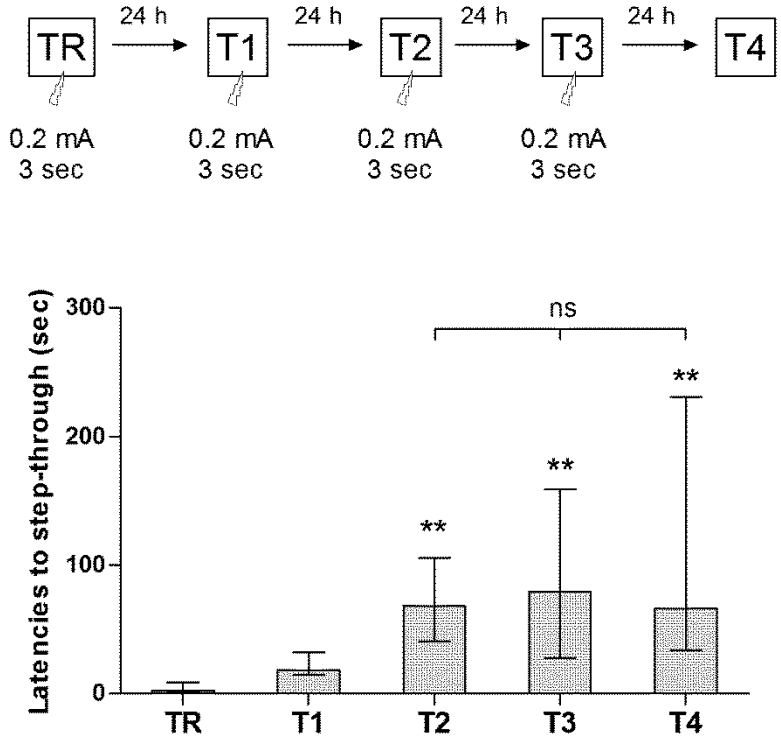

Figure 4

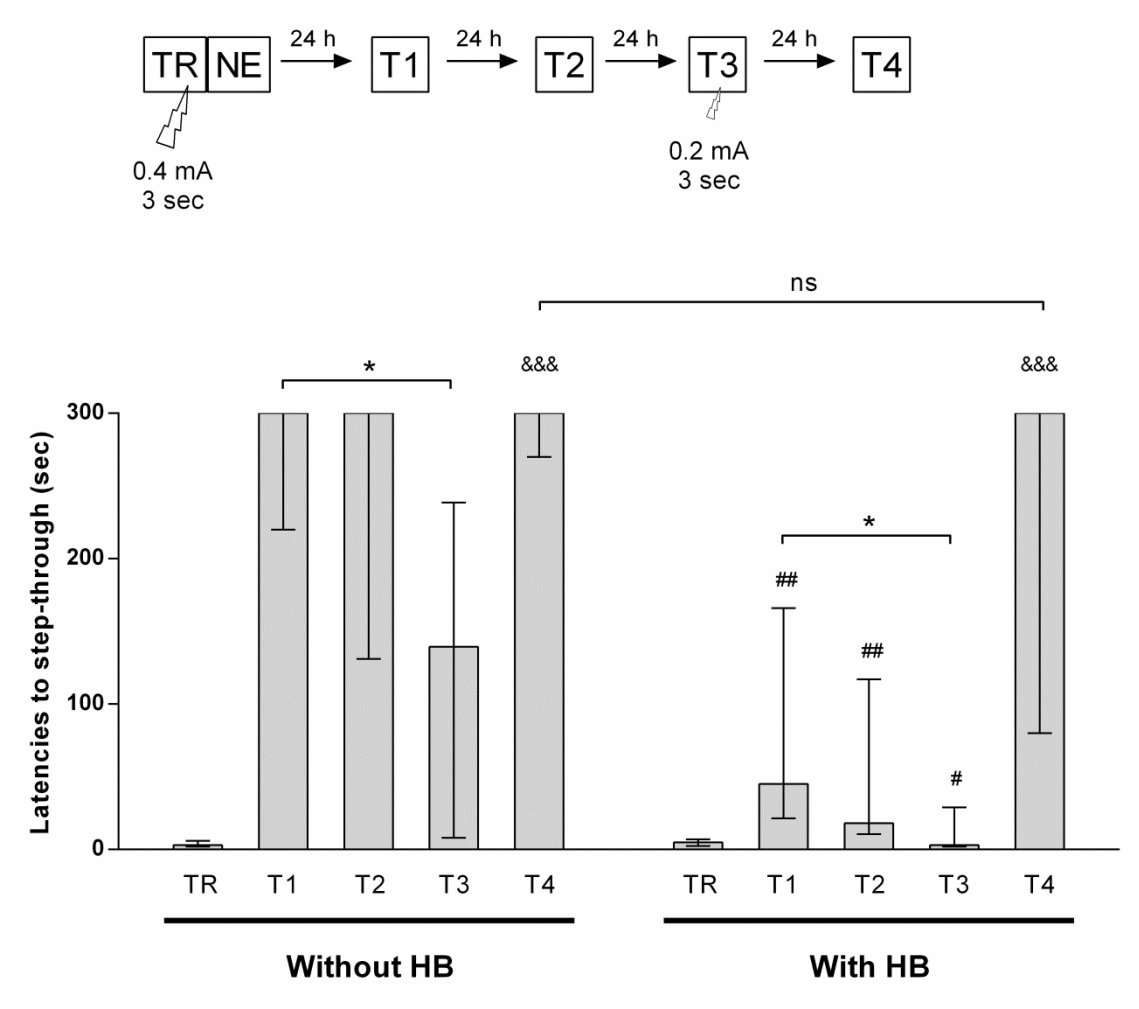

Figure 5