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ScienceDirect
Neurobiology of Learning and Memory 86 (2006) 188–196
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

Role of muscarinic M1 receptors in inhibitory avoidance and contextual fear conditioning

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Received 19 November 2005; revised 24 February 2006; accepted 25 February 2006
Available online 2 May 2006

Abstract

The objective of the present study was to observe the effects of pre-training or post-training administration of dicyclomine, a M1 muscarinic antagonist, on inhibitory avoidance (IA) and contextual fear conditioning (CFC) and to investigate if the effects observed with the pre-training administration of dicyclomine are state-dependent. For each behavioral procedure (IA and CFC) groups of Wistar male rats were treated with saline or dicyclomine either 30 min before training (pre-training), immediately after training or 30 min before training/30 min before test (pre-training/pre-test). The animals were tested 24 h after training. The acquisition of IA and CFC was impaired by pre-training administration of dicyclomine. The consolidation of both tasks was not affected by dicyclomine given immediately after training. Pre-training/pre-test administration of dicyclomine impaired both tasks, an effect similar to that observed in the group which only received pre-training administration. Pre-test treatment induced dissociation between both tasks, impairing CFC retrieval, without interfering with the animals avoidance response. These results show that the dicyclomine did not affect IA and CFC consolidation, suggesting specific involvement of M1 muscarinic receptor only in acquisition these tasks, and these effects was not state-dependent. However, it is possible that the retrieval of these tasks may be mediated, at least in part, by different neurochemical mechanisms and may be dissociated by dicyclomine.

Keywords: Inhibitory avoidance; Fear conditioning; Cholinergic; M1 muscarinic receptor; Dicyclomine

1. Introduction

A number of studies suggest that the central cholinergic system is involved in learning and memory processes (Bartus, Dean, Beer, & Lippa, 1982; Deutsch, 1971; Everitt & Robbins, 1997; Fibiger, Damsma, & Day, 1991; Van der Zee & Lutien, 1999). In particular, the cholinergic system seems to play an important role in the modulation of aversively motivated tasks, such as contextual fear conditioning and inhibitory avoidance (Fornari, Moreira, & Oliveira, 2000; Tinsley, Quinn, & Fanselow, 2004).

In contextual fear conditioning (CFC), an aversive stimulus, such as a footshock is presented in a determined environmental context. After this experience, exposure of the animal to that same context may elicit a conditioned fear response, characterized by somatomotoric immobility, known as "freezing." The inhibitory avoidance (IA) task, on the other hand, is a kind of instrumental conditioning, as the animal is punished for a response (as crossing from the light to the dark compartment of the IA apparatus), and therefore learns to inhibit that behavioral response. Similarly as in CFC, the animal goes through an aversive experience (footshock) in a determined context. However, in the IA task, the shock stimulus is contingent to the animal response, and after that experience, the animal has the possibility of avoiding that context.

Similarities between CFC and IA procedures suggest that these tasks may be modulated by common neural mechanisms. Indeed, some manipulations have been shown to affect performance in both tasks (Fornari et al., 2000). However, other studies suggest that the neural substrates

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1074-7427/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2006.02.006

underlying these two tasks may be partially distinct. Based on amygdala lesion studies, Maren (2003) suggests that this structure mediates the stimuli association required for learning a CFC response, but it would not be necessary for the stimulus-response association required in the IA task. The systematic evaluation of the effects of experimental manipulations on both tasks, performed under similar experimental conditions, may shed some light on anatomical and pharmacological mechanisms underlying emotional memory (Tinsley et al., 2004).

The involvement of cholinergic muscarinic receptors in the acquisition and consolidation of tasks comprising conditioned fear responses, such as those observed in IA and CFC, has been reported. Administration of scopolamine, a nonselective antagonist of muscarinic receptors, interferes with the acquisition of both IA and CFC when administered before the training session (Anagnostaras, Maren, & Fanselow, 1995; Anagnostaras, Maren, Sage, Goodrich, & Fanselow, 1999; Anglade, Bizot, Dodd, Baudoin, & Chapouthier, 1994; Bammer, 1982; Calhoun & Smith, 1968; Elrod & Buccafusco, 1988; Feiro & Gould, 2005; Meyers, 1965; Rudy, 1996; Rush, 1988). However, conflicting results were reported regarding the systemic post-training administration of scopolamine. While some authors reported impaired performance (Roldán, Bolaños-Badillo, González-Sánchez, Quirarte, & Prado-Alcalá, 1997; Rudy, 1996; Rush, 1988), others observed no significant effects of scopolamine on either IA or CFC (Anagnostaras et al., 1999; Calhoun & Smith, 1968; Elrod & Buccafusco, 1988). According to Tinsley et al. (2004), these inconsistencies may be due to differences in experimental procedures, such as the number of training trials (and footshocks) used in different studies. The post-training amnestic effects of scopolamine seems to be evident only in studies in which training consisted of a single footshock pairing, but not in studies using several footshocks.

Although the evidence obtained with scopolamine studies suggest that IA and CFC tasks share a common mechanism associated with the cholinergic system function, due to the lack of selectivity of scopolamine, it is not possible to infer which subtypes of muscarinic receptors would be primarily involved with. Some evidence points to an important function of the M1 muscarinic subtype of receptors in learning and memory processes (Hagan, Jansen, & Broekkamp, 1987; Hunter & Roberts, 1988; Sala et al., 1991). Furthermore, muscarinic M1 receptors are widely distributed in the amygdala, cerebral cortex, and hippocampus (Levey, Kitt, Simonds, Price, & Brann, 1991; Moreira et al., 2003; Wei, Walton, Milici, & Buccafusco, 1994), areas where the cholinergic transmission seems to be essential for learning and memory processes. Administration of selective M1 antagonists, such as pirenzepine (Caulfield, Higgins, & Straughan, 1983; Ohnuki & Nomura, 1996; Worms, Gueudet, Perio, & Soubrie, 1989), biperiden, and trihexyphenidyl (Kimura, Ohue, Kitaura, & Kihira, 1999), impairs the acquisition of IA, pointing to the involvement of M1 muscarinic receptors in the modulation of this kind of learning task.

Similarly, previous data from our laboratory have shown that dicyclomine, another antagonist with high affinity for M1 receptors, impairs both IA and CFC when administered before the training session (Fornari et al., 2000). Nonetheless, the animals were under the effect of dicyclomine not only during training, but also in the period immediately subsequent to the training session. Therefore, no conclusions could be drawn regarding whether dicyclomine affects specifically the acquisition or the consolidation of learning in IA and CFC. Studies purported to analyze the effects of selective M1 antagonists on consolidation of the IA task were reported. The systemic administration of selective M1 antagonists, such as biperiden and trihexyphenidyl in rats (Roldán et al., 1997) or dicyclomine in mice (Galeotti, Ghelardini, & Bartolini, 1998, 2000), impaired the avoidance response when administered immediately after the training session. If the activation of M1 receptors is similarly important for IA and CFC, one would expect that the administration of the selective M1 antagonist dicyclomine, both before and after training, will induce similar effects on both tasks.

Another important issue regarding pharmacological studies in which the drug is administered before the training session and the animals are later tested in a different state (without the presence of the drug), is the possibility of state-dependent learning (Overton, 1968). If this type of learning occurs in the presence of dicyclomine, it would be expected that the administration of this substance both before the training and the test sessions will not induce impairment in IA and CFC, considering that the neural mechanisms underlying both tasks would be similar.

To compare the effects of the administration of an M1 antagonist (dicyclomine) on the acquisition and consolidation of IA and CFC tasks in rats, similar parameters will be used in both tasks. Furthermore, the possibility of state-dependent learning with dicyclomine will also be analyzed.

2. Materials and methods

2.1. Subjects

Wistar male rats, 3–4 months old, bred and raised in the animal facility of the Department of Psychobiology, UNIFESP, were used for these experiments. Animals were maintained under controlled temperature (23 ± 2 °C) and 12:12-h light–dark cycle (lights on between 7:00 and 19:00 h) conditions and were provided food and water ad libitum. The procedures were taken following the Ethical Committee of UNIFESP, in accord with international rules for animal use and care.

2.2. Drugs

Dicyclomine chloride (Sigma Chemical Co.) was dissolved in 0.9% saline and injected, i.p., in a volume of 1.0 ml/kg. The doses used were 16, 32, and 64 mg/kg. The solutions were maintained at 30 °C, in a water bath, to avoid precipitation of the salt. Control animals received 0.9% saline.

The doses and the interval between administration and training were chosen on the basis of previous studies reporting no effect of 2 and 8 mg/kg of dicyclomine on acquisition of CFC and IA tasks, while 16 mg/kg resulted in memory impairments (Fornari et al., 2000).

2.3. Apparatus

The IA apparatus consisted of two compartments, each measuring 22 × 21 × 22 cm, connected by a sliding door. The walls of the safe compartment were white, whereas the other compartment, where the animals received footshock, had black walls with visual patterns (2 squares measuring 5.5 × 5.5 cm and 3 squares measuring 4.0 × 4.0 cm made of white cardboard). The tops of both compartments were covered with transparent acrylic. The floor consisted of a metal grid (0.4 cm-diameter rods placed 1.2 cm apart from each other) connected to a shock generator and control module (Ugo Basile model 7551), by which footshocks of 1 mA and 1 s long could be delivered.

The open field apparatus was 80 cm in diameter and surrounded by walls 30 cm high. The open field floor was divided into three concentric circles and subdivided by painted black lines into 18 sectors.

For tone fear conditioning (TFC) test a white cylindrical chamber, 35 cm in diameter and 30 cm height, covered with transparent acrylic was used. The floor was also made of white acrylic. A buzzer placed outside the apparatus produced a 60-dB tone. Conditioning chamber was cleaned with 30% alcohol solution, and tone test chamber with another cleaning product, to characterize each chamber with a different scent.

2.4. Behavioral procedures

2.4.1. Inhibitory avoidance task

The IA task was performed in two sessions (training and test). In the training session, the animals were placed, individually, inside the light compartment (safe side) of the avoidance apparatus. Ten seconds later the door was opened, and, as soon as the animal entered the black compartment with all four paws, the door was closed and one footshock (1 mA, 1 s) was delivered. The latency for the animal to enter the black compartment was recorded. Immediately after the footshock the animal was removed from the apparatus and returned to the homecage. The test was carried out 24 h after training. Each animal was placed again in the light compartment of the avoidance apparatus, and, 10 s later, the door was opened and the time taken by the animal to cross to the black compartment (four paws in) was recorded (test latency). If the animal did not cross within 300 s, it was removed from the apparatus and a latency of 300 s was attributed. No footshock was delivered during the test.

2.4.2. Contextual fear conditioning task

The task was carried out during two consecutive days. On the first day (training), the animals were individually placed in the black compartment of the avoidance apparatus previously described, with the sliding door closed during all CFC procedure. Two minutes later, one footshock (1 mA, 1 s) was delivered. Immediately after the footshock, the animal was removed from the apparatus and returned to its homecage. The test was performed on the second day, 24 h after the training. Each animal was placed in the same training context, that is, directly into the dark compartment of the avoidance apparatus. The sliding door remained closed and no footshock was delivered. The freezing time—defined as complete immobility of the animal, with the absence of vibrissae movements and sniffing—was recorded continuously minute by minute during 5 min with a chronometer by an experienced observer.

2.4.3. Locomotor activity in the open field

The animals were individually placed in the open field arena, and were permitted to freely explore the arena for five minutes. The total number of sectors crossed (locomotion) was recorded during 5 min, with hand-operated counters.

2.4.4. Tone fear conditioning task

In the first day (training), animals were individually placed in the black compartment of the avoidance apparatus (conditioning chamber) previously described, with the sliding door closed during all TFC procedure. Two minutes later, a tone (60 dB; the CS) sounded for 30 s, and in the last second a footshock (1.0 mA, 1 s, the US) was delivered, ending together with the tone. Immediately after the footshock, the animal was removed from the apparatus and returned to its homecage. On the second day, TFC was tested. Animals were individually placed in the cylindrical chamber (new context) for 5 min. At the end of the third minute of exposure to the apparatus, one tone (60 dB/30 s) was presented. No footshock was delivered. Freezing time was measured both before and after tone presentation.

2.5. Experimental design

2.5.1. Experiment 1: Effects of pre-training administration of dicyclomine on the acquisition of IA and CFC

To assess the effects of dicyclomine on the acquisition of IA and CFC, saline or dicyclomine (16 or 32 mg/kg) was administered 30 min before training on one of the tasks. Separate groups of animals ($n = 13$–15 per group) were run for each procedure.

2.5.2. Experiment 2: Effects of pre-training/pre-test administration of dicyclomine on IA and CFC

Four groups of rats ($n = 9$–11 per group) were used to examine the possibility that state-dependent learning occurs in the presence of dicyclomine. A factorial 2 × 2 design was employed as follows: the group sal/sal received saline before both training and test; the group dic/sal received dicyclomine (32 mg/kg) before training and saline before test; the group sal/dic received saline before training and dicyclomine (32 mg/kg) before test; and the group dic/dic received dicyclomine (32 mg/kg) before both training and test. All injections were administered 30 min before training or test. Identical designs were used for the IA and the CFC tasks, in separate groups of animals.

2.5.3. Experiment 3: Effects of administration of dicyclomine on locomotor activity (A) and Tone fear conditioning (B)

To evaluate whether dicyclomine interferes with the expression of locomotor activity of animals (Experiment 3A), different groups of rats received the same treatment of Experiment 2 (saline or 32 mg/kg dicyclomine) in two consecutive days. On day 1, the animals returned to their homecages after the injection. On the second day, 30 min after the injection each rat was placed in the open-field arena and locomotor activity was recorded for 5 min. The number of animals per group was: sal/sal ($n = 7$); dic/sal ($n = 6$); sal/dic ($n = 7$) and dic/dic ($n = 7$).

To evaluate whether dicyclomine interferes with the expression of freezing (Experiment 3B), two groups ($n = 12$ per group) of rats received saline or 32 mg/kg dicyclomine in two consecutive days. On day 1, all animals received saline. Thirty minutes after the injection each rat was submitted to TFC training, as previously described. On the second day, each rat received saline or 32 mg/kg dicyclomine 30 min before being submitted to TFC test.

2.5.4. Experiment 4: Effects of post-training administration of dicyclomine on consolidation of IA and CFC

To evaluate whether dicyclomine impairs the consolidation of IA and CFC, saline or dicyclomine (16, 32 mg/kg) were given immediately after training of one of the tasks. Separate groups of animal were run for each procedure. Rats were tested 24 h later ($n = 13$–18 per group). In two other experiments, a dose of 64 mg/kg was used to observe the effect of a higher dose on consolidation of IA and CFC.

2.6. Statistical analysis

Data from the IA and CFC tasks were analyzed by two-way ANOVA, with Group and Sessions (training, test) and for TFC with Group and Tone (before, after tone) as factors. The data from locomotor activity were analyzed by one-way ANOVA. When necessary, the analysis was followed by the Tukey honest significant difference test, with the level of significance set at $p < .05$.

3. Results

3.1. Experiment 1

3.1.1. Inhibitory avoidance

Results are shown in Fig. 1A. The two-way ANOVA with repeated measures indicated significant main effects of group [$F(2,41) = 5.35$; $p < .01$] and session [$F(1,41) = 22.46$; $p < .01$], and a significant interaction between group and session [$F(2,41) = 6.39$; $p < .01$]. Post hoc tests revealed that all animals behaved similarly in the training session ($p = .99$). On the test session, however, animals that had received 16 and 32 mg/kg dicyclomine doses before training displayed significantly lower latencies to cross to the black compartment when compared to control animals ($p < .01$).

3.1.2. Contextual fear conditioning

The same dicyclomine doses that impaired the IA also impaired the CFC test (Fig. 1B). There were significant group [$F(2,37) = 9.00$; $p < .01$] and session effects [$F(1,37) = 29.01$; $p < .01$], and a significant interaction between group and session [$F(2,37) = 8.69$; $p < .01$]. In the test session, animals injected with 16 or 32 mg/kg of dicyclomine before training displayed significantly less freezing behavior than saline-treated controls ($p < .01$, for both doses). Dicyclomine administration did not affect the freezing time of animals in the training session ($p = 1.0$).

3.2. Experiment 2

3.2.1. Inhibitory avoidance

Fig. 2A presents the latency (to cross to the black compartment of the IA apparatus) displayed by sal/sal, sal/dic, dic/sal, and dic/dic groups. The two-way ANOVA revealed significant effects for Group [$F(3,36) = 4.84$; $p < .01$] and Session [$F(1,36) = 23.18$; $p < .01$], and a significant interaction between Group and Session [$F(2,36) = 5.45$; $p < .01$]. The post hoc test showed that dic/sal and dic/dic groups showed significant lower latencies when compared to the sal/sal group ($p < .05$) in the test session. The sal/dic group did not differ from the sal/sal group ($p = .96$) in the test session. There were no group differences in the training session.

3.2.2. Contextual fear conditioning

Results are shown in Fig. 2B. The two-way ANOVA with repeated measures indicated significant main effects of Group [$F(3,37) = 4.16$; $p < .05$] and Session [$F(1,37) = 41.85$; $p < .01$], and a significant interaction between Group and Session [$F(3,37) = 3.97$; $p < .05$]. Post hoc analyses revealed that sal/dic, dic/sal, and dic/dic groups displayed significant lower freezing than sal/sal controls groups in the test session ($p < .05$).

3.3. Experiment 3

3.3.1. Locomotor activity (Experiment 3A)

Administration of dicyclomine did not affect the locomotor activity of rats. The one-way ANOVA indicated no significant effect of treatment Group [$F(3,23) = 1,30$; $p = .30$]. The means ± SEM were: sal/sal, 87.28 ± 16.27 ($n = 7$); dic/sal, 95.50 ± 9.59 ($n = 6$); dic/dic, 122.43 ± 7.51 ($n = 7$); sal/dic, 102.28 ± 16.49 ($n = 7$).

3.3.2. Tone fear conditioning (Experiment 3B)

In the TFC task, the two-way ANOVA indicated a significant effect of Tone [$F(1,22) = 11.75$; $p < .01$], but no effect of Group [$F(1,22) = .19$; $p = .66$] nor interaction between Group and Tone [$F(1,22) = .75$; $p = .40$]. The mean freezing time per minute (±SEM) before tone for each group was: sal/sal, 6.3 ± 1.8 ($n = 12$); sal/dic, 2.5 ± 1.0 ($n = 12$); and after tone were: sal/sal, 13.0 ± 3.1; sal/dic, 13.7 ± 4.7.

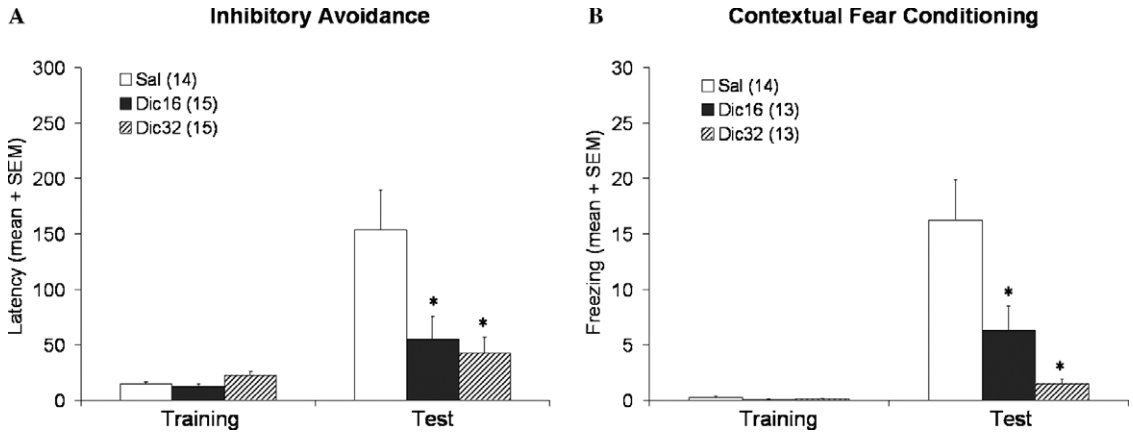

Fig. 1. Effects of pre-training administration of dicyclomine on the mean latency to cross to black compartment during training and test of the inhibitory avoidance task (A) and on mean freezing time per minute during training and test of the contextual fear conditioning task (B). Data are expressed as mean + SEM. Sal, saline; DIC16, dicyclomine 16 mg/kg; DIC32, dicyclomine 32 mg/kg. *$p < .01$ when compared to Sal group during the test. The number of animals per group is shown in parentheses after the group names.

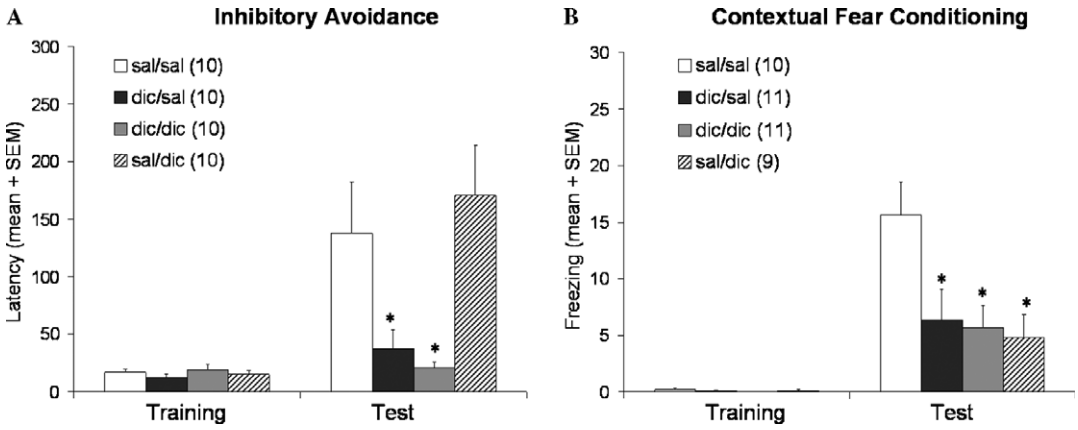

Fig. 2. Effects of pre-training/pre-test administration of dicyclomine on the mean latency to cross to black compartment during training and test of the inhibitory avoidance task (A) and on mean freezing time per minute during training and test of the contextual fear conditioning task (B). sal/sal, saline before training and test; sal/dic, saline before training and dicyclomine (32 mg/kg) before test; dic/dic, dicyclomine (32 mg/kg) before training and test; dic/sal, dicyclomine (32 mg/kg) before training and saline before test. $*p < .05$ when compared to sal/sal group during the test. Data are expressed as mean + SEM. The number of animals per group is shown in parentheses after the group names.

3.4. Experiment 4

3.4.1. Inhibitory avoidance

When animals were injected immediately after training, (16 and 32 mg/kg) dicyclomine did not affect the IA task (Fig. 3A). The two-way ANOVA indicated a significant effect of Session [$F(1,48) = 130.63$; $p < .001$], but no effect of treatment Group [$F(2,48) = 1.20$; $p = .31$] nor interaction between Group and Session [$F(2,48) = .93$; $p = .40$]. The higher dose (64 mg/kg) tested also did not affect the IA task (Fig. 3C). The two-way ANOVA indicated a significant effect of Session [$F(1,27) = 24.80$; $p < .001$], but no effect of

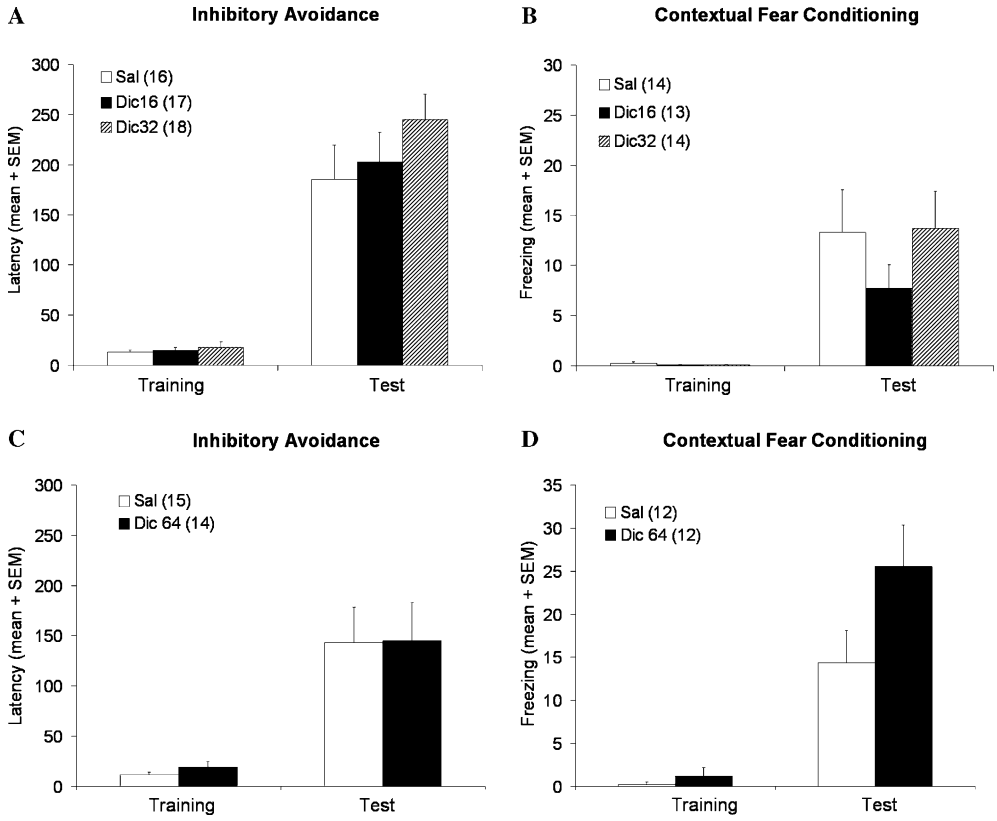

Fig. 3. Effects of post-training administration of dicyclomine on the mean latency to cross to black compartment during training and test of the inhibitory avoidance task (A and C), and on mean freezing time per minute during training and test of the contextual fear conditioning task (B and D). Sal, saline; DIC16, dicyclomine 16 mg/kg; DIC32, dicyclomine 32 mg/kg; DIC64, dicyclomine 64 mg/kg. Data are expressed as mean + SEM. The number of animals per group is shown in parentheses after the group names. No significant differences were found.

treatment Group [$F(1,27) = .04$; $p = .84$] nor interaction between Group and Session [$F(1,27) = .01$; $p = .90$].

3.4.2. Contextual fear conditioning

The post-training administration of dicyclomine also did not affect the retention 24 h later of CFC task (Figs. 3B and D). The two-way ANOVA indicated a significant effect of Session [$F(1,38) = 30.01$; $p < .001$], with no effect of treatment Group [$F(2,38) = 0.87$; $p = .43$] nor Group and Session interaction [$F(2,38) = 0.81$; $p = .45$]. The higher dose (64 mg/kg) tested did not affect the CFC task (Fig. 3D). The two-way ANOVA indicated a significant effect of Session [$F(1,22) = 45.74$; $p < .001$], with no effect of treatment Group [$F(1,22) = 3.86$; $p = .06$] nor Group and Session interaction [$F(1,22) = 3.29$; $p = .08$].

4. Discussion

The results obtained in the present study showed that: (1) administration of different doses of dicyclomine (16 and 32 mg/kg) before training impaired both IA and CFC tasks; (2) pre-training/pre-test treatment affected both tasks; (3) pre-test (without pre-training) treatment, on the other hand, induced a dissociation between the two tasks, affecting the conditioned fear response but not the avoidance response; and (4) none of the tasks was affected by the post-training administration of dicyclomine.

In the first experiment, the administration of dicyclomine before the training session reduced the retention latency in the IA task and the conditioned freezing response in the CFC task, when measured in the test session. These results agree with previous results from our laboratory, which showed impairment in the acquisition of both tasks after the administration of dicyclomine (Fornari et al., 2000); and are similar to other studies in the literature, showing that the systemic administration of M1 selective antagonists, such as pirenzepine (Caulfield et al., 1983; Ohnuki & Nomura, 1996; Worms et al., 1989), biperiden, and trihexyphenidyl (Kimura et al., 1999), impairs the acquisition of IA. These data point to an important participation of M1 receptors in the acquisition of both IA and CFC.

However, since animals were trained under the influence of the drug and were tested in a drug-free state, dicyclomine-induced impairment in IA and CFC could be due to state-dependent learning (Overton, 1968). Results from Experiment 2 suggest an absence of state-dependent learning under dicyclomine in the IA task. If state-dependent learning had occurred, rats receiving dicyclomine before the training and the test (group dic/dic) would present similar performance as the rats treated with saline before the training and the test (group sal/sal), since they were both trained and tested under the same "drug" (or saline) state. Besides, it would also be expected that animals trained and tested under different drug states (groups dic/sal and sal/dic) would present impairment. However, this was not observed in the IA task: the pre-training administration of dicyclomine affected the avoidance response, regardless of the pre-test administration of the drug. Furthermore, dicyclomine did not interfere with performance or retrieval in this task, since the group sal/dic was not impaired. These results are similar to those obtained with scopolamine, which showed absence of state-dependent learning in the IA task (Calhoun & Smith, 1968; Elrod & Buccafusco, 1988). Therefore, it seems reasonable to suggest that scopolamine effects on memory acquisition in IA may be mediated by muscarinic M1 receptors.

Similarly as in IA, the pre-training administration of dicyclomine on the training day impaired the performance of rats in CFC, regardless of the treatment received on the test day, since groups dic/sal and dic/dic presented lower freezing levels than the sal/sal group. These results suggest that the effects of dicyclomine observed in CFC are not state-dependent. On the other hand, when rats were tested under the influence of dicyclomine (group sal/dic), the freezing response to the context was impaired. This result raises the possibility that the drug might also interfere with the retrieval of what has been learned the day before or performance during the CFC test, differently from the IA task. A putative interference on locomotor activity was excluded based on results of Experiment 3, in which the administration of dicyclomine did not affect the rats' ambulation. However, the fact that dicyclomine does not interfere with locomotor activity does not rule out the possibility that it might affect specifically the expression of the freezing response, that is, although the animal might have learned the CFC task, it might have been unable to manifest any freezing, the conditioned response used to evaluate such learning. In the TFC task (Experiment 3B), it was shown that the same dose of dicyclomine that affected pre-test CFC did not affect the freezing time observed after the tone during the TFC test. Therefore, this experiment showed that the animals are able to freeze in a different situation. Suggesting that the impairment observed with pre-test administration of dicyclomine is due to a selective effect during retrieval of contextual memories and not on performance.

Interestingly, similar treatment using the IA task did not affect the avoidance response. The literature presents contradictory results concerning the effects of cholinergic system manipulation on retrieval of the IA task. Rush (1988) reported that systemic administration of scopolamine before the test session impaired the avoidance response of animals. In the same manner, administration of M1 antagonists, biperiden, and trihexyphenidyl, was also found to disrupt retrieval of this task (Kimura et al., 1999). However, no effects were obtained by other authors with the pre-test administration of scopolamine (Elrod & Buccafusco, 1988; Roldan, Cobos-Zapiain, Quirarte, & Prado-Alcala, 2001) or pirenzepine (Ohnuki & Nomura, 1996) on retrieval of the same task. Regarding the effects of muscarinic antagonists on the CFC retrieval, no reports were published until now. Therefore, more studies are necessary to investigate why the pre-test administration of dicyclomine-induced contrasting results in the two tasks.

Other factors such as drug-induced alterations in motivation or in shock sensitivity may interfere with the results when a drug is administered before training in aversively motivated tasks. However, previous studies in our laboratory showed that dicyclomine does not affect the acquisition of TFC (Fornari et al., 2000), a task that demands similar motivational and motoric abilities as CFC. These results, together with data that shows that systemic administration of dicyclomine in rats or mice did not affect pain threshold (Bartolini et al., 1992), suggest that the effects of dicyclomine are not due to interference with motivation or with shock sensitivity.

Results obtained in Experiment 4 showed that the administration of dicyclomine immediately after training did not affect the latency of rats submitted to the IA test, nor did it interfere with the conditioned freezing response to the context in the CFC task. These results suggest that dicyclomine, at least in the dose range used (16, 32, and 64 mg/kg) does not interfere with the consolidation of IA and CFC tasks. Although we have failed in observing any consolidation effect of dicyclomine after intraperitoneal administration, data from intracerebral administration studies show that the amygdalar and hippocampal cholinergic systems (Izquierdo et al., 1992; for a recent review see Power, Vazdarjanova, & McGaugh, 2003b) and possibly the M1 receptor, are important in mediating consolidation of IA (Ferreira et al., 2003; Power, McIntyre, Litmanovich, & McGaugh, 2003a). Administering pirenzepine directly into the hippocampus, Ferreira et al. (2003) observed impairment of IA consolidation. Systemic administration of other M1 antagonists such as biperiden and trihexyphenidyl immediately after training was found to impair consolidation of the IA task (Roldán et al., 1997). However, disruption was only observed with doses higher than those necessary to impair IA acquisition (Kimura et al., 1999). In the present study, the consolidation of both memory tasks was not affected, even with a dicyclomine dose four times higher (64 mg/kg) than the lower effective dose in the pre-training treatment, suggesting that no shift in dose–response would be obtained. Dicyclomine, biperiden, and trihexiphenidyl show a different affinity profile, thus it is possible that the different affinity profiles are responsible for the conflicting results. Dicyclomine, for instance, seems to act also in M3 muscarinic receptors in addition to M1 (Doods et al., 1987), so it is not possible to rule out the possibility that the effect obtained after administration of dicyclomine is due in part to the conjoint inactivation of M1 and M3 receptors.

Impairment in the IA task in mice induced by post-training (i.p.) administration of dicyclomine was reported by Galeotti et al. (1998, 2000). Some factors could acquaint for the discrepancies, such as the species used, rats in our experiments and mice in Galeotti et al. (1998, 2000). Another possible reason for the differences relies on the fact that different shock intensities can influence the outcome of the anticholinergic administration effect on consolidation in such a way that anticholinergic loses its amnesic effect when shock intensity is high (Cruz-Morales, Duran-Arevalo, Diaz Del Guante, Quirarte, & Prado-Alcala, 1992). This could be the case since Galeotti and collaborators' studies used a footshock of half the intensity as that used in the present study. Although this possibility cannot be ruled out it is interesting to note that control animals from our experiments do not show the ceiling effect that would be expected when high shock intensity is used (Cruz-Morales et al., 1992). It is also possible to argue that the avoidance procedure adopted in the present study is unusual since the shock is delivered immediately after the animal enters the black compartment and is removed from the avoidance apparatus immediately after footshock. This procedure was used to clearly contrast between two possible learning strategies in the avoidance apparatus, one based on instrumental punished response and the other based on a conditioned–unconditioned stimuli relationship such as that observed in CFC. The present procedure involves a discriminative stimulus—the safe white compartment—which is followed by the entry response which is in turn punished by the footshock. On the contrary, when the animal is allowed to explore the shock compartment and/or remains there after shock is delivered, some learning about the characteristics of that compartment should occur. Thus, the possibility exists that the anticholinergic retrograde amnestic effect appears only when learning about the spatial context where the shock is delivered. Such kind of learning can be obtained when the animals are allowed to return to the safe compartment after shock as in the procedure employed by several authors (Cruz-Morales et al., 1992; Glick & Zimmerberg, 1972; Roldán et al., 1997), when they are left in the shock compartment for some seconds after shock is delivered (Baratti, Huygens, Mino, Merlo, & Gardella, 1979), or when a step-down procedure is used (Izquierdo, 1989). In all these instances time is allowed for learning about the shock compartment characteristics. However, this line of reasoning is weakened by the fact that post-training administration of dicyclomine did not affect retrieval in a CFC task in which plenty of time was allowed for the rats to explore the environment before delivering footshock.

Another possibility is based on the fact that on other studies the animals are left for some seconds in the shock compartment after footshock. This additional time can serve as an enhancer of the avoidance learning, an effect that in turn could require cholinergic activation. The post-training cholinergic antagonism could block this enhancement effect. Accordingly, it has been shown that intra-amygdalar telenzepine, another M1 antagonist, blocks the consolidation improvement of IA induced by oxotremorine (Power et al., 2003a). In our step-through procedure the rat is immediately removed after footshock, and thus the cholinergic activation may be too low to be blocked by the anticholinergic drug. Further research is necessary to clarify this issue. Nonetheless, the avoidance procedure used in the present work was sensitive enough to the effects of pre-training dicyclomine administration. Altogether, the data here

presented suggest that acquisition and consolidation recruit the cholinergic system in different ways.

Regarding CFC, some studies have found that post-training central administration of muscarinic nonselective agents can interfere with CFC (Vazdarjanova & McGaugh, 1999; Wallenstein & Vago, 2001). To our knowledge, the present one is the first study to investigate the effect of the administration of selective M1 receptor antagonists on CFC consolidation, suggesting that, although the cholinergic system is important for consolidation of CFC, it is not through M1 receptors. Alternatively, the same reasoning used for IA can also be used here since the animals does not spend some time in the context after shock is delivered.

The results obtained in this study show a profile of effects due to systemic administration of dicyclomine, a muscarinic antagonist with high affinity for M1 receptors, in different memory phases evaluated in IA and CFC tasks. Although dicyclomine in the dose range used did not affect consolidation of either task, it significantly disrupted the acquisition of both, IA and CFC tasks. Furthermore, pre- and post-training administration of dicyclomine induced similar results in both tasks, supporting the hypothesis that the two tasks may require common neural mechanisms. However, it is possible that the retrieval of these tasks may be mediated, at least in part, by different neurochemical mechanisms dissociated by dicyclomine.

Acknowledgments

This work was supported by grants from AFIP, CAPES, CNPq, FADA, and FAPESP. The authors thank Jose Bernardo da Costa for technical help and Isabel Marian Hartman de Quadros and Orlando F. A. Bueno for text revision and helpful comments.

References

Anagnostaras, S. G., Maren, S., & Fanselow, M. S. (1995). Scopolamine selectively disrupts the acquisition of contextual fear conditioning in rats. *Neurobiology of Learning and Memory, 64*, 191–194.

Anagnostaras, S. G., Maren, S., Sage, J. R., Goodrich, S., & Fanselow, M. S. (1999). Scopolamine and pavlovian fear conditioning in rats: Dose-effect analysis. *Neuropsychopharmacology, 21*(6), 731–744.

Anglade, F., Bizot, J. C., Dodd, R. H., Baudoin, C., & Chapouthier, G. (1994). Opposite effects of cholinergic agents and benzodiazepine receptor ligands in a passive avoidance task in rats. *Neuroscience Letters, 182*, 247–250.

Bammer, G. (1982). Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: A review and some new results. *Neuroscience and Biobehavioral Reviews, 6*, 247–296.

Baratti, C. M., Huygens, P., Mino, J., Merlo, A., & Gardella, J. (1979). Memory facilitation with posttrial injection of oxotremorine and physostigmine in mice. *Psychopharmacology, 64*, 85–88.

Bartolini, A., Ghelardini, C., Fantetti, L., Malcangio, M., Malmberg-Aiello, P., & Giotti, A. (1992). Role of muscarinic receptor subtypes in central antinociception. *British Journal of Pharmacology, 105*, 77–82.

Bartus, R. T., Dean, R. L., Beer, B., & Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science, 217*, 408–417.

Calhoun, W. H., & Smith, A. A. (1968). Effects of scopolamine on acquisition of passive avoidance. *Psychopharmacologia, 13*, 201–209.

Caulfield, M. P., Higgins, G. A., & Straughan, D. W. (1983). Central administration of the muscarinic receptor subtype-selective antagonist pirenzepine selectively impairs passive avoidance learning in the mouse. *Journal of Pharmacy and Pharmacology, 35*, 131–132.

Cruz-Morales, S. E., Duran-Arevalo, M., Diaz Del Guante, M. A., Quirarte, G., & Prado-Alcala, R. A. (1992). A threshold for the protective effect of over-reinforced passive avoidance against scopolamine-induced amnesia. *Behavioral and Neural Biology, 57*, 256–259.

Deutsch, J. A. (1971). The cholinergic synapse and the site of memory. *Science, 174*, 788–794.

Doods, H. N., Mathy, M. J., Davidesko, D., van Charldorp, K. J., de Jonge, A., & van Zwieten, P. A. (1987). Selectivity of muscarinic antagonists in radioligand and in vivo experiments for the putative M1, M2 and M3 receptors. *Journal of pharmacology and experimental therapeutics, 242*, 257–262.

Elrod, K., & Buccafusco, J. J. (1988). An evaluation of the mechanism of scopolamine-induced impairment in two passive avoidance protocols. *Pharmacology, Biochemistry, and Behavior, 29*, 15–21.

Everitt, J. B., & Robbins, W. T. (1997). Central cholinegic systems and cognition. *Annual Review of Psychology, 48*, 649–684.

Feiro, O., & Gould, T. J. (2005). The interactive effects of nicotinic and muscarinic cholinergic receptor inhibition on fear conditioning in young and aged C57BL/6 mice. *Pharmacology, Biochemistry, and Behavior, 80*(2), 251–262.

Ferreira, A. R., Furstenau, L., Blanco, C., Kornisiuk, E., Sanchez, G., Daroit, D., et al. (2003). Role of hippocampal M1 and M4 muscarinic receptor subtypes in memory consolidation in the rat. *Pharmacology, Biochemistry, and Behavior, 74*(2), 411–415.

Fibiger, H. C., Damsma, G., & Day, J. C. (1991). Behavioral pharmacology and biochemistry of central cholinergic neurotransmission. *Advances in Experimental Medicine and Biology, 295*, 399–414.

Fornari, R. V., Moreira, K. M., & Oliveira, M. G. M. (2000). Effects of the selective M1 muscarinic receptor antagonist dicyclomine on emotional memory. *Learning and Memory, 7*, 287–292.

Galeotti, N., Ghelardini, C., & Bartolini, A. (1998). Role of 5-HT_4 receptors in the mouse passive avoidance test. *Journal of Pharmacology and Experimental Therapeutics, 286*, 1115–1121.

Galeotti, N., Ghelardini, C., & Bartolini, A. (2000). Role of 5-HT1A receptors in a mouse passive avoidance paradigm. *Japanese Journal of Pharmacology, 84*(4), 418–424.

Glick, S. D., & Zimmerberg, B. (1972). Amnesic effects of scopolamine. *Behavioral biology, 7*, 245–254.

Hagan, J. J., Jansen, J. H., & Broekkamp, C. L. (1987). Blockade of spatial learning by the M1 muscarinic antagonist pirenzepine. *Psychopharmacology, 93*, 470–476.

Hunter, A. J., & Roberts, F. F. (1988). The effect of pirenzepine on spatial learning in the Morris Water Maze. *Pharmacology, Biochemistry, and Behavior, 30*, 519–523.

Izquierdo, I. (1989). Mechanism of action of scopolamine as an amnestic. *Trends in Pharmacological Sciences, 10*(5), 175–177.

Izquierdo, I., Da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M. B., & Medina, J. H. (1992). Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. *Behavioral and Neural Biology, 58*, 16–26.

Kimura, Y., Ohue, M., Kitaura, T., & Kihira, K. (1999). Amnesic effects of the anticholinergic drugs, trihexyphenidyl and biperiden: Differences in binding properties to the brain muscarinic receptor. *Brain Research, 834*(1-2), 6–12.

Levey, A. I., Kitt, C., Simonds, W., Price, D., & Brann, M. R. (1991). Identification and localization of muscarinic receptor subtype proteins in rat brain. *The Journal of Neuroscience, 11*(10), 3218–3226.

Maren, S. (2003). What the amygdala does and doesn't do in aversive learning. *Learning and Memory, 10*, 306–308.

Meyers, B. (1965). Some effects of scopolamine on a passive avoidance response in rats. *Psychopharmacologia, 8*, 111–119.

Moreira, K. M., Hipolide, D. C., Nóbrega, J. N., Bueno, O. F., Tufik, S., & Oliveira, M. G. (2003). Deficits in avoidance responding after paradoxical sleep deprivation are not associated with altered [3H]pirenzepine

binding to M1 muscarinic receptors in rat brain. *Brain Research, 977*(1), 31–37.

Ohnuki, T., & Nomura, Y. (1996). Effects of selective muscarinic antagonists, pirenzepine and AF-DX 116, on passive avoidance tasks in mice. *Biological and Pharmaceutical Bulletin, 19*, 814–818.

Overton, D. A. (1968). Dissociated learning in drugs states (state dependent learning). In D. H. Efron, J. O. Cole, J. Levine, & J. R. Wittenborn (Eds.), *Psychopharmacology: A review of progress 1957–1967* (pp. 918–930). Washington: Public Health Service.

Power, A. E., McIntyre, C. K., Litmanovich, A., & McGaugh, J. L. (2003a). Cholinergic modulation of memory in the basolateral amygdala involves activation of both m1 and m2 receptors. *Behavioural Pharmacology, 14*, 207–213.

Power, A. E., Vazdarjanova, A., & McGaugh, J. L. (2003b). Muscarinic cholinergic influences in memory consolidation. *Neurobiology of Learning and Memory, 80*(3), 178–193.

Roldán, G., Bolaños-Badillo, E., González-Sánchez, H., Quirarte, G. L., & Prado-Alcalá, R. A. (1997). Selective M1 muscarinic receptor antagonists disrupt memory consolidation of inhibitory avoidance in rats. *Neuroscience Letters, 230*, 93–96.

Roldan, G., Cobos-Zapiain, G., Quirarte, G. L., & Prado-Alcala, R. A. (2001). Dose- and time-dependent scopolamine-induced recovery of an inhibitory avoidance response after its extinction in rats. *Behavioural Brain Research, 121*(1–2), 173–179.

Rudy, J. W. (1996). Scopolamine administered before and after training impairs both contextual and auditory-cue fear conditioning. *Neurobiology of Learning and Memory, 65*, 73–81.

Rush, D. K. (1988). Scopolamine amnesia of passive avoidance: A deficit of information acquisition. *Behavioral and Neural Biology, 50*, 255–274.

Sala, M., Braida, D., Calcaterra, P., Leone, M. P., Comotti, F. A., Gianola, S., et al. (1991). Effect of centrally administered atropine and pirenzepine on radial arm maze performance in the rat. *European Journal of Pharmacology, 194*, 45–49.

Tinsley, M. R., Quinn, J. J., & Fanselow, M. S. (2004). The role of muscarinic and nicotinic cholinergic neurotransmission in aversive conditioning: comparing Pavlovian fear conditioning and inhibitory avoidance. *Learning and Memory, 11*, 35–42.

Van der Zee, E. A., & Lutien, P. G. M. (1999). Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: A review of immunocytochemical localization in relation to learning and memory. *Progress in Neurobiology, 58*(5), 409–471.

Vazdarjanova, A., & McGaugh, J. L. (1999). Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. *The Journal of Neuroscience, 19*(15), 6615–6622.

Wallenstein, G. V., & Vago, D. R. (2001). Intrahippocampal scopolamine impairs both acquisition and consolidation of contextual fear conditioning. *Neurobiology of Learning and Memory, 75*, 245–252.

Wei, J., Walton, E. A., Milici, A., & Buccafusco, J. J. (1994). m1-m5 muscarinic receptor distribuition in rat CNS by RT-PCR and HPLC. *Journal of Neurochemistry, 63*, 815–821.

Worms, P., Gueudet, C., Perio, A., & Soubrie, P. (1989). Systemic injection of pirenzepine induces a deficit in passive avoidance learning in rats. *Psychopharmacology, 98*, 286–288.