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ORIGINAL INVESTIGATION

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Effects of amphetamine on the plus-maze discriminative avoidance task in mice

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Abstract *Rationale*: The contradictory amphetamine effects on memory could be due to different protocols of amphetamine administration or the well-known anxiogenic effect of the drug. *Objective*: The effects of different protocols of administration of amphetamine were investigated on mice tested in the plus-maze discriminative avoidance task (DAT), which provides simultaneous information about memory and anxiety. *Methods*: Acutely pre- or post-training, 0.3, 1.0, or 3.0 mg/kg amphetamine-treated, 10-day chronically 3.0 mg/kg amphetamine-treated, 0.3 mg/kg amphetamine plus 0.25 mg/kg scopolamine and 3.0 mg/kg amphetamine plus 3.0 mg/kg tacrine-treated mice were conditioned to choose between two enclosed arms (one of which was aversive) while avoiding two open arms. Learning/memory was evaluated by the percentage time in the aversive enclosed arm (PTAV), and anxiety by the percentage time in the open arms (PTO). *Results*: Given acutely before conditioning, amphetamine significantly decreased PTO in training, suggesting an anxiogenic effect, and significantly increased PTAV in the test, suggesting an amnestic action. Given acutely after the conditioning, no action of this drug on memory was found. After repeated treatment, the anxiogenic effect disappeared, while the amnestic effect remained. While no effects of subeffective doses of amphetamine and scopolamine co-administration were detected, tacrine attenuated the amnestic effect of amphetamine. *Conclusions*: Amphetamine has different effects on DAT when given pre- or post-training. While acute pre-training amnestic action is temporally correlated with an anxiogenic effect, there is tolerance to the anxiogenic but not to the amnestic effect after repeated administration. Because this acute amnestic effect of amphetamine is attenuated by tacrine, a possible relationship with cholinergic system cannot be discarded as a mechanism to amphetamine-induced amnesia in DAT.

Keywords Avoidance-learning · Anxiety · Amphetamine · Behavior

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Introduction

The plus-maze discriminative avoidance task (DAT) was recently developed in our laboratory (Silva et al. 1997). In this new animal model of learning/memory, mice are conditioned to choose between two enclosed arms (an aversive and a non-aversive arm) while avoiding the open arms of the apparatus. The apparatus employed is an adaptation of the conventional elevated plus-maze, which has been extensively used to assess anxiolytic and anxiogenic effects of drugs since its description and validation for rats and mice (Handley and Mithani 1984; Pellow et al. 1985; Lister 1987).

The DAT has shown to be an effective model since the effects of both memory-enhancing and amnesic drugs have been demonstrated in this task. Indeed, the administration of ganglioside GM1 (a glycosphingolipid believed to play an important role in synaptic plasticity – Bellot et al. 1996, 1997; Silva et al. 1996) was able to improve retention of normal adult mice tested in DAT (Silva et al. 1997). The performance of adult rats in DAT was also improved by neonatal GM1 administration (Silva et al. 2000). In addition, both GM1 and bovine brain phosphatidylserine (proposed as a treatment of Alzheimer's disease – Crook et al. 1992) were able to attenuate scopolamine-induced amnesia in mice tested in this DAT (Claro et al. 1999; Silva et al. 1999).

Besides being a useful model for studying learning/memory, DAT also provides simultaneous information about anxiety-like behavior of the same animals, evaluated by the time spent in the open arms of the apparatus. The well-known anxiolytic and anxiogenic effects of

chlordiazepoxide and caffeine, respectively, were clearly demonstrated by alterations in the time spent in the open arms of the plus-maze discriminative avoidance apparatus (Silva and Frussa-Filho 2000). In that study, both drugs caused retention deficits, supporting the notion that there is an important link between memory and anxiety (Izquierdo and Medina 1991; Davis et al. 1997) and suggesting that bi-directional alterations in an optimum emotional level would impair learning/memory performance in behavioral tasks. In accordance, some studies have been proposing that anxiety and memory are not just related to each other, but anxiety would be in fact a necessary step for memory formation (Mathews 1990). In this respect, although specific effects of chlordiazepoxide and caffeine on memory were not discarded, the results of our previous study raise the issue that a possible action on anxiety levels can interfere with the effects of several drugs on memory animal models.

It is important to note that, relative to other associative learning models, such as passive and active avoidance tasks, alteration in motor activity would be a less critical methodological issue concerning the interpretation of the results in the DAT, since retention is evaluated by the time spent in the aversive versus non-aversive enclosed arms.

There is extensive evidence that the psychostimulant amphetamine enhances retention of a variety of learning/memory tasks (Roffman and Lal 1971; Castellano 1973; Janak and Martinez 1992; Ventulani et al. 1993; Roozendaal et al. 1996), being the drug of choice in numerous studies of enhancement of learning by pharmacological means (Fulginiti and Cancela 1983; Carr and White 1984; Packard et al. 1994). Other studies, however, show different profiles of amphetamine effects on learning/memory, such as absence of effect (Fulginiti and Cancela 1983; Beuzen et al. 1994) or even impaired performance (Bruto et al. 1983; Gutnikov et al. 1994; McKetin and Mattick 1998; Ornstein et al. 2000). These several studies, of course, show different protocols of amphetamine administration. In some of them, the drug was acutely administered after the training session (Castellano 1973; Janak and Martinez 1992), in others administration of the drug was conducted before the training session (Roffman and Lal 1971; Fulginiti and Cancela 1983; Ventulani et al. 1993; Beuzen et al. 1994; Gutnikov et al. 1994; Roozendaal et al. 1996), and, in some, the effects of amphetamine were observed after different kinds of repeated treatment (Bruto et al. 1983; McKetin and Mattick 1998; Ornstein et al. 2000).

Although differences in protocols can be related to the contradictory findings of the effects of amphetamine on memory, other causes cannot be ruled out. Among these causes, there could be the anxiogenic effect of amphetamine that has been demonstrated in humans (Williamson et al. 1990; Hall et al. 1996) and in animal models, including in the conventional elevated plus-maze (Pellow et al. 1985; Lin et al. 1999). In addition, the activation of motor behavior acutely induced by amphetamine (Kelly 1977), as well as the increase in hyperactivity that follows its repeated administration (Kalivas and Stewart 1991), could also modify the acquisition of behavioral tasks and consequently affect retention.

The first aim of the present study was to verify the effects of acute pre- or post-training and chronic pre-training administration of amphetamine on the DAT, simultaneously evaluating memory performance, emotionality levels, and motor activity of mice. From a neurochemical point of view, while amphetamine's predominant action is to release dopamine (Sulzer et al. 1995) and to block dopamine reuptake (Jones et al. 1998), convincing evidence has accumulated in support of the importance of a dopamine–acetylcholine interaction for memory performance (Hersi et al. 1995; Nava et al. 2000; Umegaki et al. 2001). Thus, this study also investigated the effects of amphetamine co-administration with the muscarinic cholinergic antagonist scopolamine (an amnestic agent) or the cholinesterase inhibitor acrine (a memory-enhancing agent) on DAT.

Materials and methods

Three-month-old Swiss EPM-M1 male mice from our own colony were housed under conditions of controlled temperature (22–23°C) and lighting (12 h light: 12 h dark, lights on 0700 hours). Food and water were available ad libitum throughout the experiments. Animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

Amphetamine (Sigma), scopolamine (RBI), and tacrine (RBI) were diluted in saline and given i.p. in a volume of 10 ml/kg body weight.

Experiment I

Groups of ten mice were injected with 0.3, 1.0, or 3.0 mg/kg amphetamine or saline solution. Fifteen minutes later, all the animals were submitted to the DAT conditioning (a single training session), which was performed in a modified elevated plus-maze made of wood, containing two enclosed arms (28.5×7×14 cm) opposite to two open arms (28.5×7 cm). A 100-W lamp was placed exactly over the middle of one of the enclosed arms (aversive enclosed arm). Each mouse was placed in the center of the apparatus and, over a period of 10 min, every time the animal entered the enclosed arm containing the lamp, an aversive situation was produced until the animal left the arm. Thus, the animal could terminate the aversive stimuli, which were the 100-W light and an 80-dB noise at the level of the mouse. In each side of the apparatus, there were different extramaze visual cues (door, window, cupboard, and observer) that mice could use to distinguish the location of the different arms of the maze. During the test session, performed 24 h later, mice were again placed in the apparatus for 3 min, without receiving the aversive stimulation.

Experiment II

Groups of ten mice were submitted to the DAT conditioning described above. Immediately after the training, the animals were injected with 0.3, 1.0, or 3.0 mg/kg amphetamine or saline. The test was performed 24 h later, as described for experiment I.

Experiment III

Mice were treated daily i.p. with saline (n=22) or 3.0 mg/kg amphetamine (n=21) for 10 days. On day 11 (24 h after the last injec-

tion), 11 saline-treated and 11 amphetamine-treated animals received i.p. saline (groups SAL–SAL and AMPH–SAL, respectively), whereas the remaining saline and amphetamine-treated mice received 3.0 mg/kg amphetamine (groups SAL–AMPH and AMPH–AMPH, respectively). The animals were submitted to the DAT conditioning described above 15 min later, and the test session begun 24 h later (day 12).

Experiment IV

This experiment was performed to determine a subeffective dose of scopolamine with regard to learning/memory behavior in the DAT. Groups of eight mice were injected with 0.25, 0.5, or 1.0 mg/kg scopolamine or saline solution. After 20 min, all the animals were submitted to the DAT conditioning described above, and the test was performed 24 h later.

Experiment V

Mice were treated i.p. with saline (n=16) or 0.25 mg/kg scopolamine (n=16). Five minutes after this injection, eight saline-treated and eight scopolamine-treated animals received i.p. saline (groups SAL–SAL and SCO–SAL, respectively), whereas the remaining saline and scopolamine-treated mice received 0.3 mg/kg amphetamine (groups SAL–AMPH and SCO–AMPH, respectively). Fifteen minutes later, the animals were submitted to the DAT conditioning described above, and the test was performed 24 h later.

Experiment VI

Mice were treated i.p. with saline (n=15) or 3.0 mg/kg tacrine (n=16). Forty-five minutes after this injection, seven saline-treated and eight scopolamine-treated animals received i.p. saline (groups SAL–SAL and TAC–SAL, respectively), whereas the remaining saline and tacrine-treated mice received 3.0 mg/kg amphetamine (groups SAL–AMPH and TAC–AMPH, respectively). Fifteen minutes later, the animals were submitted to the DAT conditioning described above, and the test was performed 24 h later.

Statistical analysis

In all experiments, the number of entries (an arm entry was defined as the entry of all four paws into one arm) and the time spent in each type of arm (aversive enclosed arm, non-aversive enclosed arm, and open arms) were registered during training and test sessions. The time spent in the aversive enclosed arm and the time spent in the non-aversive enclosed arm were compared using two-way analysis of variance (ANOVA) followed by Duncan's test. Total number of entries in any of the arms, percentage time spent in the open arms (time spent in open arms/time spent in both open and enclosed arms), and percentage time spent in the aversive enclosed arm (time spent in the aversive enclosed arm/time spent in both enclosed arms) were calculated in each session and compared using one-way ANOVA followed by Duncan's test. Data were log transformed before analysis in order to meet assumptions for ANOVA when necessary. Memory was evaluated by the percentage time spent in the aversive arm, anxiety-like behavior was evaluated by the percentage of time spent in the open arms, and motor activity was evaluated by total number of entries.

Results

Experiment I

Two-way ANOVA with group as a between-subject factor and arm type (aversive vs non-aversive) as a within-subject factor revealed a significant arm type effect ($F_{1,76}$=819.10, P<0.001) in training. In the test, significant arm type ($F_{1,76}$=78.73, P<0.001) and group × arm type interaction ($F_{3,76}$=4.44, P<0.01) effects were found. Post-hoc analysis revealed that all the groups spent significantly less time in the aversive enclosed arm than in the non-aversive enclosed arm during both sessions (Fig. 1a, b). The effects of the interaction group × arm type in the test can be better demonstrated by the analysis of a discrimination index, i.e., the percentage time spent in the aversive arm (time in the aversive arm/time in both enclosed arms). The percentage times spent in the aversive enclosed arm by 1.0 mg/kg and 3.0 mg/kg AMP-treated mice were significantly higher than that presented by SAL-treated animals ($F_{3,36}$=2.56, P<0.05; Fig. 1c]. Percentage time in the open arms by 3.0 mg/kg AMP-treated mice was significantly lower than that presented by control mice in training ($F_{3,36}$=2.78, P<0.05).

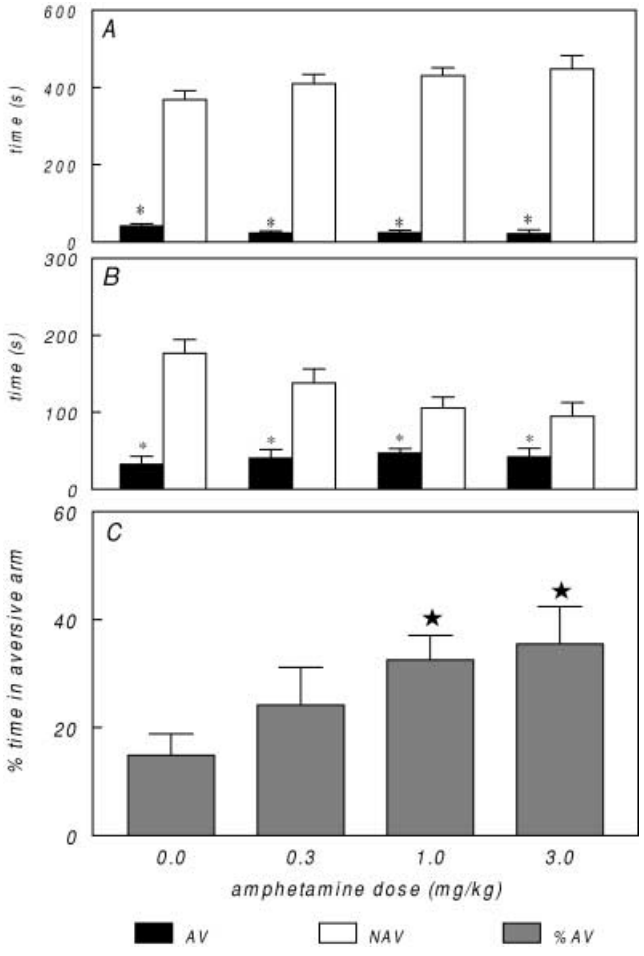

Fig. 1 Time spent in the aversive (*AV*) and non-aversive (*NAV*) enclosed arms of a plus-maze discriminative avoidance apparatus during training (**a**) and test (**b**) sessions and percentage time spent in aversive enclosed arm during the test session (**c**) presented by mice pre-training treated with saline, 0.3, 1.0, or 3.0 mg/kg amphetamine (mean±SEM). *P<0.05 compared with time spent in non-aversive arm (two-way ANOVA and Duncan's test). ★P<0.05 compared with saline group (ANOVA and Duncan's test)

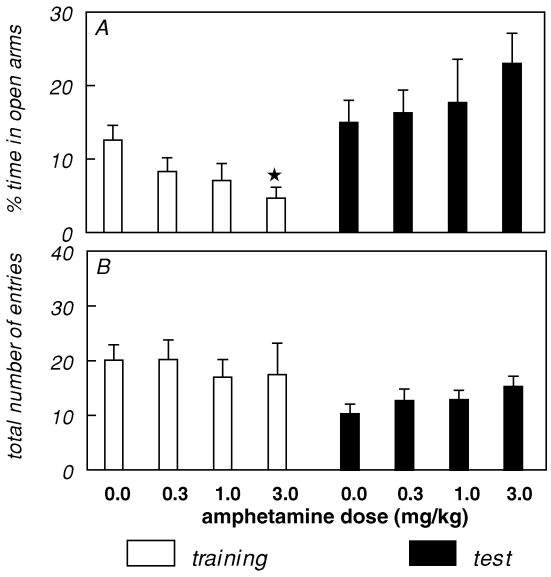

Fig. 2 Percentage time spent in the open arms (**a**) and total number of arm entries (**b**) during training and test sessions presented by mice pre-training treated with saline, 0.3, 1.0, or 3.0 mg/kg amphetamine in a plus-maze discriminative avoidance apparatus (mean±SEM). ★P<0.05 compared with saline group (ANOVA and Duncan's test)

No differences were found for percentage time spent in the open arms of the apparatus in the test (Fig. 2a). No differences were found in total number of entries in both sessions (Fig. 2b).

Experiment II

Two-way ANOVA with group as a between-subject factor and arm type as a within-subject factor revealed only a significant arm type effect in training ($F_{1,76}$=534.05, P<0.001) and test ($F_{1,76}$=109.05, P<0.001). No group × arm type interaction effects were detected. Post-hoc analysis revealed that all the groups spent significantly less time in the aversive enclosed arm than in the non-aversive enclosed arm during both sessions (data not shown). No differences were found in percentage time spent in the open arms or total number of entries in both sessions (data not shown).

Experiment III

Two-way ANOVA with group as a between-subject factor and arm type as a within-subject factor revealed a significant arm type effect ($F_{1,78}$=565.69, P<0.001) in training. In the test, significant arm type ($F_{1,78}$=495.87, P<0.001) and group × arm type interaction ($F_{3,78}$=7.22, P<0.001) effects were found. Post-hoc analysis revealed that all the groups spent significantly less time in the aversive enclosed arm than in the non-aversive enclosed arm during both sessions (Fig. 3a, b). In line with the

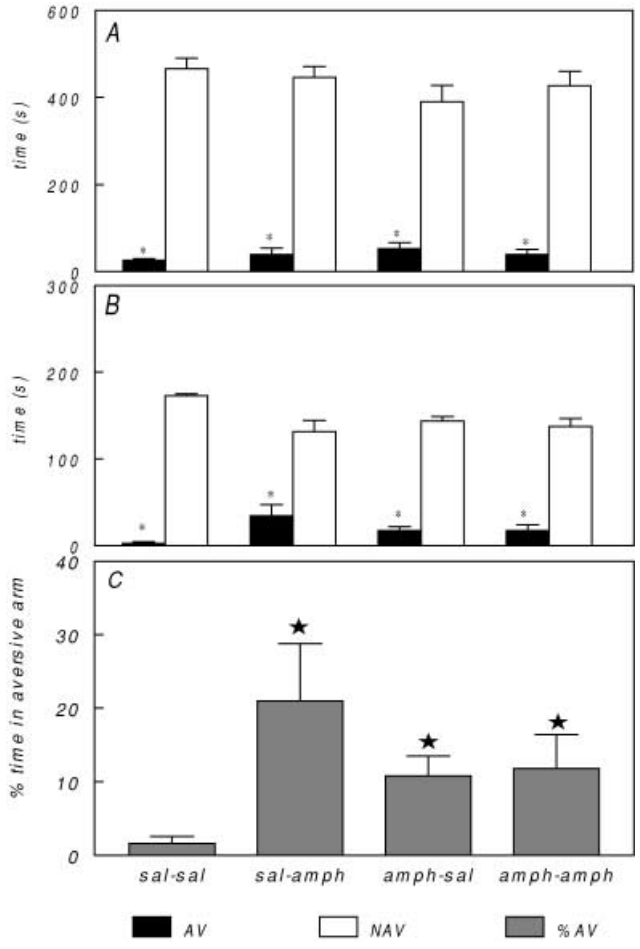

Fig. 3 Time spent in the aversive (*AV*) and non-aversive (*NAV*) enclosed arms of a plus-maze discriminative avoidance apparatus during training (**a**) and test (**b**) sessions and percentage time spent in aversive enclosed arm (**c**) during the test session presented by mice treated with saline (*sal*-) or 3.0 mg/kg amphetamine (*amph*-) for 10 days and challenged with saline (-*sal*) or 3.0 mg/kg amphetamine (-*amph*) 15 min before training session (mean±SEM). *P<0.05 compared with time spent in non-aversive arm (two-way ANOVA and Duncan's test). ★P<0.05 compared with sal–sal group (ANOVA and Duncan's test)

group × arm type interaction effect, percentage times spent in the aversive enclosed arm by SAL–AMPH, AMPH–SAL, and AMPH–AMPH mice were significantly higher than that presented by SAL–SAL animals during the test session ($F_{3,39}$=2.84, P<0.05 – Fig. 3c). In training, percentage time in the open arms by SAL–AMPH mice was significantly lower than that presented by control mice, and the percentage time in the open arms in the AMPH–SAL and AMPH–AMPH groups increased relative to the SAL–AMPH group ($F_{3,39}$=4.78, P<0.01). Percentage times in the open arms of the apparatus presented by AMPH–SAL and AMPH–AMPH groups during the test session were significantly higher than those presented by SAL–SAL and SAL–AMP groups ($F_{3,39}$=3.57, P<0.01; Fig. 4a). The total number of entries in any of the arms of the apparatus showed by AMPH–AMPH group in training was significantly

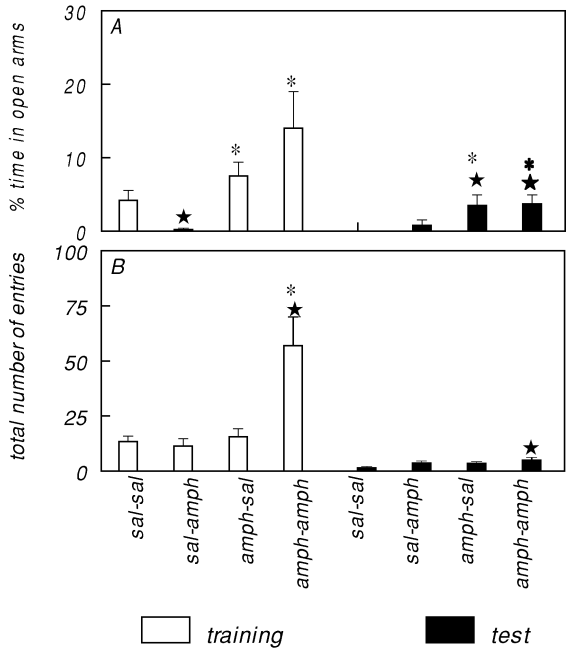

Fig. 4 Percentage time spent in the open arms (**a**) and total number of arm entries (**b**) during training and test sessions presented by mice treated with saline (*sal*-) or 3.0 mg/kg amphetamine (*amph*-) for 10 days and challenged with saline (-*sal*) or 3.0 mg/kg amphetamine (-*amph*) 15 min before training session, in a plus-maze discriminative avoidance apparatus (mean±SEM). ★P<0.05 compared with sal–sal group; *P<0.05 compared with sal-amph group (ANOVA and Duncan's test)

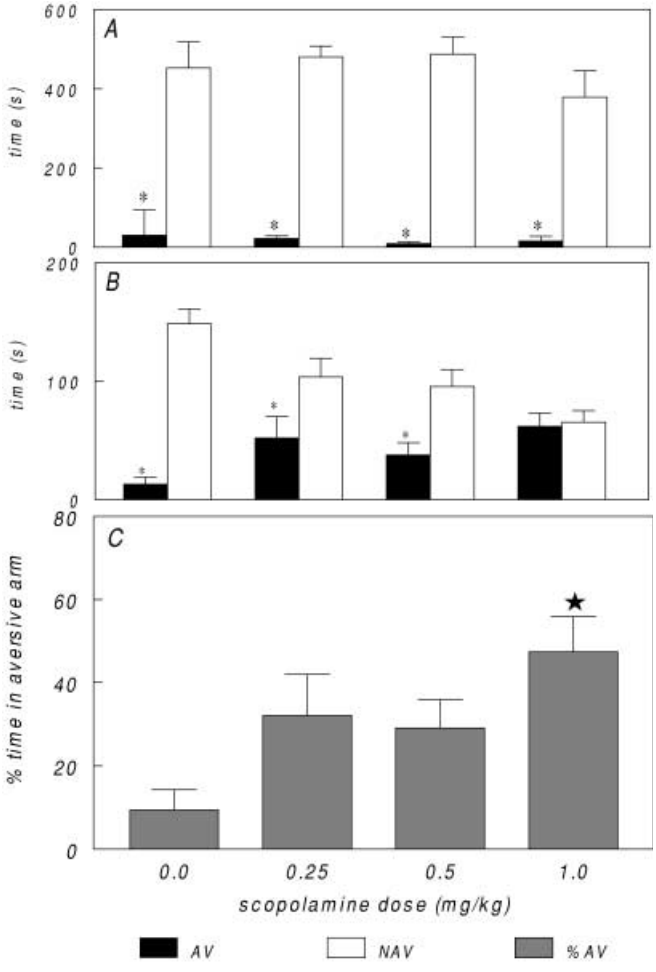

Fig. 5 Time spent in the aversive (*AV*) and non-aversive (*NAV*) enclosed arms of a plus-maze discriminative avoidance apparatus during training (**a**) and test (**b**) sessions and percentage time spent in aversive enclosed arm (**c**) during the test session presented by mice treated with saline or 0.25, 0.5, or 1.0 mg/kg scopolamine 20 min before training session (mean±SEM). *P<0.05 compared with time spent in non-aversive arm (two-way ANOVA and Duncan's test). ★P<0.05 compared with sal group (ANOVA and Duncan's test)

higher than that of all the other groups ($F_{3,39}$=10.06, P<0.0001). In the test, the AMP–AMP group presented a total number of entries significantly higher than that of the control group ($F_{3,39}$=3.27, P<0.05; Fig. 4b).

Experiment IV

Two-way ANOVA with group as a between-subject factor and arm type as a within-subject factor revealed only a significant arm type effect in training ($F_{1,60}$=173.02, P<0.001). Post-hoc analysis revealed that, in this session, all the groups spent significantly less time in the aversive enclosed arm than in the non-aversive enclosed arm (Fig. 5a). In the test, significant arm type ($F_{1,60}$=47.28, P<0.001) and group × arm type interaction ($F_{3,60}$=9.14, P<0.001) effects were found. Post-hoc analysis revealed that saline, 0.25 mg/kg and 0.5 mg/kg scopolamine- but not 1.0 mg/kg scopolamine-treated mice spent significantly less time in the aversive enclosed arm than in the non-aversive enclosed arm (Fig. 5b). In line with the group × arm type interaction effect, percentage time in the aversive enclosed arm by 1.0 mg/kg scopolamine-treated mice was significantly higher than that presented by saline-treated animals in the test ($F_{3,27}$= 3.24, P<0.05). Percentage times in aversive enclosed arm presented by 0.25 mg/kg and 0.5 mg/kg scopolamine-treated animals were not significantly different from that of control animals (Fig. 5c). No differences were found in percentage time in the open arms during both sessions or in total number of entries in training. Interestingly, 1.0 mg/kg scopolamine-treated mice presented an increased total number of entries when compared with the control group in the test, which could be related to the amnesic effect induced by this dose of scopolamine (data not shown).

Experiment V

Two-way ANOVA with group as a between-subject factor and arm type as a within-subject factor revealed only a significant arm type effect in training ($F_{1,60}$=845.77, P<0.001) and test ($F_{1,60}$=40.40, P<0.001). No group × arm type interaction effects were detected. Post-hoc

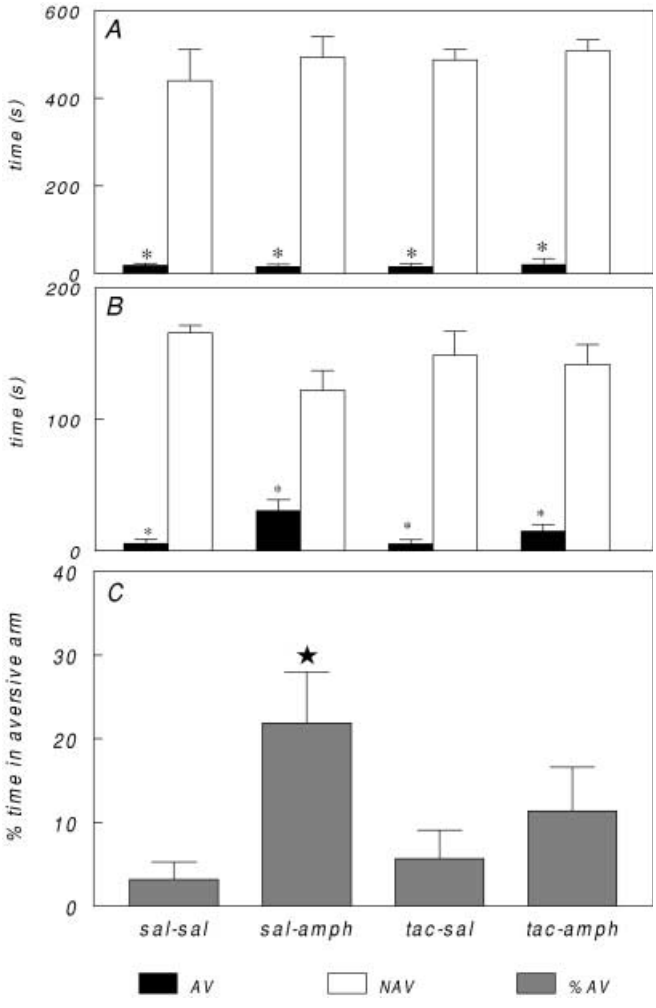

Fig. 6 Time spent in the aversive (*AV*) and non-aversive (*NAV*) enclosed arms of a plus-maze discriminative avoidance apparatus during training (**a**) and test (**b**) sessions and percentage time spent in aversive enclosed arm (**c**) in the test session presented by mice treated with saline (*sal*-) or 3.0 mg/kg tacrine (*tac*-) and with saline (-*sal*) or 3.0 mg/kg amphetamine (-*amph*) 60 min and 15 min before training session, respectively (mean±SEM). **P*<0.05 compared with time spent in non-aversive arm (two-way ANOVA and Duncan's test). ★*P*<0.05 compared with sal–sal and tac–sal groups (ANOVA and Duncan's test)

analysis revealed that all the groups spent significantly less time in the aversive enclosed arm than in the non-aversive enclosed arm in both sessions (data not shown). No differences were found in the percentage time spent in the open arms and total number of entries in both sessions (data not shown).

Experiment VI

Two-way ANOVA with group as a between-subject factor and arm type as a within-subject factor revealed a significant arm type effect ($F_{1,58}$=422.34, P<0.001) in training. In the test, significant arm type ($F_{1,58}$=273.84, P<0.001) and group × arm type interaction ($F_{3,58}$=3.46, P<0.05) effects were found. Post-hoc analysis revealed that all the groups spent significantly less time in the aversive enclosed arm than in the non-aversive enclosed arm in both sessions (Fig. 6a, b). In line with the group × arm type interaction effect, percentage time in the aversive enclosed arm by SAL–AMPH mice was significantly higher than that presented by SAL–SAL and TAC–SAL animals in the test ($F_{3,27}$=3.24, P<0.05). Percentage time in the aversive enclosed arm presented by TAC–ANF animals was not significantly different from that of control animals (Fig. 6c). No differences were found in percentage time in the open arms or total number of entries in both sessions (data not shown).

Discussion

In the DAT, an inhibitory effect on learning/memory can be demonstrated by reduced avoidance of the aversive enclosed arm, i.e., the time spent in this arm was not significantly different from time spent in the non-aversive enclosed arm in the test. This kind of inhibitory effect has been reported for scopolamine (Claro et al. 1999; Silva et al. 1999) and chlordiazepoxide (Silva and Frussa-Filho 2000) pre-training administration. Alternatively, learning/memory reduction can be evaluated by an increase in the percentage time spent in the aversive enclosed arm (time in the aversive enclosed arm/time in both enclosed arms) in the test, even if the animals were still able to avoid the aversive enclosed arms. This kind of inhibitory effect has been reported for caffeine pre-training administration (Silva and Frussa-Filho 2000). In this context, the results obtained in experiment I showed that the administration of amphetamine 15 min before the training session impaired the retention performance of the animals. Indeed, mice treated with 1.0 mg/kg or 3.0 mg/kg amphetamine spent more percentage time in the aversive arm than control animals in the test, although both saline- and amphetamine-treated animals avoided the aversive arm. However, when mice were submitted to the same behavioral task, but injected after the conditioning (experiment II), amphetamine-treated animals did not present any alterations in retention.

Previous studies have shown that amphetamine promotes memory improvement mostly when given after the conditioning (see Introduction), supporting the idea that this effect is specifically related to memory consolidation. Accordingly, long-term potentiation of synaptic transmission, which is believed to underlie information storage in the brain (Bliss and Collingridge 1993), can be enhanced by peripheral administration of amphetamine (Delanoy et al. 1983; Gold et al. 1984). Although improving effects of amphetamine, probably related to consolidation, have been reported, we were not able to detect any action of post-training administration of this drug on learning and memory evaluated by the plus-maze DAT. This discrepancy between previous findings and our results may reflect neuroanatomical and neurotransmission differences between discriminative and

other kinds of learning/memory tasks. Alternatively, the possibility that the improving effect of amphetamine would be detected in this discriminative task if some kind of procedure leading to learning difficulties or memory deficits were introduced (shortening the training session, performing the test session at longer time points, or using an amnestic agent, for example) cannot be discarded. From another standpoint, the lack of improving effects of post-training administration on this particular task may have facilitated the detection of the amnestic effect induced by the pre-training administration of the drug. Indeed, it is important to note that, when amphetamine was given 15 min before the training session, its pharmacological action was probably still present after conditioning. Thus, a hypothetical improving effect of amphetamine action after conditioning would inhibit or even abolish the retention deficit induced by the drug administration before conditioning.

Other different behavioral effects of amphetamine, not specifically related to the consolidation process, could lead to the decreased retention induced by the drug administration before conditioning. One of these effects, as mentioned before, could be the increase in the levels of anxiety-like behaviors of the animals during the training session, here demonstrated by a decreased percentage time in open arms relative to the control group during the training session of experiment I. Although this pre-treatment did not interfere with the acquisition performance of the animals (percentage time in the aversive arm was not modified relative to control group during the training session of experiment I), increased anxiety levels during the training session could be interfering with the performance of the animals during the test session. This hypothesis is corroborated by our previous work, demonstrating that alterations in the anxiety levels (increase or decrease) during the plus-maze discriminative avoidance conditioning may lead to retention deficits during the test session (Silva and Frussa-Filho 2000). In that study, pre-training administration of caffeine decreased the percentage time in open arms in the training session and did not modify the acquisition performance. Caffeine pre-training treatment, however, did lead to performance deficits during the test session, which were counteracted by simultaneous pre-training chlordiazepoxide administration, as was the anxiogenic effect (percentage time in open arms in training session). In addition, it has been demonstrated that biochemical events involved in memory formation are regulated by hormonal and neurohumoral mechanisms related to stress and anxiety (Korneyev 1997). Corticotrophin, glucocorticoids, vasopressin, epinephrine, and norepinephrine have been found to facilitate memory at low doses but impair memory at high doses (Izquierdo 1989; Gold 1995; McGaugh et al. 1995). It was also demonstrated that low to moderate levels of arousal could facilitate learning, whereas high levels of anxiety have an impairment effect (Gold 1995).

Concerning another methodological issue, although both anxiety-like and memory/learning tasks of the plus-maze discriminative avoidance model are avoidance tasks, memory/learning and anxiety can be separated using this model since the avoidance task related to memory/learning does not seem to be modified by anxiety level of the animal during the training session (Silva and Frussa-Filho 2000 and present data). In this respect, there is a fundamental difference between these two kinds of avoidance tasks. Since the aversive light/sound stimuli are presented only after the animal enters the aversive enclosed arm, they produce an active avoidance response (escape). In contrast, the open arms of the apparatus can be passively avoided. Within this context, it is interesting to note that in an elevated T-maze paradigm, while diazepam decreased the time of withdrawal from the enclosed arm toward the open arms (passive avoidance), the benzodiazepine did not affect the latency of withdrawal from one of the open arms toward the enclosed arm (active avoidance; Graeff et al. 1993). In addition, both chlordiazepoxide and caffeine, although inducing increase and decrease, respectively, in percentage time spent in the open arms, did not cause significant differences in percentage time spent in the aversive enclosed arm during the training session of the plus-maze DAT (Silva and Frussa-Filho 2000). This could be an indication that the conditioned aversion caused by light/noise in this arm is different from the innate fear caused by exposition to the open arms, and that this last condition would better be related to anxiety, since it was modified by the anxiolytic and the anxiogenic drugs.

In experiment III, once again, the acutely treated animals presented impaired retention and increased levels of anxiety-like behavior when compared with controls (group SAL–AMPH; Fig. 3 and Fig. 4). Although increased levels of anxiety-like behavior during the training session could lead to an impaired retention following acute amphetamine administration, the anxiogenic effect induced by this drug does not seem to be related to the deficits presented by chronically treated animals. Indeed, repeatedly amphetamine-treated animals (AMPH–AMPH group) did not show any difference in percentage time spent in the open arms when compared with control animals (Fig. 4a), suggesting that, when given repeatedly, the anxiogenic action of amphetamine was tolerated. However, this experimental group still demonstrated impaired retention when compared with saline-treated animals. These results are in line with other studies showing that repeated treatment with amphetamine, or methamphetamine, induces performance impairments in learning/memory animal models (Bruto et al. 1983; Yamamura et al. 1992). Accordingly, amphetamine abusers have been reported to present several kinds of memory impairment, considered to be a result of amphetamine neurotoxicity (McKetin and Mattick 1998; Ornstein et al. 2000). In this respect, it has been suggested that chronic methamphetamine-induced damages on learning/memory are related to the important alterations in dopamine turnover promoted by this drug, which were still present 11 days after methamphetamine withdrawal (Yamamura et al. 1992). Although less toxic than methamphetamine,

amphetamine has a similar pharmacological profile (Hotchkiss and Gib 1980), and this same mechanism could be underlying the effects reported here. In fact, after 24 h of withdrawal, the amnestic effect was still present (group AMPH–SAL; Fig. 3).

Finally, among the behavioral effects induced by amphetamine possibly related to its effects on learning/memory could be the activation of motor behavior (Kelly 1977). This effect has been shown in several animal models, including the conventional elevated plus-maze, where the administration of 4.0 mg/kg amphetamine increased the total number of entries in open or enclosed arms (Lister 1987). The motor stimulating effect could interfere with the acquisition of different behavioral tasks and consequently affect retention in pre-training amphetamine-treated animals, and this issue has been discussed in some studies, most of them excluding this possibility (Roffman and Lal 1971; Ishikawa and Saito 1976; Roozendaal et al. 1996). In the present study, however, this well-known effect of amphetamine was not verified, since there was no change in the total number of entries due to amphetamine treatment in the experiment-I training session. The motor stimulating effect was tested for our laboratory conditions in an open-field arena, and 3.0 mg/kg amphetamine, given 15 min before the exposition to the apparatus, caused an increase in the number of squares crossed by the animals (data not shown). This discrepancy between the plus-maze and open-field findings may suggest that total arm entries in the former test does not represent a very sensitive index of locomotor activation. In this respect, factor analyses have suggested the superiority of closed arm entries (vs total entries) as an index of locomotor activity in the plus-maze apparatus (Rodgers et al. 1997). However, no differences were found in closed arm entries between saline and amphetamine groups during the experiment-I training session (data not shown). Thus, an alternative possibility is that, in the case of the plus-maze DAT, the experimental situation could have caused the suppression of the motor stimulating effect of amphetamine.

The anxiogenic action of the drug plus the presence of the aversive stimuli during the training session could have induced motor inhibition, which prevented the observation of the stimulating effect. In accordance with this hypothesis, when amphetamine was chronically administered, the anxiogenic effect was no longer present, and the stimulating motor effect of this drug could be observed (group AMPH–AMPH; Fig. 6b). In this respect, the question arises as to the involvement of this finding in the phenomenon of behavioral sensitization. Indeed, repeated exposure to psychostimulants such as amphetamine and cocaine produces behavioral sensitization, which is characterized by an augmented locomotor response to a subsequent psychostimulant challenge injection and has been suggested to be associated with mechanisms that underlie both pharmacological psychosis and compulsive drug intake (Pierce and Kalivas 1997). The present data not only demonstrate that behavioral sensitization to amphetamine can be evaluated by plus-maze behavior but also indicate that this phenomenon is correlated with tolerance to the anxiogenic effect of the drug. Clearly, however, more extensive experimentation is necessary to characterize the importance of this possible link.

Interestingly, both AMPH–AMPH and AMPH–SAL groups, i.e., mice repeatedly treated with amphetamine and pre-training challenged with amphetamine or saline, respectively, presented an increased percentage time spent in the open arms during the test session, which suggests lower anxiety at this time point. While this finding could be interpreted on the basis of a rebound effect to the anxiogenic effect of amphetamine, one must be wary of discussing open arm data in mice previously exposed to the apparatus, because there is extensive literature showing that the animal's motivational state upon re-exposure to the plus-maze is very different from that upon initial exposure. Specifically, despite its widespread appeal to evaluate anxiety-like behavior, the plus-maze model has an intriguing feature: the phenomenon of "one-trial tolerance". Indeed, there is marked attenuation or even abolition of the anxiolytic effect of benzodiazepines by a single previous experience on the maze (Lister 1987; Rodgers and Shepherd 1993; Gonzales and File 1997). In this respect, it has been suggested that the phenomenon of one-trial tolerance might reflect a relative absence of an approach/avoid conflict on trial 2 (Rodgers and Shepherd 1993). In line with this possibility, the phenomenon is abolished by the introduction of a motivational conflict situation on trial 2: rendering the enclosed arms of the apparatus aversive on trial 1 (Pereira et al. 1999). In this context, it might therefore be hypothesized that the anxiolytic effect presented by AMPH–AMPH and AMPH–SAL groups during the test session could be detected in the present study due to the similarities between the present protocol and that of Pereira et al. (1999).

An interesting finding of the present study to be discussed is the increase in the total number of entries presented by the AMPH–AMPH group during the test session. Clearly, more extensive experimentation is necessary before this question can be answered. However, while there is substantial evidence for classical conditioning of the behavioral effects of psychostimulants to environmental stimuli (Pierce and Kalivas 1997), the AMP–AMPH group was the only one to present increased total number of entries in the training session, thereby suggesting a possible role of environmental conditioning in the locomotor increase observed for this group during the test session.

Experiments IV, V, and VI were performed to verify whether the retention deficit induced by acute pre-training amphetamine administration on the plus-maze DAT could be modified by memory-enhancing and -disrupting drugs. Experiment IV was performed to determine sub-effective doses of scopolamine on learning/memory performance of mice in the plus-maze DAT. In line with previous reports (Claro et al. 1999; Silva et al. 1999), 1.0 mg/kg scopolamine was effective in inducing a memory deficit in this model. Conversely, the lower

doses of 0.25 mg/kg and 0.5 mg/kg were not. In order to avoid the possibility of an amnestic effect of scopolamine per se, the lowest dose of the muscarinic antagonist (0.25 mg/kg) was used in combination with a subeffective dose of amphetamine (0.3 mg/kg) in experiment V. However, we were not able to find a synergistic amnestic action between these subeffective doses of amphetamine and scopolamine. In contrast, in experiment VI, the administration of tacrine was able to attenuate amphetamine-induced amnesia. In fact, while 3.0 mg/kg amphetamine-treated mice presented increased percentage time in the aversive arm during the test session, tacrine plus amphetamine-treated animals' data were not different from data presented by control animals. It is important to note that, as mentioned before, both scopolamine and tacrine exert their actions via cholinergic mechanisms. Several studies have shown that dopaminergic and cholinergic systems interact in a complex manner with regard to cognitive function. For example, both dopamine receptors agonists and antagonists attenuate cognitive deficits promoted by decreased cholinergic function (Flexner et al. 1992; McGurk et al. 1992; Gasbarri et al. 1993). In this respect, nicotinic receptor- and muscarinic receptor-mediated cholinergic transmissions can be differentially modified by dopaminergic drugs (Kim and Levin 1996), which might explain why scopolamine (a muscarinic receptor blocker) did not potentiate the amnestic effect of amphetamine on the plus-maze inhibitory avoidance task, while tacrine (which exerts its cholinergic agonistic action by blocking acetylcholinesterase) attenuates the amnestic action in the task.

The results related to anxiety-like behavior from experiment VI did not reproduce those from experiments I and III. In fact, the previously observed anxiogenic effect (decreased percentage time in open arms) induced by acute 3.0 mg/kg amphetamine in training (Fig. 2A and Fig. 4A) was not presented by saline plus amphetamine-treated mice in experiment V. In this experiment, however, unlike experiments I and III, two injections were given to each animal within 1 h before the behavioral session. In this way, it might be suggested that the differences between the protocols were responsible for the different results obtained. In fact, variations of the experimental procedures, such as pre-session manipulations, can modify not only spontaneous anxiety-like behavior, but also alterations in the anxiety levels induced by drugs measured in the conventional elevated plus-maze (Hogg 1996; Rodgers et al. 1997). Anyway, this is extra evidence that the anxiogenic effect of acute pre-training amphetamine administration is not causatively related to its amnestic effect on the DAT.

In conclusion, this study shows that amphetamine (at the same dose range) has an amnestic effect on a DAT when administered acutely pre- but not post-training. While this acute amnestic action is temporally correlated with an anxiogenic effect, after repeated treatment there is tolerance to the latter but not to the former effect. A possible relationship with the cholinergic system cannot be discarded as a mechanism to amphetamine-induced amnesia in the DAT since it was attenuated by tacrine co-administration. This is a provocative-working hypothesis that will be addressed in the future.

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References

Bellot RG, Camarini R, Vital MABF, Palermo-Neto J, Leyton V, Frussa-Filho R (1996) Monosialoganglioside administration attenuates the excitatory and behavioural sensitization effects of ethanol. Eur J Pharmacol 313:175–179

Bellot RG, Vital MABF, Palermo-Neto J, Frussa-Filho R (1997) Repeated monosialoganglioside administration attenuates behavioral sensitization to amphetamine. Brain Res 747:169–172

Beuzen A, Belzung C, Roullet P (1994) Drug effects in a radial maze designed for dissociation of cues used by mice. Pharmacol Biochem Behav 48:23–29

Bliss TVP, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39

Bruto V, Kokkinidis L, Anisman H (1983) Attenuation of perseverative behavior after repeated amphetamine treatment: tolerance or attentional deficits? Pharmacol Biochem Behav 19:497–504

Carr GD, White NM (1984) The relationship between stereotypy and memory improvement produced by amphetamine. Psychopharmacology 82:203–209

Castellano C (1974) Cocaine, pemoline and amphetamine on learning and retention of a discrimination test in mice. Psychopharmacologia 36:67–76

Claro FT, Silva RH, Frussa-Filho R (1999) Bovine brain phosphatidylserine attenuates scopolamine induced amnesia. Physiol Behav 67:551–554

Crook T, Petrie W, Wells C, Massari DC (1991) Effects of phosphatidylserine in Alzheimer's disease. Psychopharmacol Bull 28:61–66

Davis M, Walker DL, Lee Y (1997) Amygdala and bed nucleus of the stria terminalis: differential roles in fear and anxiety measured with the acoustic startle reflex. Philos Trans R Soc Lond B Biol Sci 352:1675–1678

Delanoy RL, Tucci DL, Gold PE (1983) Amphetamine effects on long term potentiation in dentate granule cells. Pharmacol Biochem Behav 18:137–139

Flexner JB, Church AC, Flexner LB (1992) Long-term suppression of the development of complementary memory storage sites in mice: functional interdependence of acetylcholine and dopamine. Pharmacol Biochem Behav 43:617–619

Fulginiti S, Cancela LM (1983) Effect of naltrexone and amphetamine on acquisition and memory consolidation of active avoidance responses in rats. Psychopharmacology 79:45–48

Gasbarri A, Introini-Collison IB, Packard MG, Pacitti C, McGaugh JL (1993) Interaction of cholinergic-dopaminergic systems in the regulation of memory storage in aversively motivated learning tasks. Brain Res 627:72–78

Gold PE (1995) Modulation of emotional and non emotional memories: same pharmacological systems, different anatomical systems. In: McGaugh JL, Weinberger N, Lynch G (eds) Brain and memory: modulation and mediation of neural plasticity. Oxford University Press, New York, pp 41–74

Gold PE, Delanoy RL, Merrin J (1984) Moculation of long-term potentiation by peripherally administered amphetamine and epinephrine. Brain Res 305:103–107

Gonzales LE, File SE (1997) A five minute experience in the elevated plus-maze alters the state of the benzodiazepine receptor in the dorsal raphe nucleus. J Neursoci 17:1505–1511

Graeff FG, Viana MB, Tomaz C (1993) The elevated T maze, a new experimental model of anxiety and memory, effects of diazepam. Brazilian J Med Biol Res 26:67–70

Gutnikov AS, Barnes JC, Rawlins JNP (1994) Working memory tasks in five-choice operant chambers. Use of relative and absolute spatial memories. Behav Neurosci 108:899–910

Hall W, Hando J, Darke S, Ross J (1996) Psychological morbidity and route of administration among amphetamine users in Sydney, Australia. Addiction 91:81–87

Handley SL, Mitani S (1984) Effects of alfa-adrenoceptor agonists and antagonists in a maze exploration model of 'fear'-motivated behavior. Naunyn Schmiedebergs Arch Pharmacol 327:1–5

Hersi AI, Rowe W, Gaudreau P, Quirion R (1995) Dopamine D1 receptor ligands modulate cognitive performance and hippocampal acetycholine release in memory-impaired aged rats. Neuroscience 69:1067–1074

Hogg S (1996) A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 54:21–30

Hotchkiss AJ, Gibb JW (1980) Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. J Pharmacol Exp Ther 214:257–262

Ishikawa K, Saito S (1976) Differences in the effects of post-trial chlorpromazine, reserpine and amphetamine on discrimination learning in rats. Psychopharmacology 48:45–51

Izquierdo I (1989) Different forms of posttraining memory processing. Behav Neural Biol 51:171–202

Izquierdo I, Medina JH (1991) $GABA_A$ receptor modulation of memory: the role of endogenous benzodiazepines. Trends Pharmacol Sci 12:260–265

Janak PH, Martinez JL (1992) Cocaine and amphetamine facilitate retention of jump-up responding in rats. Pharmacol Biochem Behav 41:837–840

Jones SR, Gainetdninov RR, Wightman RM, Caron MG (1998) Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J Neurosci 18:1979–1986

Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 16:223–244

Kelly PH (1977) Drug-induced motor behavior. In: Iversen LL, Iversen SD, Snyder SH (eds) Handbook of psychopharmacology, vol. 8. Plenum, New York, pp 295–331

Kim JS, Levin ED (1996) Nicotinic, muscarinic and dopaminergic actions in the ventral hippocampus and the nucleus accumbens: effects on spatial working memory in rats. Brain Res 725:231–240

Korneyev AY (1997) The role of the hypothalamic-pituitary-adrenocortical axis in memory-related effects of anxiolytics. Neurobiol Learn Mem 67:1–13

Lin HQ, Burden PM, Christie MJ, Johnston AR (1999) The anxiogenic-like and ansiolytic-like effects of MDMA on mice in the elevated plus-maze: a comparison with amphetamine. Pharmacol Biochem Behav 62:403–408

Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92:180–185

Mathews A (1990) Why worry? The cognitive function of anxiety. Behav Res Ther 28:455–568

McGaugh JL, Cahill L, Parent MB, Mesches MH, Coleman-Mesches K, Salinas JÁ (1995) Involvement of the amygdala in the regulation of memory storage. In: McGaugh JL, Bermúdez-Rattoni F, Prado-Alcalá RA (eds) Plasticity in the central nervous system: learning and memory. Erlbaum, Mahwah, NJ, pp 17–39

McGurk SR, Levin ED, Butcher LL (1992) Dopaminergic drugs reverse the impairment of radial-arm maze performance caused by lesions involving the cholinergic medial pathway. Neuroscience 50:129–135

McKetin R, Mattick RP (1998) Attention and memory in illicit amphetamine users: comparison with non-drug-using controls. Drug Alcohol Depend 50:181–184

Nava F, Carta G, Battasi AM, Gessa GL (2000) D2 dopamine receptors enable Δ9-tetrahydrocannabinol induced memory impairment and reduction of hippocampal extracellular acetylcholine concentration. Br J Pharmacol 130:1201–1210

Ornstein TJ, Iddon JL, Baldacchino AM, Sahakian BJ, London M, Everitt BJ, Robbins TW (2000) Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers. Neuropsychopharmacology 23:113–126

Packard MG, Cahill L, McGaugh JL (1994) Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. Proc Natl Acad Sci U S A 91:8477–8481

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167

Pereira JKD, Vieira RJ, Konishi CT, Ribeiro R de A, Frussa-Filho R (1999) The phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze is abolished by the introduction of a motivational conflict situation. Life Sci 65:PL101–PL107

Pierce RC, Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Brain Res Rev 25:192–216

Rodgers RJ, Shepherd JK (1993) Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light;dark tests of anxiety in mice. Psychopharmacology 113:237–242

Rodgers RJ, Cao BJ, Dalvi A, Holmes A (1997) Animal models of anxiety: an ethological perspective. Braz J Med Biol Res 30:289–304

Roffman M, Lal H (1971) Facilitatory effect of amphetamine on learning and recall of an avoidance response in rats. Arch Int Pharmacodynam 193:87–91

Roozendaal B, Carmi O, McGaugh JL (1996) Adrenocortical suppression blocks the memory-enhancing effects of amphetamine and epinhephrine. Proc Natl Acad Sci USA 93:1429–1433

Silva RH, Frussa-Filho R (2000) The plus-maze discriminative avoidance task: a new model to study memory-anxiety interactions. Effects of chlordiazepoxide and caffeine. J Neurosci Methods 102:117–125

Silva RH, Felicio LF, Nasello AG, Vital MABF, Frussa-Filho R (1996) Effects of ganglioside (GM1) on memory in senescent rats. Neurobiol Aging 14:582–586

Silva RH, Bellot R, Vital MABF, Frussa-Filho R (1997) Effects of long-term ganglioside GM1 administration on a new discriminative avoidance test in normal adult mice. Psychopharmacology 129:322–328

Silva RH, Felicio LF, Frussa-Filho R (1999) Ganglioside GM1 attenuates scopolamine-induced amnesia. Psychopharmacology 141:111–117

Silva RH, Bergamo M, Frussa-Filho R (2000) Effects of neonatal gangliosde GM1 administration on memory in adult and old rats. Pharmacol Toxicol 87:120–125

Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, Ewing A (1995) Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. J Neurosci 15:4102–4108

Umegaki H, Munoz J, Meyer RC, Spangler EL, Yoshimura J, Ikari H, Iguchi A, Ingram DK (2001) Involvement of dopamine D2 receptors in complex maze learning and acetylcholine release in ventral hippocampus of rats. Neuroscience 103:27–33

Ventulani J, Batagglia M, Castellano C, Sansone M (1993) Facilitation of shuttle-box avoidance behavior in mice treated with nifedipine in combination with amphetamine. Psychopharmacology 113:217–221

Williamson S, Gossop M, Powis B, Griffiths P, Fountain J, Strang J (1997) Adverse effects of stimulant drugs in a community sample of drug users. Drug Alcohol Depend 44:87–94

Yamamura T, Hishida S, Hatake K, Taniguchi T, Ouchi H (1992) Effects of methamphetamine and ethanol on learning and brain neurotransmitters in rats. Pharmacol Biochem Behav 42:389–400