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Dimebon enhances hippocampus-dependent learning in both appetitive and inhibitory memory tasks in mice

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ABSTRACT

Pre-clinical and clinical studies on dimebon (dimebolin or latrepirdine) have demonstrated its use as a cognitive enhancer. Here, we show that dimebon administered to 3-month-old C57BL6N mice 15 min prior to training in both appetitive and inhibitory learning tasks via repeated (0.1 mg/kg) and acute (0.5 mg/kg) i.p. injections, respectively, increases memory scores. Acute treatment with dimebon was found to enhance inhibitory learning, as also shown in the step-down avoidance paradigm in 7-month-old mice. Bolus administration of dimebon did not affect the animals' locomotion, exploration or anxiety-like behaviour, with the exception of exploratory behaviour in older mice in the novel cage test. In a model of appetitive learning, a spatial version of the Y-maze, dimebon increased the rate of correct choices and decreased the latency of accessing a water reward after water deprivation, and increased the duration of drinking behaviour during training/testing procedures. Repeated treatment with dimebon did not alter the behaviours in other tests or water consumption. Acute treatment of water-deprived and non-water-deprived mice with dimebon also did not affect their water intake. Our data suggest that dimebon enhances hippocampus-dependent learning in both appetitive and inhibitory tasks in mice.

1. Introduction

Dimebon is a candidate for therapeutics against Alzheimer's disease, and its clinical activity is currently under investigation. It was originally developed in 1983 in Russia where it was used as an antihistamine. Ongoing clinical trials are re-assessing contradictory results (Miller, 2010) concerning the previously shown efficacy of dimebon in the improvement of thinking processes and functioning in patients with mild to moderate Alzheimer's disease (Bachurin et al., 2001; Doody et al., 2008; O'Brien, 2008; Gura, 2008) and its curative effects in patients with Huntington's disease (Kieburtz et al., 2010), also during co-application with other therapeutics. Evaluation of dimebon against a set of biochemical targets indicated that dimebon inhibits alpha-adrenergic receptors (alpha1A, alpha1B, and alpha1D, and alpha2A, alpha 2B, and alpha 2C), histamine H1 and H2 receptors and serotonin receptors (5-HT-2A, 5-HT2B, 5-HT2C, 5-HT5A, 5-HT6, and 5-HT7), dopamine receptors (D, D2S, and D3), and imidazoline I2 receptors (Schaffhauser et al., 2009; Giorgetti et al., 2010). At low concentrations, dimebon potentiates the activity of AMPA-receptors and blocks NMDA-receptors in neurons (Grigorev et al., 2003).

Pre-clinical studies revealed a number of activities of dimebon in *in vitro* and *in vivo* assays. Dimebon prevents the opening of mitochondrial pores induced by neurotoxins, which is regarded as the major pathogenetic factor of neurodegeneration (Bachurin et al., 2003; Hung, 2008), elevates extracellular levels of amyloid beta in cell culture and the hippocampus of freely moving Tg2576 mice (Steele et al., 2009), promotes neurite outgrowth in cultured hippocampal and cortical neurons (Protter et al., 2009; Bernales et al., 2009), and enhances hippocampal neurogenesis (Pieper et al., 2010).

Bolus administration of dimebon in rats resulted in enhancement of short-term learning in the social recognition paradigm (10 mg/kg, i.p.;

Abbreviations: H1, H2 receptors, histamine type 1 and 2 receptors; 5-HT-2A, 5-HT2B, 5-HT2C, 5-HT5A, 5-HT6 and 5-HT7 receptors, 5-hydroxy-tryptamine (serotonin) receptors types 2A, 2B, 2C, 5A, 6 and 7; D, D2S and D3 receptors, dopamine receptors types 2, 2S (short form) and 3; I2, imidazoline receptor type 2; AMPA, alfa-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; NMDA, N-Methyl-D-Aspartate; AF64A, ethylcholine aziridinium; Tg2576 mice, transgenic mouse with K670N/M671L mutation in APP; C57BL/6N, inbred mouse inbred mouse strain; ANOVA, analysis of variance; LTP, long-term potentiation; CA1, Cornu Ammonis 1; K1, constant of association of the drug with receptor; Ach, acetylcholine; AChE, acetylcholinesterase; BrdU, bromodeoxyuridine.

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Schaffhauser et al., 2009) and the novel object recognition task (0.05, 0.5 and 5 mg/kg, p.o.; Giorgetti et al., 2010). In rats treated with the neurotoxin AF64A, which selectively lesions cholinergic neurons and impairs active avoidance, 10-day administration of dimebon reversed these deficits (1 mg/kg/day, i.p.; Lermontova et al., 2000). Similar results were obtained in the Morris water maze in rats subjected to intracerebroventricular administration of AF64: chronic administration of dimebon at the dose of 0.05 mg/kg rescued spatial learning, which was disrupted by chemical lesion of the dorsal hippocampus (Bachurin et al., 2006). A single administration of dimebon increased the memory for new object localization in young C57BL/6N mice at the dose of 0.1 mg/kg, while in a separate experiment, this treatment did not alter the animals' exploratory activity estimated under the same testing conditions (Bolkunov et al., in preparation). Performance in a new object localization model in rodents was shown to be disrupted by selective lesions of the hippocampus (Galani et al., 1998). Thus, dimebon has been shown to facilitate learning in various animal models, including paradigms of hippocampus-dependent learning at a range of concentrations.

Here, we studied whether dimebon applied at the dose that is efficient in a mouse model of new object localization memory, interferes with the appetitive learning of mice in the Y-maze. Studies that assessed dimebon's activity in assays of this type have not been reported to date. We used a protocol of the Y-maze in mice that was previously validated as a test for spatial learning (Dolgov et al., 2005; Gorenkova et al., 2005). In this paradigm, water-deprived C57BL/6N mice were allowed to orientate themselves by distant visual cues when selecting the arm of the maze with a filled bottle. As a model of spatial learning, the Y-maze paradigm is considered to be a test for hippocampus-dependent memory (Gerlai, 2001; Liu et al., 2001; Finger et al., 2010; Alhassan et al., 2009; Tan et al., 2010). We also tested whether dimebon affects memory in a single trial inhibitory learning paradigm using the step-down avoidance paradigm, another model for hippocampus-dependent memory (Lorenzini et al., 1996; Izquierdo and Medina, 1997; Strekalova et al., 2001, 2001) in young and middle-aged mice. Additionally, we investigated the potential effects of the drug treatment applied in both memory tests on parameters of anxiety, exploration and water intake.

The selection of dimebon doses used in this study are based on previous reports, which demonstrate that a single peripheral administration of the drug at a dose of 0.05–0.5 mg/kg evokes a memory-enhancing effect in rats, and results in an effective dimebon brain concentration of 1.7–14 nM/g, that was suggested to trigger neurochemical processes associated with cognitive function (Bachurin et al., 2006; Giorgetti et al., 2010). Recent studies provide evidence for a stimulatory effect by dimebon on neurogenesis in the dentate gyrus of the hippocampus in rats, observed after 1-week of intraperitoneal injections of the drug applied at the same range of concentrations: 0.1 MKM/kg (0.32 mg/kg; Pieper et al., 2010). Therefore, we anticipated that repeated dosing with dimebon at a dose of 0.1 mg/kg, and single administration of a dose of 0.5 mg/kg, would evoke similar neurobiological effects as described in the literature.

We demonstrated that administration of dimebon shortly before training enhances hippocampus-dependent learning in both appetitive and inhibitory learning tasks in C57BL/6N mice. Acute treatment with dimebon was also shown to enhance inhibitory step-down avoidance in older animals. In the Y-maze, dimebon increased the duration of drinking behaviour. Together, our findings suggest the memory-enhancing effects of dimebon in two memory paradigms, which are based on the biologically opposite motivations of positive reward and aversive stimulation.

2. Methods

2.1. Animals and general conditions of testing

Male C57 BL/6N mice aged 3 and 7 months were used. After transportation to the experimental facilities, the animals were housed individually for ten days before the start of experiments under a reverse 12 h:12 h light–dark cycle (lights on: 22:00 h) in standard laboratory conditions. Mice were tested during the dark period of the light cycle in a lab protected from noise. Experimenters were blinded to the treatment. In order to minimize the possible influence of the environment, animals from vehicle- and drug-treated groups were tested alternately. All experiments were carried out in accordance with the European Communities Council Directive for the care and use of laboratory animals following approval by the local governmental bodies for animal care and welfare.

2.2. Study design

2.2.1. Effects of repeated dimebon administration in the Y-maze

In the first study, we investigated the effects of repeated administration of dimebon on learning to access a bottle with water after drinking deprivation in a spatial version of the Y-maze. The dislocation of visual cues impaired the performance of previously trained mice in the Y-maze protocol, which validated this test as a model of spatial learning (Gorenkova et al., 2005; Gorenkova and Strekalova, unpublished results). Naïve 3-month-old male mice were treated daily either with dimebon (0.1 mg/kg/day) or vehicle ($n=8$ and $n=8$, respectively) and 15 min thereafter were trained in two consequent trials spaced one hour apart; the training lasted 5 days (Fig. 1A); their latencies for reaching the bottle of water, the percentage of correct choices and the duration of drinking were scored (*see below*). Additionally, the potential effects of acute and 3-day repeated treatment of dimebon on behaviour in the O-maze and novel cage were subsequently evaluated 15 min after the injection of 0.1 mg/kg of dimebon ($n=8$) or vehicle ($n=8$). On the same day, a 24-h long water consumption test was carried out in repeatedly dosed animals (Fig. 1C). In a separate experiment, we further addressed the question of whether dimebon increases thirst in water-deprived animals (which would elevate their reward during training and explain the observed accelerated memory acquisition in the Y-maze) (Fig. 1D). Mice were either not deprived from water or deprived from drinking for 18 h prior to the test. For each condition, 10 animals were treated with dimebon at a dose of 0.1 mg/kg 15 min before the onset of the test and, respectively, 10 and 9 mice were treated by a vehicle. The amount of consumed water was evaluated 1, 3 and 24 h after the beginning of the test.

2.2.2. Effects of acute treatment with dimebon in the step-down avoidance model

As repeated administration of dimebon (0.1 mg/kg) was found to enhance performance in the Y-maze (*see below*), we addressed the question of whether acute treatment with dimebon at this dose affects hippocampus-dependent learning in a single trial paradigm. Besides, behavioural changes in dimebon-treated mice were observed in the Y-maze during the very first hours after drug administration. Therefore, naïve 3-month-old male mice were treated either with dimebon (dose 0.1 mg/kg) or vehicle and 15 min later were subjected to a training session in the step-down avoidance model ($n=9$ and $n=8$, respectively); the test for recall was carried out 1 and 24 h later (Fig. 1B, *also see below*). Because of the lack of any effects in this experiment, a higher dose of dimebon (0.5 mg/kg: $n=16$ and $n=17$) was tested in the same study design. Since in this assay, in which young animals were used, the higher dose of dimebon enhanced memory, the efficacy of dimebon at a dose of 0.5 mg/kg was further tested in 7-month-old mice, which received a bolus i.p. injection of dimebon or vehicle ($n=13$ in each group) and were tested in the same protocol of step-down avoidance as used for the younger animals. In a separate study, 3- and 7-month-old mice were given an i.p. injection of dimebon at a dose of 0.5 mg/kg ($n=8$ and $n=8$ for each age group, respectively) and 15 min later were tested in the O-maze and novel cage tests (Fig. 1C; *see below)* to address the possible

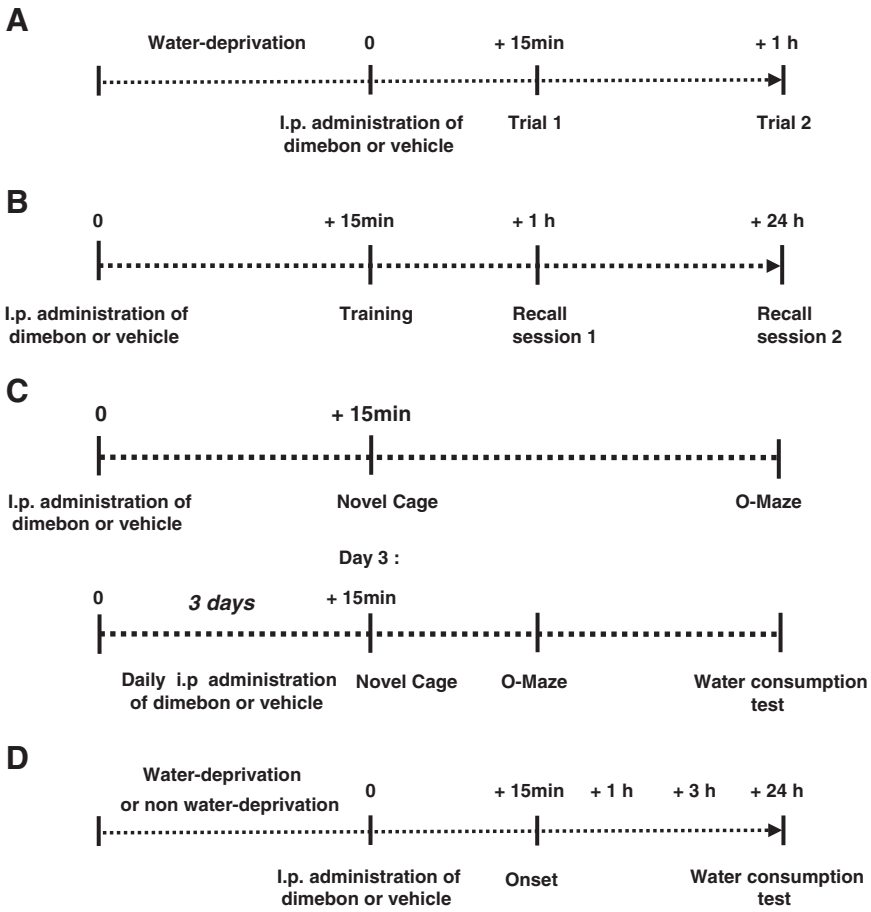

Fig. 1. Schemes of treatment and behavioural testing. (A) In the Y-Maze paradigm, two-trial training protocol was applied 15 min after an i.p. administration of dimebon or vehicle during 5 consecutive days. (B) In the step-down avoidance test, single training session was carried out 15 min after an i.p. administration of dimebon or vehicle; mice were tested for memory recall 1 and 24 h thereafter. (C) Timeline of testing of anxiety-like and exploratory behaviours, and water intake after bolus or repeated administration of dimebon or vehicle. (D) Timeline of testing the potential effects of bolus dimebon administration on 1-, 3- and 24-h water intake in water-deprived and non-water-deprived mice.

non-specific effects of dimebon on parameters of anxiety and exploration/locomotion.

2.2.3. Apparatus and experimental procedures

2.2.3.1. Y-maze. The apparatus used was a symmetrical Y-shaped construction made from black Plexiglas, which consisted of three arms (40×6×10 cm) with an angle of 120° between each arm (Technosmart, Rome, Italy). Figures of different shapes (approx. size 20×40 and 20×30 cm) were placed on the walls of the room to allow spatial orientation. Validation studies showed that the 180°-rotation of cues around the Y-maze apparatus disrupted the performance of previously trained mice (Gorenkova et al., 2005; Gorenkova and Strekalova, unpublished results). Two bottles, one filled with water and another empty, were placed at the ends of the arms in a position adjusted to allow drinking. The illumination strength was 25 lx.

Before the first training session, mice were water-deprived for 18 h. Two 15-min trials per day spaced one hour apart were carried out for 5 days in a row. The administration of either dimebon or vehicle was performed 15 min prior to the first training session on each day. During the trial, a mouse was placed at the starting point of the apparatus (where no bottle was presented) and allowed to access either arm of the maze containing the bottles, one of which was filled with water. Half of each experimental group was trained to receive a water reward from either the lefthand or righthand bottle. Each mouse was allowed to drink for up to 15 min in each trial; when no drinking behaviour was observed by the end of the training day, free access to water in the home cages was allowed for 30 min one hour after the termination of behavioural testing. The animals' body weight was monitored throughout the testing period; previous studies showed this Y-maze protocol to be optimal for training and demonstrated a lack of negative effects of the drinking schedule on body weight (Gorenkova et al., 2005, Gorenkova and Strekalova, unpublished results). The latency to reach the filled bottle and the percentage of correct choices for the arm containing this bottle were taken as indicators of learning of the task. The total duration of drinking during each trial was also measured.

2.2.3.2. Step-down avoidance test. Fifteen minutes after the i.p. administration of drug or vehicle, mice were trained in a step-down avoidance paradigm. The step-down apparatus (Evolocus LLC Tarrytown, NY, USA and Technosmart, Rome, Italy) consisted of a transparent plastic cubicle (25 cm×25 cm×50 cm) with a stainless-steel grid floor (33 rods 2 mm in diameter), onto which a square wooden platform (7 cm×7 cm×1.5 cm) was placed. A shocker was used to deliver an alternating electric current (AC, 50 Hz). The illumination strength was 25 lx. In this paradigm, animals will be trained not to step down from a platform onto a grid floor to avoid an electric shock.

During the training session, mice were placed onto the platform inside a transparent cylinder for 30 s to prevent them from stepping down immediately. After removal of the cylinder, the time until the animal left the platform with all four paws was measured as baseline latency of step down. Immediately after step down, mice received a single electric foot-shock (0.5 mA, 2 s) and returned to their home cages. One hour and twenty-four hours later, during the recall trial

session, animals were exposed to the apparatus again by being handled the same way as in the training session; no foot shock was delivered. Latency of step down with all four paws was measured until 180 s elapsed. Accordingly to a previously validated criterion of the acquisition of the step down avoidance task (Strekalova and Steinbusch, 2009, 2010), animals that showed latencies of more than 30 s in the recall session were considered as good learners. The behaviour of individual mice, which showed during a recall session the escape latency 1 s or lower while baseline latency exceeded 5 s, was regarded as panic response to aversive context, these animals were discarded from the experiment.

2.2.3.3. O-maze. The apparatus, which consisted of a circular path (runway width 5.5 cm, diameter 46 cm) was placed 50 cm above the floor. Two opposing arms were protected by walls (height 10 cm), and the illumination strength was 25 lx. The apparatus was placed on the dark surface in order to reduce a reflection and keep a control over lighting conditions during testing. The anxiety-like behaviour was assessed in applied protocol of the O-maze test using parameters, which were validated earlier (Strekalova et al., 2004, 2005). Mice were placed in one of the close arms compartments of the apparatus. The latency of the first exit to the anxiety-related open compartments of the maze and total duration of time spent therein were scored during a 5 min observation period.

2.2.3.4. Novel cage test. The novel cage test was performed to assess exploration of a new environment (Strekalova et al., 2001, 2004). Mice were introduced into a standard plastic cage filled with fresh sawdust. The number of exploratory rearings was counted under red light during a 5 min period.

2.2.3.5. Water consumption test in repeatedly treated mice. A 24-h drinking test was carried out to assess potential effects of repeated administration of dimebon on water intake (17.00–17.00). Consumption of regular drinking water was evaluated by weighing bottles before and after the test.

2.2.3.6. Water consumption test in acutely treated mice. To further evaluate the possible effects of acute dimebon administration on water intake in water-deprived animals, mice were either not deprived of water or were deprived from drinking for 18 h. Dimebon or vehicle was administrated 15 min prior to the test, which was started at the onset of the dark phase of the light cycle (10.00). Intake of regular drinking water was measured by weighing bottles 1, 3 and 24 h after the beginning of the test.

2.2.4. Drugs and drug administration

Dimebon (obtained from the Institute of Physiologically Active Compounds, Chernogolovka, Russia) was dissolved in isotonic NaCl solution for delivery in a volume of 0.01 ml/g of body weight by intraperitoneal injection at doses of 0.1 mg/kg and 0.5 mg/kg; vehicle was administered in the same volume.

2.2.5. Statistical analysis

Data were analyzed with a statistical software package (Prizm 3, Chicago, IL). Values of latencies to reach water reward in the Y-maze had normal distribution, therefore, repeated measurements data were compared by repeated measures ANOVA test followed by the Tukey' post-hoc test, independent measurements were treated by one way ANOVA analysis followed by unpaired *t*-test; differences of variances were assessed by the F test. Since values of the duration of drinking behaviour in the Y-maze model in many cases were assigned to a period of observation, these data were considered as arbitrarily measured and, therefore, treated by the non-parametric analysis: Friedman test followed by the Dunn's multiple comparison test was applied for repeated measurements; Kruskal–Wallis test followed by Mann–Whitney *U*-test was used for a group comparison. Percentage of correct choices was analyzed by a Fischer's exact test. Independent data sets obtained in the step-down avoidance and O-maze test were treated with non-parametric analysis, even though the populations were Gaussian, since in essential percentage of animals behavioural parameters were measured arbitrary. This was due to the cut off in the behavioural scoring: several values were assigned to the observation periods elapsed. Mann–Whitney test was used for the analysis of independent data sets. Data obtained in the novel cage and water intake tests had normal distribution and were analyzed by the *t*-test. The level of confidence was set at 95% ($p<0.05$).

3. Results

3.1. Repeated dimebon administration enhances learning in the Y-maze

The effects of daily administration of dimebon on the selection of the correct arm of the Y-maze baited with a filled bottle and the latency to reach the water reward were measured to assess spatial learning. A repeated ANOVA revealed significant changes in the latter parameter in the course of the 5-day training period both in the control ($p=0.006$, $F=2.92$, $R^2=0.29$) and dimebon-treated groups ($p<0.0001$, 10.64, $R^2=0.60$); a decrease in the latency to reach the water reward (Fig. 2A,B) suggested that mice from both groups acquired the task. Compared to the mean latency to reach the water reward measured on day 1 (trial 1), the dimebon-treated group had a significant reduction in this parameter on day 2, while vehicle-treated mice showed this effect of training only on day 5 ($p<0.05$; Fig. 2A,B). ANOVA revealed significant differences between dimebon- and vehicle-treated groups in the overall comparison of latencies to reach the reward ($p<0.0001$, $F=3.77$, $R^2=0.34$). The percentage of correct choices in the course of nine testing trials (the training trial 1 on day 1 was excluded from the analysis), was significantly higher in the dimebon-treated group (77.8%) than in vehicle-treated mice (52.78%, $p=0.001$, Fisher's exact test, Fig. 2C) and chance values ($p=0.0004$); the vehicle-treated group showed no significant difference from the chance values for correct choices ($p=0.43$). Together, repeated treatment with dimebon accelerated the acquisition of the Y-maze memory task.

The Friedman test revealed significant differences in the duration of drinking between the trials both in dimebon- and vehicle-treated mice which spent more time drinking as the experiment progressed from day 1 to day 5 ($p<0.0001$ for each group; Fig. 3A,B). This suggests that animals gradually learnt the location of the water source and became less anxious in the course of training. The duration of drinking was significantly higher in the dimebon-treated group than in the vehicle-treated group (on day 1, trial 1: $p=0.01$, $U=11.0$; day 2, trials 1 and 2: $p=0.003$, $U=7.5$ and $p=0.0005$, $U=3.0$, respectively; on day 3, trial 2: $p=0.01$, $U=10.5$; Mann–Whitney *U* test; Fig. 3B). A tendency to increased time spent drinking in the dimebon-treated mice compared to control animals was revealed in trial 1, day 3 ($p=0.06$, $U=17.0$) and in trial 1, day 4 ($p=0.06$, $U=17.5$). Starting from day 4, trial 2 and until the end of the training period, the duration of drinking did not differ between groups ($p>0.05$, Mann–Whitney *U* test). Thus, in a novel environment, which is well documented to induce anxiety and suppress consummatory behaviour (Loiseau et al., 2003; Dulawa and Hen, 2005), mice repeatedly treated with dimebon showed elevated water intake. The latter finding suggested that drug administration might induce an anxiolytic-like effect and/or change the physiological need for water. To rule out these factors, additional experiments were carried out.

Since significant effects of dimebon on the latency to reach a reward and the duration of drinking behaviour were revealed both after repeated treatments, the potential effects of repeated dimebon administration was assessed in additional tests. It was found that the 3-day treatment with dimebon at a dose of 0.1 mg/kg, compared to a

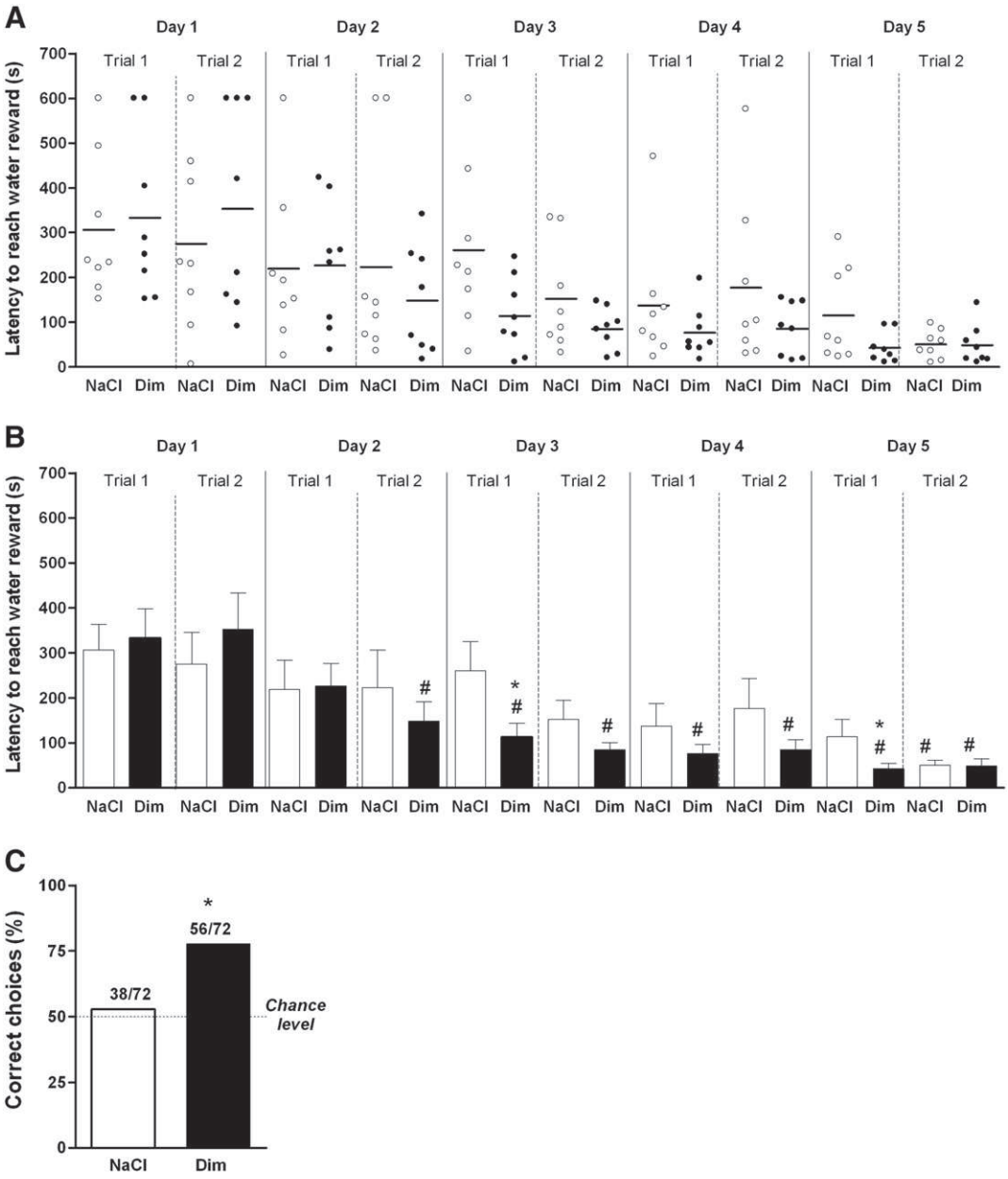

Fig. 2. Repeated dimebon administration accelerates learning in the Y-Maze. (A) Individual and (B) group data demonstrate a significant reduction of the latency to reach the water reward in dimebon-treated group on days 2–5 of training, while vehicle-treated group showed such difference on day 5 ($^{\#}p<0.05$ vs. training values on Day 1 Trial 1, Tukey's posthoc test). On days 3 and 5, this parameter was significantly lower in dimebon-treated animals than in vehicle-treated mice ($^{*}p<0.05$, unpaired *t*-test). Bars represent the means; each column represents the mean ± SEM. (C) Percentage of correct choices for the arm with a filled bottle from nine testing trials overall carried out for 8 animals was significantly higher in dimebon-treated group than in control group ($^{*}p<0.05$, Fischer's exact test). Absolute numbers of correct choices are indicated above the bars. NaCl: vehicle-treated group; and Dim: dimebon-treated group.

vehicle-treated group, did not change the latency of exit, the time spent in the open arms or the number of exits in the O-maze ($p>0.05$ and $p>0.05$ respectively, Mann–Whitney *U* test; Fig. 3C). In particular, the difference in time spent in the open arms of the O-maze between the dimebon- and vehicle-treated group was far from statistically significant ($p=0.79$, $U=29$). No difference was found between vehicle- and dimebon-treated groups in the number of exploratory rearings in the novel cage either ($p>0.05$ and $p>0.05$ respectively, unpaired *t*-test; Fig. 3D).

Repeated dosing with dimebon did not alter the 24-h water intake ($p>0.05$, unpaired *t*-test; Fig. 3E). In addition, in order to determine whether acute administration of dimebon can increase thirst in water-deprived animals, we studied the effects of dimebon on mice under normal and water-deprivation conditions. Bolus injection of dimebon did not affect 1-h, 3-h or 24-h water consumption regardless of previous water deprivation ($p>0.05$, two-way ANOVA; Fig. 3F). The amount of water consumed did not differ between vehicle-treated and dimebon-treated mice not deprived of water, or between animals previously deprived of water ($p>0.05$ and $p>0.05$ respectively, Mann–Whitney *U* test; Fig. 3F). A two-way ANOVA showed that water-deprivation increased water intake at three time points ($p<0.05$, two-way ANOVA). Together, our results suggest that treatment with dimebon at a dose of 0.1 mg/kg accelerates spatial learning of the task based on water reward without affecting the metabolic requirement for water, locomotion or exploration in mice. However, dimebon seemed to have an anxiolytic-like effect in the model of spatial learning, which may have contributed to the improvement of performance under the testing conditions employed.

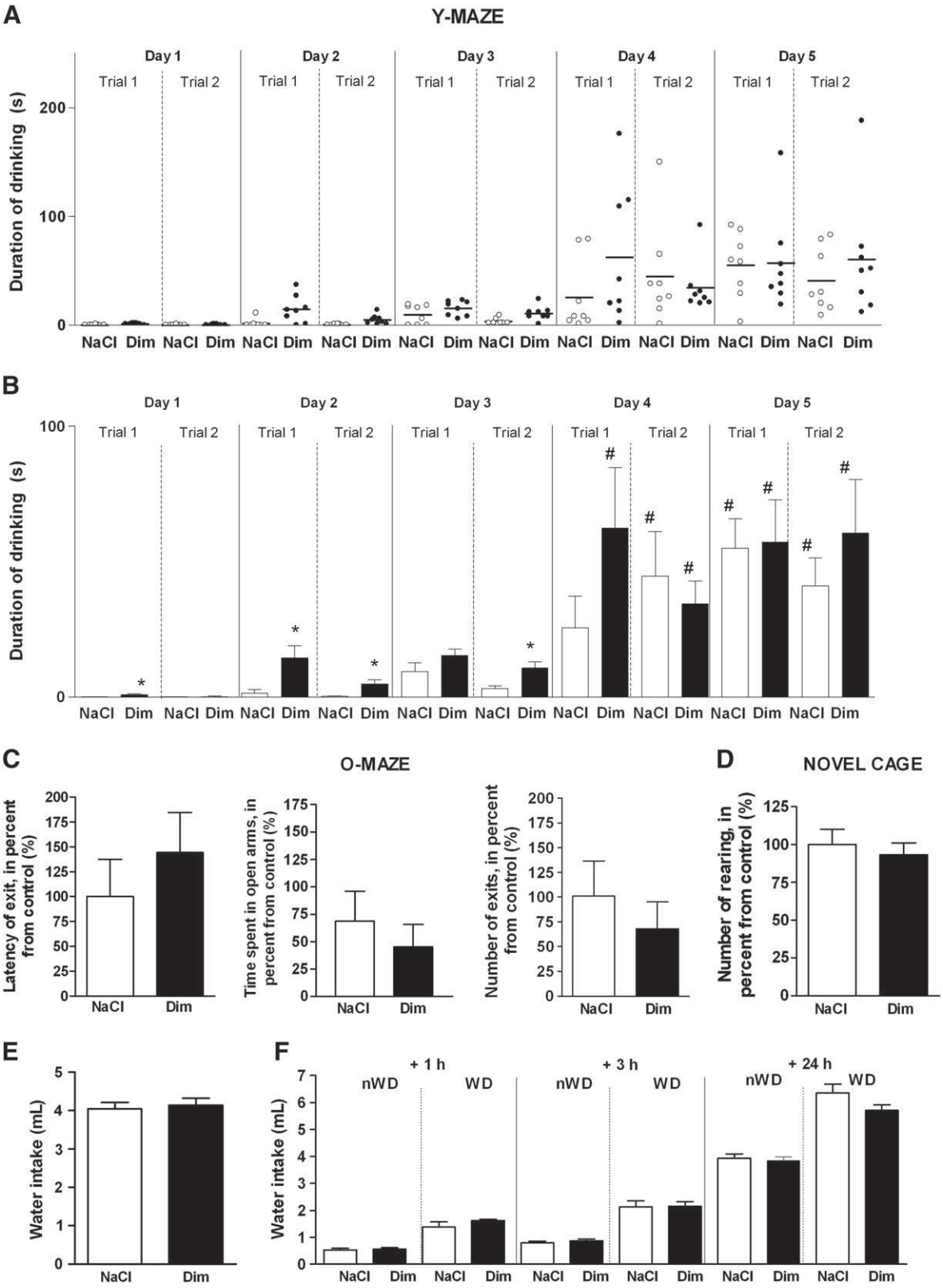

Fig. 3. Effects of repeated administration of dimebon on parameters of anxiety, locomotion, exploration and drinking. (A) Individual and (B) group data evidence significant increase of drinking behaviour in a course of training experiment in the Y-Maze ($^{\#}p<0.05$ vs. training values on Day 1 Trial 1, Dunn's multiple comparison test). Duration of drinking was significantly higher in dimebon-treated mice than in control group on days 1–3 ($^{*}p<0.05$, Kruskal–Wallis test). Bars represent the medians. Each column represents the mean ± SEM. Mice, repeatedly treated with dimebon did not differ from vehicle-treated animals in (C) latency of exit to open arms and time spent therein, as well as number of exits in the O-maze, (D) number of exploratory rearings in the novel cage test, (E) 24-h water intake; $p>0.05$, Mann–Whitney *U*-test. (F) Bolus treatment with dimebon does not affect 1-, 3- and 24-h water intake, regardless of preceding water deprivation ($p>0.05$, two-way ANOVA), which increased 24-h water consumption both in vehicle- and dimebon-treated groups (*see text*). Each column represents the mean ± SEM. NaCl: vehicle-treated group; Dim: dimebon-treated group; WD: water-deprived; and nWD: non-water-deprived.

3.2. Acute treatment with dimebon increases performance in the step-down avoidance model

In the above-described experiment, dimebon-treated mice showed behavioural changes during the course of a repeated training/dosing protocol and also immediately after the very first drug administration (within a 2-h period), comprising a significant increase in the duration of drinking behaviour (Figs. 2,3). Therefore, we addressed the question of whether a bolus injection of dimebon at the dose of 0.1 mg/kg can affect learning in a single trial memory model in 3-month-old mice using the step-down avoidance paradigm. The vehicle- and dimebon-treated groups showed similar baseline latencies ($p=0.35$, U = 21.00, Mann–Whitney *U* test) suggesting that dimebon injection is unlikely to affect locomotion, exploration or anxiety-like behaviours in the step-down avoidance test. In comparison to the baseline values, the latencies of step down were similarly increased in vehicle- and dimebon-treated groups during the first recall session ($p=0.02$ and $p=0.04$, respectively, Wilcoxon test; Fig. 4A,B) and the second recall session ($p=0.01$ and $p=0.01$ respectively), indicating that both experimental groups acquired the task.

We found no differences between the groups in the latency of step down in either of the two recall sessions, carried out 1 or 24 h after training ($p=0.28$, U = 19.0 and $P=0.45$, U = 23.5 respectively, Mann–Whitney *U* test; Fig. 4B). According to our exclusion criteria (*see Methods section*) three mice were discarded from the experiment (one from the control group and two from the dimebon-treated group). The number of animals that were classified as good learners according to the 30-sec criterion of the task acquisition (*see Methods section*) among vehicle- and dimebon-treated mice was similar during

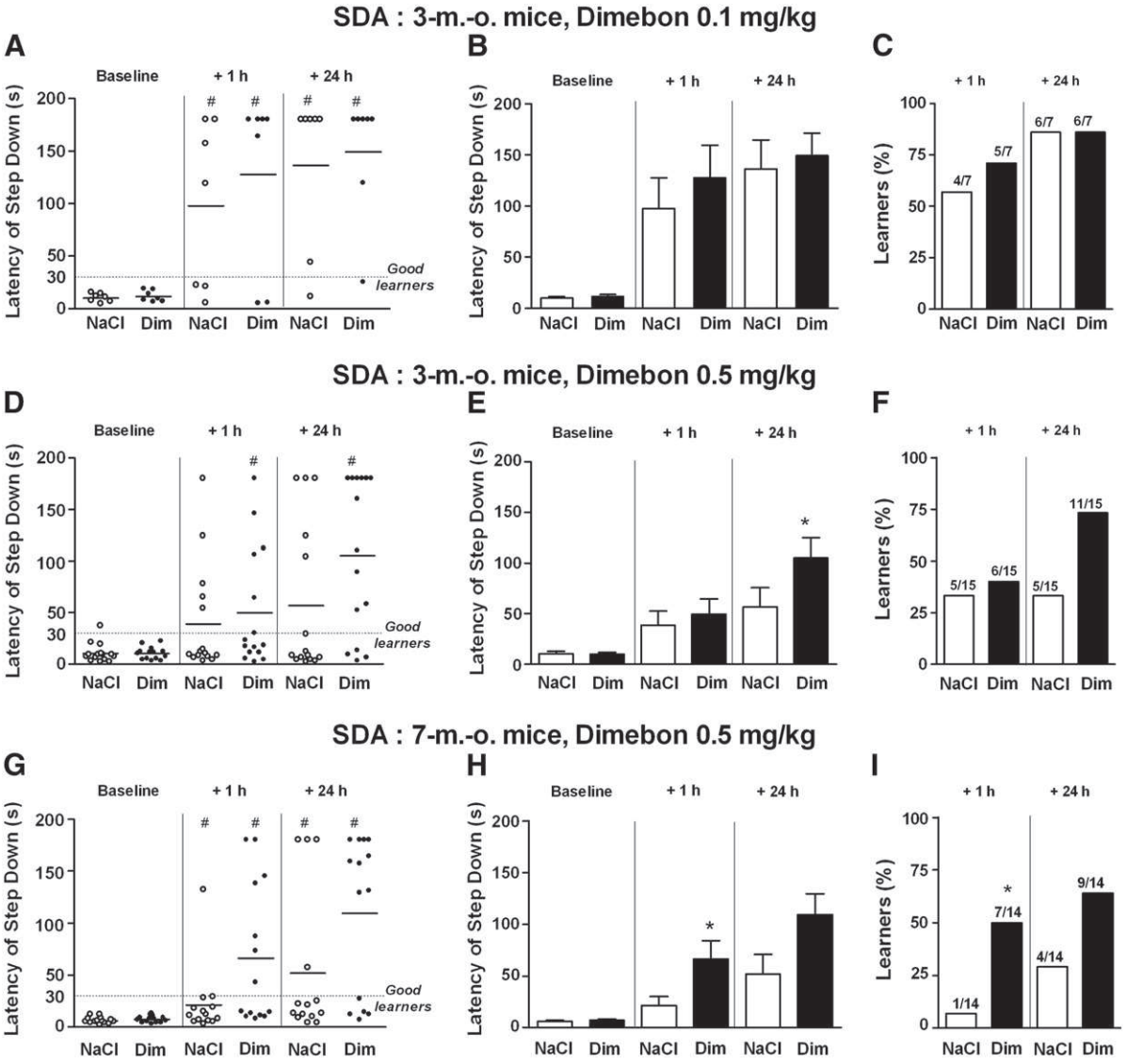

Fig. 4. Acute treatment with dimebon enhances performance in the step-down avoidance paradigm: effects in 3- and 7-month old mice. (A, D, G) Individual and (B, E, H) group data show an increase of the latencies of step down measured during two recall sessions(+1 h and +24 h) in comparison to the training session (baseline) in vehicle and dimebon-treated group suggesting that mice acquired the task ($^{\#}p<0.05$ vs. training values, Wilcoxon test). Three- and seven-month old mice treated with dimebon at the dose of 0.5 mg/kg revealed significant increase in the latency of step down evaluated 24 h after training as compared to vehicle-treated group ($^{*}p<0.05$, Mann–Whitney *U*-test); no such difference was observed in 3-month old mice treated by 0.1 mg/kg of dimebon ($p>0.05$, Mann–Whitney *U*-test). Seven-month old mice treated with dimebon showed also a significant increase of this parameter 1 h after training, as compared to vehicle-treated animals ($^{**}p<0.05$, Mann–Whitney *U*-test). (C, F, I) Three- and seven-month old mice treated with dimebon at the dose of 0.5 mg/kg had significantly higher percentage of good learners estimated 24 h after training as compared to vehicle-treated group ($^{*}p<0.05$, Fischer's exact test); no such difference was observed in 3-month old mice treated by 0.1 mg/kg of dimebon ($p>0.05$, Fischer's exact test). Seven-month old mice treated with dimebon showed also a significant increase of this parameter 1 h after training, as compared to vehicle-treated animals ($^{**}p<0.05$, Fischer's exact test). Each column represents the mean ± SEM. NaCl: vehicle-treated group; and Dim: dimebon-treated group.

the first and second recall sessions ($p = 0.50$ and $p = 0.77$ respectively, Fischer's exact test; Fig. 4C). Thus, acute injection of dimebon at the dose of 0.1 mg/kg does not alter short-term and contextual learning in mice trained in the step-down avoidance task.

In the next experiment, we studied whether a higher dose of dimebon (0.5 mg/kg) would change the performance in the step-down avoidance model assessed in the same experimental design. As in a previous study, drug administration did not affect the baseline latencies of step down, which were similar in vehicle-treated and dimebon-treated mice ($p > 0.05$, Mann–Whitney *U* test; Fig. 4D,E). The latencies of step down were increased during recall sessions in both groups in comparison to baseline values, both during the first recall trial ($p = 0.05$ and $p = 0.001$ for vehicle- and dimebon-treated groups, respectively, Wilcoxon; Fig. 4D,E) and during the second recall session ($p = 0.06$ and $p = 0.01$, respectively). Whereas no difference in the latency of step down between the groups was observed during the first recall session ($p = 0.18$, U = 90.0, Mann–Whitney *U* test; Fig. 4D, E), the dimebon-treated group showed a significantly longer latency of step down during the second recall session compared to vehicle-treated mice ($p = 0.03$, U = 67.5, Mann–Whitney *U* test). In addition, the percentage of good learners was significantly higher among dimebon-treated animals than in vehicle-treated mice during the second recall session ($p = 0.03$, Fischer's exact test, Fig. 4F). Three mice were discarded from the experiment (one from the control group and two from the dimebon-treated group), according to the exclusion criteria (*see Methods section*). The results of this experiment demonstrate improved scores of long-term memory of mice treated with a bolus i.p. injection of dimebon at a dose of 0.5 mg/kg in a step-down avoidance paradigm.

To verify these effects of dimebon in slightly different conditions, we used 7-month-old mice in the same study design. The administration of dimebon did not alter the baseline latencies of step down, which did not differ significantly between the vehicle and dimebon-treated mice ($p > 0.05$, Mann–Whitney *U* test; Fig. 4G,H). In vehicle- and dimebon-treated groups, the latencies of step down were elevated during recall sessions in comparison to baseline values, both during the first recall trial ($p = 0.0008$ and $p < 0.0001$ for respectively, Wilcoxon; Fig. 4D,E) and during the second recall session ($p < 0.0001$ and $p = 0.0004$, respectively; Fig. 4G,H). The dimebon-treated group showed a significantly higher latency of step down during the second recall session than vehicle-treated mice ($p = 0.03$, U = 63.0; Mann–Whitney *U* test; Fig. 4G,H) and during the first recall session ($p = 0.03$, U = 67.0). The percentage of good learners was significantly higher in the dimebon-treated than control mice during the first and second recall sessions ($p = 0.04$ and $p = 0.01$, respectively; Fisher's exact test, Fig. I). According to the exclusion criteria (*see Methods section*) five mice were excluded from the experiment (one from the control group and four from the dimebon-treated group). Thus, bolus i.p. injection of dimebon at a dose of 0.5 mg/kg also elevates memory scores in a step-down avoidance paradigm in 7-month-old mice. These results further demonstrate the memory-enhancing effects of a single administration of dimebon at a dose of 0.5 mg/kg in a step-down avoidance model.

To rule out the possibility that dimebon, at a dose that affects performance in the step-down avoidance model, interferes with anxiety-like behaviour and exploration, supplementary tests were carried out in young and middle-aged mice. Application of a battery of tests 15 min after intraperitoneal administration of dimebon and vehicle in 3-month-old and 7-month-old mice at a dose of 0.5 mg/kg revealed a lack of differences between the groups in the latency of exit, time spent in the open arms and the number of exits in the O-maze ($p > 0.05$, Mann–Whitney *U* test, Fig. 5A,D). Of note, while 3- and 7-month-old dimebon-treated mice show a graphical trend towards decreased time spent in the open arms of the O-maze, the difference in this parameter from the vehicle-treated group is far from statistically significant ($p = 0.88$, U = 30 and $p = 0.28$, U = 21.5, respectively).

The number of exploratory rearings in the novel cage was unchanged in young mice treated with dimebon ($p > 0.05$, unpaired *t*-test; Fig. 5B) and was significantly lower in older mice after dimebon administration ($p = 0.04$, unpaired *t*-test; Fig. 5E). Thus, the statistically significant increase in the latency of step down in 7-month-old mice treated with dimebon during the first recall session can be accounted for by the general suppressive effect of the treatment on exploration/locomotion; these effects were observed in 3-month-old animals. Together, our results suggest that similar to the experiment on young animals, bolus treatment with dimebon at a dose of 0.5 mg/kg enhances long-term contextual learning in the step-down model in middle-aged mice.

4. Discussion

The present data suggest that the administration of dimebon 15 min prior to training in the Y-maze and step-down avoidance via repeated (0.1 mg/kg) and acute (0.5 mg/kg) i.p. injections, respectively, increases learning scores in C57BL/6N mice while affecting other behaviours in some test situations, as well (Table 1). Bolus administration of dimebon at the dose of 0.1 mg/kg did not alter the learning of the step-down avoidance task in 3-month-old mice. Acute treatment with dimebon was also shown to enhance inhibitory learning in the 7-month-old mice. Bolus administration of dimebon did not affect locomotion, exploration or anxiety-like behaviour in additional experiments in young and old mice, except rearing activity in 7-month-old mice in a novel cage test, which was decreased by the treatment. No effects of 3-day treatment with dimebon on locomotion, exploration, O-maze behaviour or water consumption were found either. Repeated administration of dimebon increased the duration of drinking behaviour in the Y-maze. Together, our data suggest that in mice, dimebon increases hippocampus-dependent learning in both appetitive and inhibitory tasks. Thus, dimebon enhances memory based on the biologically opposite situations of positive reward and aversive stimulation.

In the course of training in the Y-maze, dimebon-treated mice showed overall shorter latencies of reaching the water reward than control mice (Fig. 2A,B). Dimebon-treated mice revealed a significant reduction in this parameter compared to the values measured on day 1, trial 1, starting from day 2, trial 2 of the training procedure, while control animals demonstrated such an effect of training only on day 5. On days 3 and 5, the dimebon-treated group had significantly shorter latencies to reach the water reward than the vehicle-treated group. The percentage of correct choices was significantly higher in the dimebon-treated group than in control mice (Fig. 2D). Together, our data suggest that repeated treatment with dimebon accelerated the acquisition of the spatial task in the Y-maze test.

Importantly, acute and chronic administrations of dimebon significantly increased the duration of drinking during the first experimental session and in the course of daily training (Fig. 3A,B). As anxiolytic drugs are well-documented to increase novelty-suppressed consummatory behaviour (Loiseau et al., 2003; Dulawa and Hen, 2005) and the anxiolytic effects of dimebon were observed in two classical tests for anxiety-like behaviour and open field tests (Bachurin and Grigoriev, 2009), our findings might be considered to be an indication of the anti-anxiety properties of dimebon. In the latter study, bolus intraperitoneal administration of dimebon at a dose of 2 mg/kg, 40 min prior to testing was found to induce an anxiolytic effect in a rat Vogel conflict model, dark/light box paradigm, and an open field test, that was similar to the effects of diazepam applied at the same dose. Lower doses of dimebon (0.05–0.1 mg/kg) evoked anxiolytic-like changes in some, but not all behavioural measures in this study. In our experiments, repeated 3-day treatments with dimebon did not alter anxiety-like behaviour in the O-maze (Fig. 3C), and similar results were found after bolus treatment with dimebon at a dose of 0.5 mg/kg in 3-month-old and 7-month-old mice (Fig. 5A,C,

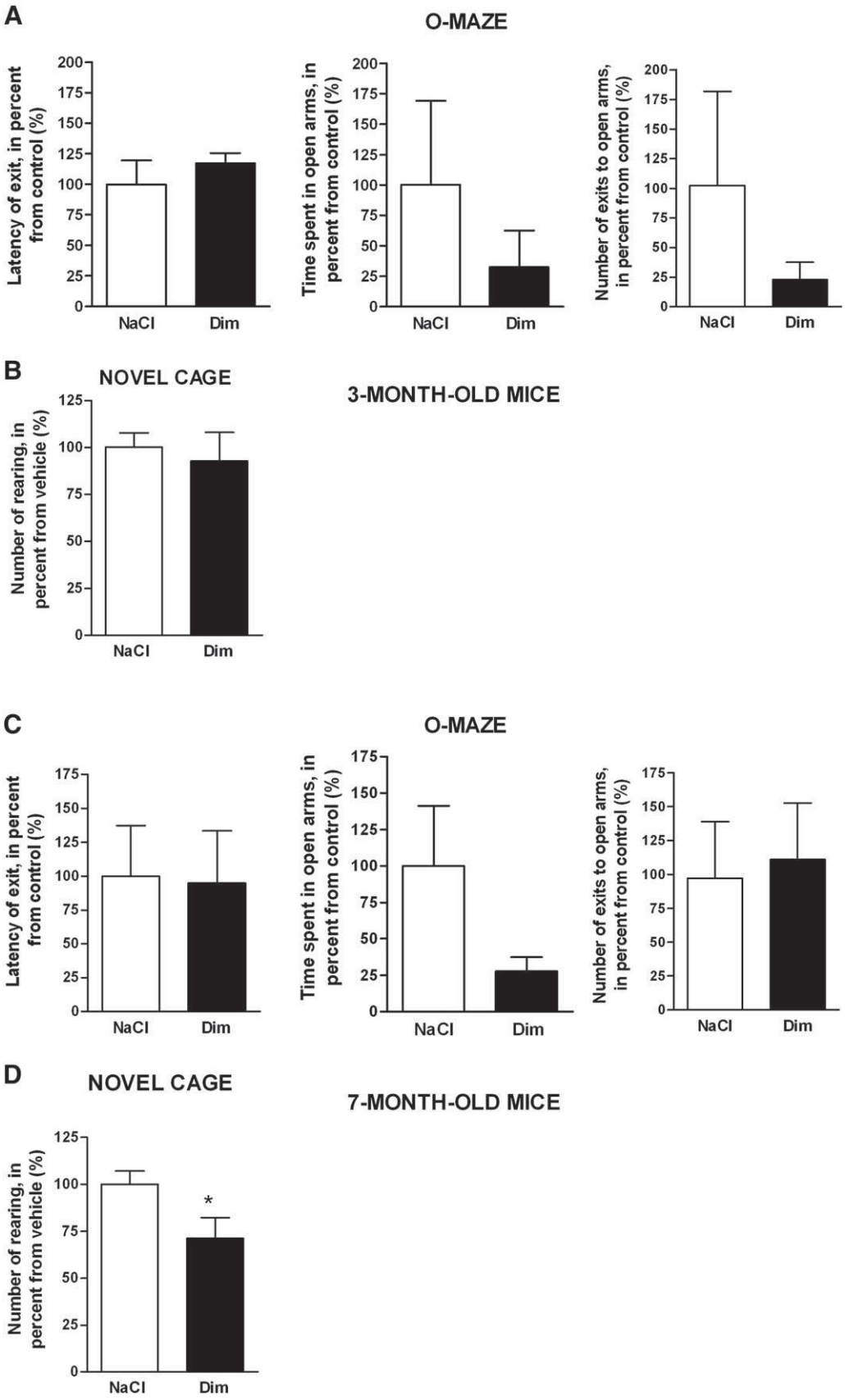

Fig. 5. Bolus injection of dimebon and parameters of anxiety, locomotion and exploration. Three-month old mice, treated with dimebon did not differ from vehicle-treated animals in (A) latency of exit to open arms and time spent therein, as well as number of exits in the O-maze ($p>0.05$, Mann–Whitney *U*-test) and (B) number of exploratory rearings in the novel cage test ($p>0.05$, unpaired *t*-test). (C) Seven-month old mice, treated with dimebon did not differ from vehicle-treated animals in the latency of exit to open arms and time spent in the open arms, as well as in the number of exits in the O-maze ($p>0.05$, Mann–Whitney *U*-test), but (B) showed reduced number of exploratory rearings in the novel cage test ($^{*}p<0.05$, unpaired *t*-test). Each column represents the mean ± SEM. NaCl: vehicle-treated group; and Dim: dimebon-treated group.

Table 1
Memory enhancing action and other behavioural effects of acute and repeated treatment with dimebon. Dimebon induced memory enhancing effect at the dose of 0.1 when injected repeatedly and at the dose of 0.5 when applied acutely. Both types of treatment induced also other behavioural effects (*see text*; n.a.: non-applicable).

			Acute treatment		Repeated treatment	
			Memory enhancing	Other effects	Memory enhancing	Other effects
Y-maze	3-m-o 0.1 mg/kg		–	+	+	+
Step down avoidance	3-m-o	0.1 mg/kg	–	–		
		0.5 mg/kg	+	–		
	7-m-o 0.5 mg/kg		+	+	n.a.	n.a.

see also below). Mice treated with dimebon demonstrated an insignificant decrease in the duration of time spent in the O-maze; statistically, this difference was far from significant, and is due to the presence of a few outliers. The latency of exit to open arms, and the number of exits had similar values in vehicle- and dimebon-treated groups, thus additionally suggesting a lack of effect by dimebon on anxiety-related behaviour under these testing conditions.

The differences between our experimental results and the above described study might be due to differences in dimebon doses, species and anxiety paradigms employed, as well as distinct animal testing times with respect to dosing. In order to rule out potential confounds in the evaluation of the cognitive effects of dimebon, mice were tested in O- and Y-mazes using consistent time schedules, 15 min after drug administration in both tests. In contrast to this testing protocol, changes in the anxiety-like behaviour of dimebon-treated rats were investigated at a time point which is believed to be closer to the peak of dimebon concentration in the brain, as other studies have revealed maximal levels of the drug in the rat brain 50–60 min after its intragastrical administration at doses of 0.05–1 mg/kg (Giorgetti et al., 2010). As these doses of dimebon were similar to those used in the present study, we assume that the concentration of dimebon in the brain peaks during a comparable time period, i.e. 30 min–1 h 30 min after dosing. Thus, it can be speculated that among other possible reasons, the anti-anxiety effects of dimebon in the O-maze experiment were not observed because of lower concentrations of the drug in the mouse brain at the moment of testing in this paradigm. In support of this explanation, we found that in the Y-maze, a significant prolongation of drinking behaviour in dimebon-treated mice was detected on Day 1, Trial 2, i.e. approximately 1 h after the first drug administration, but not during testing in Trial 1, which was carried out only 15 min after treatment (Fig. 3A,B). Together, our results in the Y- and O-maze paradigms, and previously obtained data on the inconsistent appearance of the anti-anxiety effects of dimebon in rats treated with low drug doses, led us to suggest that the proposed anxiolytic-like action of this compound does not occur in all test situations. Similar results were found for other drugs as well (Haller et al., 2000; Merali et al., 2003; Reddy and Devi, 2006).

Suggested anxiolytic-like effect of dimebon in the Y-maze might contribute to the animals' performance by the facilitation of mouse exploration in the anxiogenic situation of novelty, which generally increases the chances of finding a bottle with water and receiving a reward. In addition, the enhanced rewarding impact of the training procedure may be due to the prolongation of water intake in water-deprived mice; increased reward during training may accelerate the acquisition of the Y-maze appetitive task above and beyond the immediate effects of dimebon as a cognitive enhancer.

Supplementary tests rule out the possibility that dimebon merely increases exploratory behaviour (Fig. 3D) and elevates the metabolic need for water; the latter factor was assessed for repeated bolus administration of dimebon in both water-deprived mice and under normal drinking conditions (Fig. 3E,F). Bolus injection of dimebon did not increase 1-, 3- or 24-h water intake, regardless of previous water deprivation. Moreover, after acute treatment with dimebon, 24-h water intake was insignificantly decreased in non-water deprived animals in this study (Fig. 3F), in line with earlier reported suppressive effects of other drugs with inhibitory action on various behaviours in rodents, e.g., citalopram, on water consumption (Strekalova et al., 2006). Hence, possible changes in exploration and thirst did not seem to interfere with enhanced performance in the Y-maze and increased duration of drinking behaviour of animals treated with dimebon.

Thus, dimebon enhances spatial learning in the memory model based on positive reward. Because novelty exploration is also considered to be a rewarding stimulus, the memory enhancing effects of dimebon in the new object exploration/localization paradigms mentioned above and described by other groups (Chuhan and Taukulis, 2006; Giorgetti et al., 2010) indirectly support our findings.

Since in the Y-maze, the very first administration of dimebon induced behavioural changes (increasing the duration of drinking behaviour; Fig. 3A,B), we tested whether dimebon affected learning after a bolus injection. To achieve consistency with the Y-maze study, we selected the step-down avoidance paradigm, since this is a single trial memory test in which the animals' performance is well known to depend on intact hippocampal function (Lorenzini et al., 1996; Izquierdo et al., 2006). The object recognition test was not selected, since there are discrepancies concerning the role of the hippocampus in this task (Albasser et al., 2010).

We found that dimebon delivered at the dose of 0.5, but not 0.1 mg/kg significantly increased the latency of step down in a recall session carried out 24 h after the training session (Fig. 4A,B,D,E), as well as the number of animals classified as good learners (Fig. 4C,F) in 3-month-old mice, i.e., it evoked memory-enhancing effects, according to previously validated criteria of memory acquisition in this task (Strekalova and Steinbusch, 2009, 2010). Changes in the latency to step down under conditions of intrahippocampal administration of various active compounds and the induction of stress-induced anhedonia have been shown to correlate with other parameters of hippocampal plasticity, such as induction of the LTP in the CA1 area of the hippocampal formation (Strekalova et al., 2001, 2002; Strekalova and Steinbusch, 2010; Tokarski et al., under revision). The effective dose of dimebon also increased learning scores in the step-down avoidance test in 7-month-old mice (Fig. 4G–I). No changes in the latency to step down were found in dimebon-treated mice tested 1 h after the training session in young mice, while in 7-month-old animals it was significantly increased in the dimebon-treated group (Fig. 4E,H).

However, dimebon at the dose of 0.5 mg/kg inhibited exploration/locomotion in the novel cage test in the older, but not younger animals (Fig. 5A,D). Given the lack of memory-enhancing effects on short-term memory in young mice, these data suggest that a significant increase in the latency to step down revealed in 7-month-old mice treated with dimebon during the first recall session was due to its non-specific inhibitory effects on locomotion. Data on the suppressive effects of dimebon on exploratory vertical activity in the novel cage are in line with the well-documented sedative effects of histamine receptor blockers (Passani et al., 2007; Van Ruitenbeek et

al., 2010), which are more subtle with dimebon treatment than with other antihistamines (Iliyuchenok and Matveeva, 1989). Similarly to our study, dimebon was found to decrease exploratory behaviour in rats at a dose of 30 mg/kg (Schaffhauser et al., 2009). Interestingly, the inhibitory action of dimebon on exploratory rearing activity in the novel cage was age-dependent. Other studies have revealed differential effects of psychotropic drugs, including compounds with sedative activity, on younger vs. older rodents (Smith et al., 2002; Takase et al., 2009). The distinct locomotory effects of dimebon on 3- and 7-month-old mice in the present work might be accounted for by altered receptor sensitivity and slower drug metabolism, resulting in elevated dimebon concentrations in the brains of older animals.

Inhibitory behavioural effects of dimebon in 7 month-old-mice on exploratory rearing activity and step down avoidance behaviour measured shortly after training were detected 0.5 h and 1 h 15 min after the treatment, respectively, thus, the occurrence of these effects of dimebon corresponded a proposed time window of maximal brain concentrations of the drug in the mouse brain. No such effects of the treatment were revealed at earlier time points relative to the treatment, as no changes were observed in the latency of baseline step down behaviour and parameters reflecting animals' locomotion in the O-maze, which were assessed 15 min after injection of a dose dimebon.

The fact that the dimebon- and vehicle-treated groups in both age groups showed no difference in their baseline latencies of step down which demonstrates the absence of its effects on anxiety and locomotion under conditions of testing in the step-down avoidance apparatus. In line with these data, most of the supplementary tests in mice of both age groups revealed no effects of dimebon on the parameters of anxiety-like behaviour in the O-maze test (Fig. 5A,C) or exploratory behaviour in the novel cage test (Fig. 5B) with the above-mentioned exception of the behavioural inhibition of 7-month-old mice in the latter paradigm (Fig. 5D). Similarly to the study results on the effect of repeated dimebon administration, both 3- and 7-month-old animals acutely treated with dimebon demonstrated an insignificant decrease in the duration of time spent in the O-maze; these differences were far from the level of statistical significance. Single outliers, which might represent individual mice with increased sensitivity to the drug or/and testing procedures, could contribute to these insignificant changes. Of note, the latency of exit to open arms and the number of exits were virtually the same between the groups, again suggesting a lack of effect by dimebon on anxiety-related behaviour in the O-maze in our study. Thus, these data rule out the possibility of non-specific effects of dimebon on mouse performance in the step-down avoidance model for young animals.

All together, presented data suggest that dimebon enhances learning in both appetitive and inhibitory tasks of the hippocampus-dependent memory in mice. As it was mentioned above, dimebon administrated to a rat at the dose of 0.05–1 mg/kg was found to have a half-life in the plasma over 2 h (Giorgetti et al., 2010); similar doses used in the present work led to expect comparable pharmacokinetics of dimebon in employed here battery of memory tests. Together with the fact that dimebon was delivered 15 min prior to training, this let to propose that dimebon was present at an effective concentration in the mouse brain during phases of early and intermediate memory consolidation in both the Y-maze and step-down avoidance models (Gerlai, 2001; Cammarota et al., 2005; Da Silva Costa et al., 2009; Benchenane et al., 2010). The relatively short half-life of dimebon in rodents suggests that the non-specific effects of dimebon observed within 30 min after drug administration in the novel cage test are unlikely to underlie an increase in the latency of step down documented 24 h after training in the step-down avoidance paradigm. These changes are very likely to reflect the memory-enhancing effects of dimebon administration.

The mechanism underlying the memory enhancing effects of dimebon remains elusive. During the last few decades, the concept of multi-target drug activity has been proposed (Wong et al., 2008; Cavalli et al., 2008; Combarros et al., 2009). According to this concept, the mechanisms responsible for the beneficial actions of drugs can be realised via multiple actions of the compound on a number of receptors; in this case, effective changes in the concentrations of neurotransmitters may be much lower than if only one receptor signaling system underlies the drug's activity. On one hand, dimebon was shown to interact with a broad spectrum of neuronal receptors, which are involved in synaptic plasticity and cognitive functions (Lermontova et al., 2001; Schaffhauser et al., 2009; Grigoriev, 2009; Giorgetti et al., 2010; Okun et al., 2010). At the same time, effects from dimebon on pre-clinical and clinical measures of cognition were found at doses corresponding to brain concentrations much lower than the K1 values for many receptors which were found to be effective in models of learning and memory (Gold, 2006). Thus, it can be speculated that, in the mouse test battery employed in this study, dimebon affects a number of receptors and may act as a multi-target drug, i.e. the concentrations used in this study, which correspond to sub-threshold levels for receptor activation via a mono-target mechanism, are only able to evoke physiological effects by synergistic activation of several neurotransmitter systems (Youdim and Buccafusco, 2005; Cavalli et al., 2008). The memory-enhancing effects of dimebon may indicate simultaneous activity toward AMPA, NMDA, dopamine and serotonin receptors, all of which have been involved in inhibitory and appetitive hippocampus-dependent learning (Ungerer et al., 1998; Orsetti et al., 2001; Rogawski and Wenk, 2003; LaLumiere et al., 2003; Lynch and Gall, 2006; Balschun et al., 2006; Da Silva Costa et al., 2009; Benchenane et al., 2010). In particular, it has been hypothesised that dimebon's activity as a positive modulator of AMPA receptors and low affinity non-competitive blocker of NMDA receptors via a multi-drug mechanism, can explain the pro-cognitive action of this compound (Grigorev et al., 2003; Grigoriev 2009).

The last data suggest that it is unlikely that the effects of dimebon on memory presented here and in other reports occur via its action on neurotransmitter systems via a “one drug–one molecule” mechanism. For instance, a recent study showed that intragastrically delivered dimebon at a dose of 0.05–5.0 mg/kg is inefficient at the inhibition of AChE or blockade of the NMDA receptor; these doses, however, evoked pronounced improvement of new object recognition memory in rats. This dose range of dimebon did not affect the turnover of ACh in the hippocampus and prefrontal cortex, and is ineffective at blocking the NMDA-induced calcium influx; both effects observed at higher dimebon concentrations (Giorgetti et al., 2010; Wu et al., 2008). In another study, dimebon was found to bind to 5-HT6 receptor, where it enhanced social recognition memory; however, the weak binding affinity and the relatively low drug concentrations employed in the study cannot link the observed behavioural effects to changes in serotonin transmission (Schaffhauser et al., 2009). In addition, dimebon was shown to bind to a number of receptors, such as histamine, dopamine, norepinephrine and serotonin receptors, at concentrations which are not comparable to those used *in vivo* (Wu et al., 2008; Schaffhauser et al., 2009; Giorgetti et al., 2010). In sum, at present, a consistent view on the neurotransmitter mechanism that underlies the memory-enhancing effects of dimebon is lacking.

Interestingly, a recent study reported that dimebon had remarkable effects on hippocampal neurogenesis, at doses comparable to those used in the present study (Pieper et al., 2010). One-week treatment of rats with dimebon at a dose of 0.1 mkM/kg (0.32 mg/kg) increased the number of BrdU-positive cells in the dentate gyrus. Because activation of structural plasticity and neurogenesis in the hippocampus is well demonstrated to be implicated in the mechanisms of contextual and spatial learning (Epp et al., 2007; Yang et al., 2008; Li et al., 2010; Pieper et al., 2010), we suggest that this effect might be one of the potential mechanisms which underlie the memory enhancing action of dimebon observed in the current study. Further experiments are required to address the possible link

between the mnemonic effects of dimebon and its properties as a proneurogenic compound.

5. Conclusion

Recently, a series of new functional analogues of dimebon with predominant action on several proposed mechanisms discussed above was developed (Lermontova et al. 2003; Perlovich et al., 2009; Bachurin et al., unpublished results). A comparative analysis of these compounds in the battery of tests employed in the current study will hopefully help to elucidate its mechanism of memory-enhancing action in the future. Since the hippocampus was shown to be a primary brain structure, the function of which is compromised during early stages of Alzheimer's disease (Foerstl, 2009), this suggests the usefulness of the battery of mouse models of hippocampus-dependent memory employed in the present work for such studies as well as, in general, for fundamental and pre-clinical aspects of testing drug candidates for the treatment of this pathology.

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References

Albasser MM, Chapman RJ, Amin E, Iordanova MD, Vann SD, Aggleton JP. New behavioral protocols to extend our knowledge of rodent object recognition memory. Learn Mem 2010;17:407–19.
Alhassan AW, Bauchi ZM, Adebisi SS, Mabrouk MA, Sabo AM. The effect of early administration of glucocorticoids on learning and spatial memory. Niger J Physiol Sci 2009;24:137–9.
Bachurin S, Bukatina E, Lermontova N, Tkachenko S, Afanasiev A, Grigoriev V, et al. Antihistamine agent dimebon as a novel neuroprotector and a cognition enhancer. Ann NY Acad Sci 2001;939:425–35.
Bachurin S, Shevtsova E, Kireeva E, Oxenkrug G, Sablin S. Mitochondria as a target for neurotoxins and neuroprotective agents. Ann NY Acad Sci 2003;993:334–44.
Bachurin S, Serkova T, Lermontova N, Ivanova T, Beznosko B, Vankin G, et al. Comparative study of aricept, memantine and dimebon effect on memory and cognition in neurotoxin-induced animal model of Alzheimer's disease. Abstracts of 10th International Conference on Alzheimer's Disease and Related Disorder, July 15–20, Chicago; 2006.
Bachurin S.O, Grigoriev V.V. A drug demonstrating anxiolytic effect based on hydrogenated pyrido (4,3-B) indoles, its pharmacological compound and application method WO2009005771 (A1).
Balschun D, Zuschratter W, Wetzel W. Allosteric enhancement of metabotropic glutamate receptor 5 function promotes spatial memory. Neuroscience 2006;142:691–702.
Benchenane K, Peyrache A, Khamassi M, Tierney PL, Gioanni Y, Battaglia FP, et al. Coherent theta oscillations and reorganization of spike timing in the hippocampal-prefrontal network upon learning. Neuron 2010;66:921–36.
Bernales S, Alarcon R, Guerrero J, Higaki JN, Protter AA. Dimebon induces neurite outgrowth from hippocampal, spinal, and cortical neurons. Neurology 2009;72:A:385.
Bolkunov A, Beznosko B, Bachurin S. Effects of dimebon in the new object recognition test in mice. (in preparation)
Cammarota M, Bevilaqua LRM, Rossato JI, Ramirez M, Medina JH, Izquierdo I. Relationship between short- and long-term memory and short- and long-term extinction. Neurobiol Learn Mem 2005;84:25–32.
Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, et al. Multi-target-directed ligands to combat neurodegenerative diseases. J Med Chem 2008;5:347–72.
Chuhan YS, Taukulis HK. Impairment of single-trial memory formation by oral methylphenidate in the rat. Neurobiol Learn Mem 2006;85:125–31.
Combarros O, Rodríguez-Rodríguez E, Mateo I, Vázquez-Higuera JL, Infante J, Berciano J, Sánchez-Juan P. Neurobiol Aging 2009 [Epub ahead of print].
Da Silva Costa V, Duchatelle P, Boulouard M, Dauphin F. Selective 5-HT6 receptor blockade improves spatial recognition memory and reverses age-related deficits in spatial recognition memory in the mouse. Neuropsychopharm 2009;34:488–500.
Dolgov O, Gorenkova N, Bartsch D, Strekalova T. Distinct behavioral and cognitive correlates of hedonic deficit in a new model of stress-induced anhedonia in mice. Proceedings of the 6th Meeting of the German Neuroscience Society; 2005. http://www.neuro.uni-goettingen.de.
Doody RS, Gavrilova SI, Sano M, Thomas RG, Aisen PS, Bachurin SO, et al. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. Lancet 2008;372:207–15.
Dulawa SC, Hen R. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. Neurosci Biobehav Rev 2005;29:771–83.
Epp JR, Spritzer MD, Galea LA. Hippocampus-dependent learning promotes survival of new neurons in the dentate gyrus at a specific time during cell maturation. Neurosci 2007;149:273–85.
Finger BC, Dinan TG, Cryan JF. Leptin-deficient mice retain normal appetitive spatial learning yet exhibit marked increases in anxiety-related behaviours. Psychopharmacol Berl 2010;210:559–68.
Foerstl H. Neuro-enhancement. Brain doping. Nervenarzt 2009;80:840–6.
Galani R, Weiss I, Cassel JC, Kelche C. Spatial memory, habituation, and reactions to spatial and nonspatial changes in rats with selective lesions of the hippocampus, the entorhinal cortex or the subiculum. Behav Brain Res 1998;96:1-12.
Gerlai R. Behavioral tests of hippocampal function: simple paradigms complex problems. Behav Brain Res 2001;125:269–77.
Giorgetti M, Gibbons JA, Bernales S, Alfaro IE, La Rochelle CD, Cremers T, et al. Cognition enhancing properties of dimebon in a rat novel object recognition task are unlikely to be associated with acetylcholinesterase inhibition or NMDA receptor antagonism. J Pharmacol Exp Ther 2010;333:748–57.
Gold PE. The many faces of amnesia. Learn Mem 2006;13:506–14.
Gorenkova N, Kunert J, Berger S, Strekalova T, Bartsch D. Gene expression profiling in sterssed mice with and without depressive-like syndrome. Proceedings of EMBO conference of Young Investigator Awardees; 2005. http://www. embo. Org/about _embo/EMBO_AR_2005.pdf.
Grigorev VV, Dranyi OA, Bachurin SO. Comparative study of action mechanisms of dimebon and memantine on AMPA- and NMDA-subtypes glutamate receptors in rat cerebral neurons. Bull Exp Biol Med 2003;136:474–7.
Grigoriev VV. A Development of the System of Identification of Correction of Cognitive Impairments during Neurodegenerative Disorders among Glutamate Receptor Ligands. Moscow: Max Press; 2009. 1–49.
Gura T. Hope in Alzheimer's fight emerges from unexpected places. Nat Med 2008;14:894.
Haller J, Halász J, Makara GB. Housing conditions and the anxiolytic efficacy of buspirone: the relationship between main and side effects. Behav Pharmacol 2000;5:403–12.
Hung D. Dimebon: A phase 3 investigational agent for Alzheimer's disease with a novel mitochondrial mechanism of action. Alzheimers Dement 2008;4:182–3.
Iliyuchenok TI, Matveeva IA. Dimebon — a novel antihistaminergic agent. New Pharm agents 1989;4:12–6 [Article in Russian].
Izquierdo I, Bevilaqua LRM, Rossato JI, Bonini JS, Medina JH, Cammarota M. Different molecular cascades in different sites of the brain control memory consolidation. Trends Neurosci 2006;29:496–505.
Izquierdo Y, Medina JH. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structure. Neurobiol Learn Mem 1997;68:285–316.
Kieburtz K, McDermott MP, Voss TS, Corey-Bloom J, Deuel LM, Dorsey ER, et al. A randomized, placebo-controlled trial of latrepirdine in Huntington disease. Arch Neurol 2010;67:154–60.
LaLumiere RT, Buen TV, McGaugh JL. Post-training intra-basolateral amygdala infusions of norepinephrine enhance consolidation of memory for contextual fear conditioning. J Neurosci 2003;23:6754–8.
Lermontova NN, Lukoyanov NV, Serkova TP, Lukoyanova EA, Bachurin SO. Dimebon improves learning in animals with experimental Alzheimer's disease. Bull Exp Biol Med 2000;129:544–6.
Lermontova NN, Redkozubov AE, Shevtsova EF, Serkova TP, Kireeva EG, Bachurin SO. Dimebon and tacrine inhibit neurotoxic action of beta-amyloid in culture and block L-type Ca(2+) channels. Bull Exp Biol Med 2001;132:1079–83.
Lermontova NN, Mukhina TV, Vankin GI, Serkova TP, Bachurin SO. Comparison of the Effect of NT-0409 and antidementia drugs on learning and memory in rats with chronic cerebral cholinergic dficiency. Bull Exp Biol Med 2003;135:58–61.
Li B, Wanka L, Blanchard J, Liu F, Chohan MO, Iqbal K, et al. Neurotrophic peptides incorporating adamantane improve learning and memory, promote neurogenesis and synaptic plasticity in mice. FEBS Lett 2010;584:3359–65.
Liu P, Jarrard LE, Bilkey DK. Excitotoxic lesions of the pre- and parasubiculum disrupt object recognition and spatial memory processes. Behav Neurosci 2001;115:112–24.
Loiseau F, Le Bihan C, Hamon M, Thiébot MH. Distinct effects of diazepam and NK1 receptor antagonists in two conflict procedures in rats. Behav Pharmacol 2003;14:447–55.
Lorenzini CA, Baldi E, Bucherelli C, Sacchetti B, Tassoni G. Role of hippocampus acquisition, consolidation and retrieval of rat's passive avoidance response: a tetrodoxin functional inactivation study. Brain Res 1996;730:32–9.
Lynch G, Gall CM. Ampakines and the threefold path to cognitive enhancement. Trends Neurosci 2006;10:554–62.
Merali Z, Levac C, Anisman H. Validation of a simple, ethologically relevant paradigm for assessing anxiety in mice. Biol Psychiatry 2003;54:552–65.
Miller G. The puzzling rise and fall of a dark-horse Alzheimer's drug. Science 2010;327(5971):1309.

O'Brien JT. A promising new treatment for Alzheimer's disease? Lancet Neurol 2008;7: 768–9.
Okun I, Tkachenko SE, Khvat A, Mitkin O, Kazey V, Ivachtchenko AV. From anti-allergic to anti-Alzheimer's: molecular pharmacology of dimebon. Curr Alzheimer Res 2010;7:97-112.
Orsetti M, Ghi P, Di Carlo G. Histamine H(3)-receptor antagonism improves memory retention and reverses the cognitive deficit induced by scopolamine in a two-trial place recognition task. Behav Brain Res 2001;124:235–42.
Passani MB, Giannoni P, Bucherelli C, Baldi E, Blandina P. Histamine in the brain: beyond sleep and memory. Biochem Pharmacol 2007;73:1113–22.
Perlovich GL, Volkova TV, Kurkov SV, Proshin AN, Petrova LN, Bachurin SO. Novel salts of dibenzylamine derivative isothiourea as potent neuroprotectors and cognition-enhancers: synthesis, biological and physicochemical properties. J Med Chem 2009;52:1845–52.
Pieper AA, Xie S, Capota E, Estill SJ, Zhong J, Long JM, et al. Discovery of a proneurogenic, neuroprotective chemical. Cell 2010;142:39–51.
Protter A, Vartiainen V, Yrjanheikki J, Bernales S. Neurite outgrowth and mitochondrial function in dimebon treated rat cortical cultures. Neurodegener Dis 2009;6 Abstract 1536.
Reddy PV, Devi K. Intrastrain variations in anxiolytic effect of nitrazepam in mice. Indian J Physiol Pharmacol 2006;50:309–12.
Rogawski MA, Wenk GL. The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease. CNS Drug Rev 2003;I;3:275–308.
Schaffhauser H, Mathiasen JR, Dicamillo A, Huffman MJ, Lu LD, McKenna BA, et al. Dimebolin is a 5-HT6 antagonist with acute cognition enhancing activities. Biochem Pharmacol 2009;78:1035–42.
Smith JW, Al-Khamees O, Costall B, Naylor RJ, Smythe JW. Chronic aspirin ingestion improves spatial learning in adult and aged rats. Pharmacol Biochem Behav 2002;71:233–8.
Steele JW, Kim SH, Cirrito JR, Verges DK, Restivo JL, Westaway D, et al. Acute dosing of latrepirdine (Dimebon), a possible Alzheimer therapeutic, elevates extracellular amyloid-beta levels in vitro and in vivo. Mol Neurodegener 2009;17:51.
Strekalova T, Sun M, Sibbe M, Evers M, Dityatev A, Gass P, et al. Fibronectin domains of extracellular matrix molecule tenascin-C modulate hippocampal learning and synaptic plasticity. Mol Cell Neurosci 2002;21:173–87.
Strekalova T, Wotjak C, Schachner M. Intrahippocampal administration of an antibody against the HNK-1 carbohydrate impairs memory consolidation in an inhibitory learning task in mice. Mol Cell Neurosci 2001;17:1102–13.
Strekalova T, Spanagel R, Bartsch D, Henn F, Gass P. Stressed-induced anhedonia in mice is associated with deficits in forced swimming and exploration. Neuropsychopharm 2004;11:2007–17.
Strekalova T, Spanagel R, Dolgov O, Bartsch D. Stress-induced hyperlocomotion as a confounding factor in anxiety and depression models in mice. Behav Pharm 2005;16:171–80.
Strekalova T, Gorenkova N, Schunk E, Dolgov O, Bartsch D. Selective effects of citalopram in the mouse model of stress-induced anhedonia with control effects for chronic stress. Behav Pharm 2006;17:271–87.
Strekalova T, Steinbusch H. Factors of reproducibility of stress-induced anhedonia in chronic stress depression models in mice. In: Gould T, editor. Mood and Anxiety Related Phenotypes in Mice: Characterization using Behavioral Tests. Totowa, NJ: Humana Press; 2009. p. 153–76.
Strekalova T, Steinbusch HWM. Measuring behavior in mice with chronic stress depression paradigm. Progr Neuro Psychopharm Biol Psych 2010;34:348–61.
Tan WF, Cao XZ, Wang JK, Lv HW, Wu BY, Ma H. Protective effects of lithium treatment against spatial memory deficits induced by tau hyperphosphorylation in splenectomized rats. Clin Exp Pharmacol Physiol 2010;37:1010–5.
Takase LF, Fornal CA, Jacobs BL. Effects of the hypnotic drug zolpidem on cell proliferation and survival in the dentate gyrus of young and old rats. Brain Res 2009;1259:26–31.
Tokarski K, Draguhn A, Gorenkova N, Schunk E, Kunchulia, Dolgov O, Steinbusch H, Strekalova T. Hippocampal plasticity in mice with and without hedonic deficit after stress and pre-treatment by citalopram. PLoSOne (under revision).
Ungerer A, Mathis C, Mélan C. Are glutamate receptors specifically implicated in some forms of memory processes? Exp Brain Res 1998;123:45–51.
Van Ruitenbeek P, Vermeeren A, Riedel WJ. Memory in humans is unaffected by central H1-antagonism, while objectively and subjectively measured sedation is increased. Eur Neuropsychopharmacol 2010;4:226–35.
Wong EH, Nikam SS, Shahid M. Multi- and single-target agents for major psychiatric diseases: therapeutic opportunities and challenges. Curr Opin Investig Drugs 2008;9:28–36.
Wu J, Li Q, Bezprozvanny I. Evaluation of dimebon in cellular model of Huntington's disease. Mol Neurodegener 2008;3:15.
Yang WM, Shim KJ, Choi MJ, Park SY, Choi BJ, Chang MS, et al. Novel effects of Nelumbo nucifera rhizome extract on memory and neurogenesis in the dentate gyrus of the rat hippocampus. Neurosci Lett 2008;443:104–7.
Youdim MB, Buccafusco JJ. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. Trends Pharmacol Sci 2005;26:27–35.