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Spatial learning deficit in dopamine D_1 receptor knockout mice

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Abstract

Dopamine D_1 receptors are expressed in the hippocampus and prefrontal cortex, suggesting a role in cognition. Dopamine D_1 receptor-deficient mice ($D_1-/-$) were used to investigate the role of this receptor in spatial learning and memory. Using the Morris water maze, mice were trained to locate a hidden platform. Subsequently, the platform was removed from the maze and mice were scored for the percentage of time spent in the target quadrant and the number of crossings through the target position. $D_1-/-$ mice had significantly longer escape latencies compared to wild-type ($D_1+/+$) and heterozygous ($D_1+/-$) littermates and showed absence of spatial bias during the probe trials. In a visually cued task, $D_1-/-$ mice performed better than on the hidden platform trials, but maintained slightly higher escape latencies than $D_1+/+$ and $D_1+/-$ mice. Naive $D_1-/-$ mice exposed only to the cued task eventually acquired identical escape latencies as the $D_1+/+$ and $D_1+/-$ mice. Sensorimotor reflexes, locomotor activity, spontaneous alternation and contextual learning were not different among the groups. These results indicate that $D_1-/-$ mice have a deficit in spatial learning without visual or motor impairment, suggesting that dopamine D_1 receptors are involved in at least one form of the cognitive processes.

Keywords: Morris water maze; Spatial learning; Dopamine D_1 receptor-deficient mouse; Spontaneous alternation; Locomotor activity; Passive avoidance behavior

1. Introduction

The hippocampus and the prefrontal cortex are important brain structures implicated in various types of cognitive function since lesions in these regions impair spatial learning and memory processes (Morris et al., 1982; Sutherland et al., 1982; Whishaw and Kolb, 1984; Winocur and Moscovitch, 1990; Squire, 1992; Jarrard, 1993). Moreover, impaired learning and memory loss in aged rats has been attributed to cortical and hippocampal dysfunction (Winocur, 1992). The hippocampal formation (hippocampus, dentate gyrus, and subicular cortex) is connected to diverse subcortical structures, including the nucleus accumbens (Kelley and Domesick, 1982); lesions of the nucleus accumbens and different components of the hippocampal formation have been shown to cause deficits in spatial performance (Olton and Papas, 1979; Annett et al., 1989).

Dopamine, along with several other neurotransmitters, innervates the hippocampus and the prefrontal cortex (Baulac et al., 1986; Gasbarri et al., 1994; Law-Tho et al., 1994; Seamans et al., 1998) and modulates working memory function (Goldman-Rakic, 1990). In support of the evidence that dopamine plays a modulatory role in learning and memory (Simon et al., 1986; Packard and White, 1989; Yamamuro et al., 1994), dopamine has also been shown to facilitate in vivo hippocampal and cortical acetylcholine release (Day and Fibiger, 1994; Hersi et al., 1995a,b), modulate glutamatergic/cortical neurotransmission, as well as *N*-methyl-D-aspartate (NMDA) receptor-

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mediated responses (Levine et al., 1996; Mele et al., 1996; Verma and Moghaddam, 1996). Depletion or dysfunction of dopamine in the prefrontal cortex or lesions of the mesohippocampal dopaminergic system alters spatial learning and working memory in rodents and nonhuman primates (Brozoski et al., 1979; Hagan et al., 1983; Whishaw and Dunnett, 1985; Gasbarri et al., 1996) and has been correlated with age-related cognitive dysfunction in nonhuman primates (Arnsten 1993; Murphy et al., 1996) and aged rats (Lee et al., 1994). Cognitive deficits are prominent and important clinical manifestations of Alzheimer's disease (De Keyser et al., 1990), Parkinson's disease (Bradley et al., 1990), schizophrenia (Goldberg et al., 1989; Berman and Weinberger, 1990; Park and Holzman, 1992; Okubo et al., 1997) and attention deficit hyperactivity disorder (Russell et al., 1995), all of which are linked to dopaminergic dysfunction.

Animal studies using nonhuman primates and rodents have indicated that optimal prefrontal cortex cognitive function depends on a critical range of dopamine D_1 receptor activation, above or below which impairment is evident (Murphy et al., 1996; Zahrt et al., 1997). To date, five dopamine receptors have been cloned, termed D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) (reviewed in O'Dowd et al., 1994). Both dopamine D_1 and D_2 receptors have been implicated in various learning and memory processes (Packard and White, 1989; Ichihara et al., 1992; Bernabeu et al., 1997; Izquierdo et al., 1998; Wilkerson and Levin, 1999). The fact that dopamine D_1 receptor is expressed more abundantly than the dopamine D_2 receptor in the hippocampus and prefrontal cortex of nonhuman primates (Lidow et al., 1991) and rodents (Dubois et al., 1986) suggests a more dominant role for this receptor subtype in cognition. While evidence based on pharmacological manipulations (local infusion or systemic administration) in monkeys is consistent with this hypothesis (Sawaguchi and Goldman-Rakic, 1991; Arnsten et al., 1994; Williams and Goldman-Rakic, 1995; Cai and Arnsten, 1997), studies in rodents are very limited and have not firmly established such a role. Dopamine D_1 receptor antagonist has been shown to differentially affect several types of learning as well as short and long-term memory in rats (Ichihara et al., 1989; Didriksen, 1995; Murphy et al., 1996, Bernabeu et al., 1997; Izquierdo et al., 1998, Wilkerson and Levin, 1999), or have no overall effects on spatial learning in aged rats (Hersi et al., 1995b). Dopamine D_1 receptor agonists were found to enhance passive avoidance (Bernabeu et al., 1997) and improve cognitive performance in rats (Hersi et al., 1995b; Steele et al., 1996) and mice (Bach et al., 1999) or have no effect on learning (Packard and White, 1989; Wilkerson and Levin, 1999).

In the present study, we used $D_1-/-$ mice homozygous for dopamine D_1 receptor gene deletion (Drago et al., 1994) to study the role of this receptor in cognition. These mice were tested for their ability to perform several cognitive tasks. These include place navigation using the Morris water maze, a task that is especially sensitive to manipulation of hippocampal function (Morris, 1984), spontaneous alternation in a Y-maze, proposed to reflect working memory (Sarter et al., 1988), a process which involves the prefrontal cortex. In addition, two different fear-conditioning paradigms proposed to involve hippocampus-dependent associative learning were also used: passive avoidance and contextual conditioning.

2. Materials and methods

2.1. Animals

Mice lacking the dopamine D_1 receptor were generated by homologous recombination as described previously (Drago et al., 1994). Wild-type ($D_1+/+$), homozygote ($D_1-/-$) and heterozygote ($D_1+/-$) offspring used in this study were derived from the mating of heterozygous mice. Genotype was determined by Southern blot analyses of genomic DNA (Drago et al., 1994). We have previously reported no specific dopamine D_1 receptor binding in $D_1-/-$ mice, ~50% lower dopamine D_1 receptor binding in $D_1+/-$ mice as compared to $D_1+/+$ mice (El-Ghundi et al., 1998). All mice were 3–5 months of age, and were housed in groups of three per cage in a temperature-controlled room (22°C), maintained on a reversed 12 h dark–light cycle (lights off 7 AM–7 PM). All mice were given free access to food pellets and water in their home cages. In addition to food pellets, $D_1-/-$ mice were fed hydrated mouse meal (mash) at weaning age. Prior to the start of the experiment, all mice were fed mash to control for the feeding variables. $D_1-/-$ mice were smaller (by 20–30%) than $D_1+/+$ or $D_1+/-$ littermates, fertile and exhibited normal home cage behavior. All experiments were conducted during the dark light phase in a sound-attenuated room. Animal care was according to guidelines approved by the Canadian Council for Animal Care (CCAC).

2.2. Experiment 1: Morris water maze

Spatial learning and memory were assessed using the Morris water maze. Three groups of adult male mice were used, $D_1-/-$ mice ($n=15$), $D_1+/-$ mice ($n=8$) and $D_1+/+$ ($n=15$) siblings. The apparatus consisted of a circular tank (80 cm high × 140 cm diameter) filled with water (up to 60 cm deep) maintained at room temperature (26°C) and made opaque with powdered milk. A hidden circular escape platform (15 cm diameter × 59 cm high), made of roughened Plexiglas, was submerged 1 cm under water in one of four designated positions within the tank. The tank was located in a sound-attenuated, well-lit room with many external cues that could be seen from the water tank.

During acquisition trials (day 1–3), mice were trained to escape from water by swimming from variable starting

points around the tank to the hidden platform. A total of 16 training trials were given (six trials per day for 2 days followed by four trials for 1 day, with an intertrial interval of 5–7 min). On test days, mice were put in individual cages and transferred to the room where the water maze was located. At the start of each trial, mice were held facing the tank wall and released into the water from one of six random starting points around the tank. Mice failing to find the platform within 90 s were guided to the platform and placed on it for 30 s. After each trial, the mouse was dried and returned to its cage and left there for 5–7 min until the next trial. All sessions were recorded by a video camera located above the tank. The escape latency (time taken to climb onto the platform) for each mouse was recorded immediately. A probe trial (Probe Trial 1) was conducted 24 h after the last acquisition trial (day 4), to measure spatial learning and memory. The platform was removed and mice were allowed to swim for 90 s. The duration of time spent in the target quadrant where the platform was previously located and the number of annulus crossings through the previous platform location were determined later by viewing the videotape. Only the first 60 s of this trial were analyzed, since control mice were consistently found to shift their search strategy during the 60–90 s period. The swimming speed was measured within 90 s and expressed as cm per min.

Following Probe Trial 1, all mice were given reversal trials (day 5–6) identical to those during the acquisition phase except that the hidden platform was relocated in a different position (diagonal to the previous position). A total of 12 trials over two consecutive sessions (six trials per day) were given and escape latencies were recorded. Following the reversal trials, the platform was again removed and all mice were given Probe Trial 2 (day 7) to test their abilities to memorize the new position of the hidden platform, as described for Probe Trial 1.

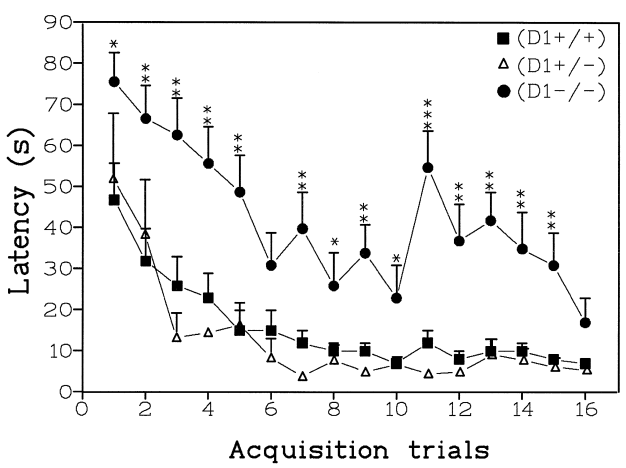

Fig.1. Escape latencies to locate a hidden platform over 90 s during the acquisition training trials. $D_1-/-$ mice displayed significantly longer escape latencies than control groups on all trials. Data shown are mean values ± S.E.M. *, **, ***, Significantly different from $D_1+/+$ and $D_1+/-$ mice ($P < 0.05$–0.0001).

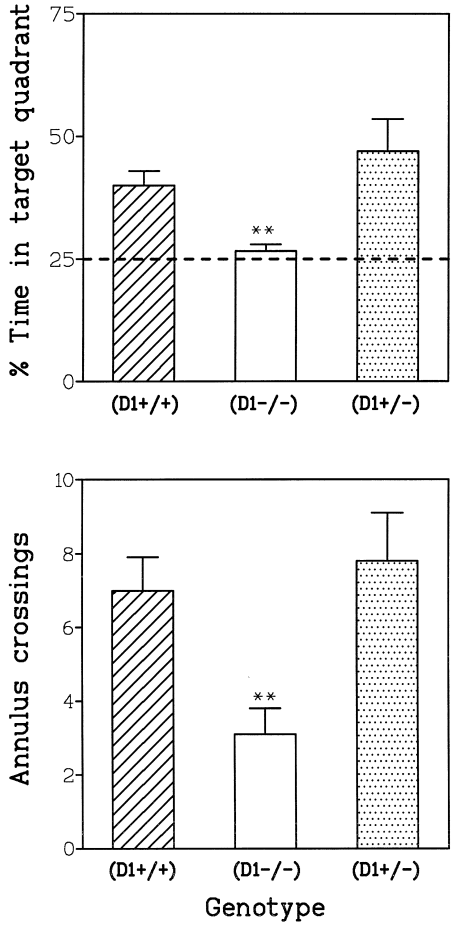

Fig. 2. Probe Trial 1 given after the last acquisition trial to test the memory of the previous platform position. The platform was removed and the mice were scored for the percent time spent in the target quadrant and the number of annulus crossings through the previous platform location. $D_1-/-$ mice spent significantly less time (25% chance level) in the target quadrant and displayed significantly fewer direct crosses over the previous platform position compared to $D_1+/+$ and $D_1+/-$ mice. Data shown are mean values ± S.E.M. **, Significantly different from $D_1+/+$ and $D_1+/-$ mice ($P < 0.001$).

Following Probe Trial 2, mice were subjected to cued training trials (day 8–9) to test their nonspatial learning ability, motivation and sensorimotor coordination. Mice were trained to find and escape onto a submerged platform marked with a local visible cue (15-cm-high × 2.5-cm-diameter black cylinder attached to the platform). From trial to trial, different platform and starting positions were used. All mice were given a series of 12 trials (intertrial interval 5–7 min) over two consecutive days (six trials per day). Similarly, additional naive groups of $D_1-/-$ and $D_1+/+$ mice, that had never been exposed to the water tank, were given identical but extended cue training trials (six trials per day for 5 days).

2.3. Experiment 2: locomotor activity

Naive $D_1-/-$ ($n = 10$), $D_1+/-$ ($n = 10$) and $D_1+/+$ ($n = 10$) littermates were used. Basal locomotor

activity was monitored in four Plexiglas chambers (Med Associates, St. Albans, VT) measuring $40 \times 40 \times 28$ cm. Horizontal movement was detected by two arrays of 16 infrared beams, while a third array positioned 4 cm above the floor detected vertical movement. The software allowed a distinction to be made between repetitive interruptions of the same photobeam, and interruptions of adjacent photobeams. This latter measure was used as an index of ambulatory activity.

Mice were placed in the activity monitors for a period of 1 h on each of 8 days. After each trial, the floors of all chambers were wiped with a sponge, rinsed with water and dried before starting the next group of mice. All sessions were conducted in a sound-attenuated room illuminated with a dim red light.

2.4. Experiment 3: sensorimotor tasks

Naive mice from the three genotypes ($n = 15$ per group) were subjected to a series of sensorimotor tasks designed to assess their visual acuity, muscle strength, coordination and equilibrium as described by Lamberty and Gower (1990). Visual acuity was assessed by the ability of a mouse to extend its forepaws when lowered gently by the tail towards a flat surface. Muscle strength was assessed by the ability of a mouse to grasp a horizontal bar (3 mm diameter, elevated to a height of 25 cm) with its forepaws and remain so suspended for 5 s. At the same time, while still suspended, the ability of the mouse to raise one hind limb to reach the wire within 5 s was taken as a measure of equilibrium and muscle tone and strength. Finally, the mouse's ability to balance and walk along a wooden horizontal bar (0.8 cm diameter, elevated 40 cm above the floor) within 3 min was a measure of psychomotor integration and equilibrium.

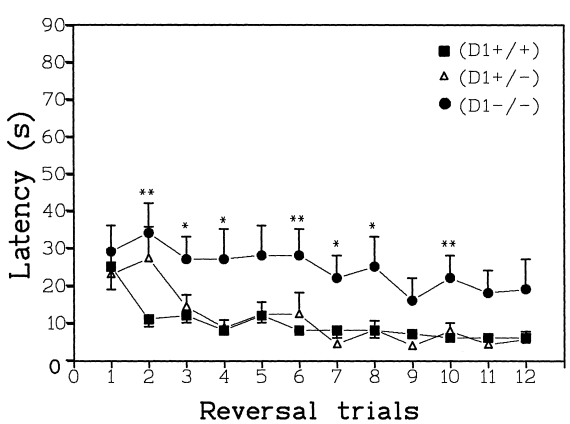

Fig. 3. Escape latencies to locate a hidden platform over 90 s during platform reversal trials. The platform was relocated to a different position and the same mice were trained to find the hidden platform at the new location. $D_1-/-$ mice displayed significantly longer escape latencies than control groups on most trials. Data shown are mean values ± S.E.M. *, **, Significantly different from $D_1+/+$ and $D_1+/-$ mice ($P < 0.05$–0.001).

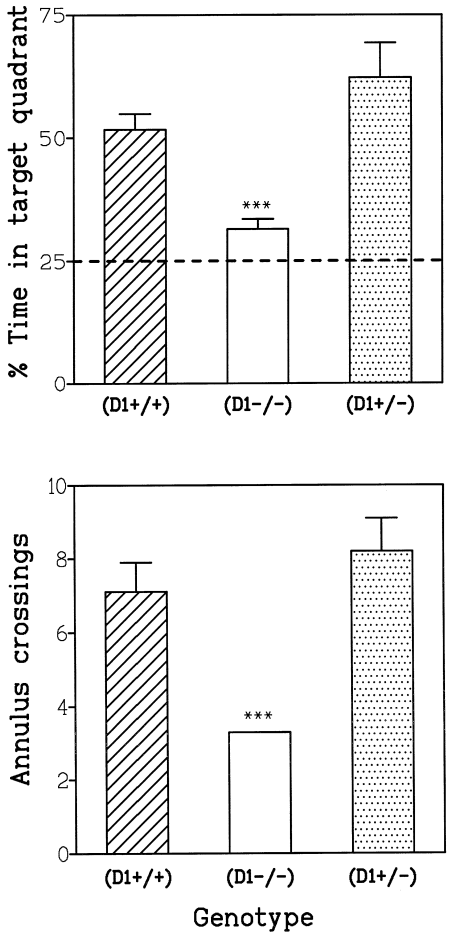

Fig. 4. Probe Trial 2 conducted after the last reversal trial to assess the spatial ability to learn the new position of the relocated platform. The platform was removed and all mice were scored for the percent time spent in the previous platform quadrant and the number of annulus crossings through the previous platform location. $D_1-/-$ mice spent significantly less time in the target quadrant and displayed significantly less direct crosses over the previous platform position than $D_1+/+$ and $D_1+/-$ mice. Data shown are mean values ± S.E.M. ***, Significantly different from $D_1+/+$ and $D_1+/-$ mice ($P < 0.0001$).

2.5. Experiment 4: spontaneous alternation

Naive $D_1-/-$ mice ($n = 20$) and $D_1+/+$ mice ($n = 17$) were used to assess working memory. Spontaneous alternation was assessed using a wooden Y maze. Each arm was $40 \times 15 \times 12$ cm. The floor of the maze was lined with paper which was replaced after each mouse. All sessions were recorded by a video camera placed above the maze. The testing procedure was according to that described by Sarter et al. (1988). Naive mice were placed singly at the center of the maze and allowed to move freely for an 8-min test session each day for 4 days. The sequences of entries into the three arms and the spontaneous alternations (defined as an entry into two or three arms on consecutive choices) were recorded manually. Reentry into an already visited arm during a trial was

recorded as an error. The number of maximum alternations was defined as the total number of arms entered −2, and the percentage alternation was defined as the ratio of actual alternations to maximum alternations multiplied by 100.

2.6. Experiment 5: fear conditioning

Mice were trained, in two different fear-conditioning paradigms, to learn to associate a conditioning chamber with an aversive stimulus and tested for contextual conditioning 5 min and 24 h later.

2.6.1. Passive avoidance conditioning

Naive $D_1-/-$ mice and their $D_1+/+$ and $D_1+/-$ siblings ($n=8$ per group), were conditioned in a one-trial step-through passive avoidance task to fear a novel context through the use of aversive footshocks. The conditioning chamber was divided by a sliding door into light and dark compartments equipped with a stainless steel grid floor. Mice were allowed to explore both compartments for 120 s, then were given two consecutive trials separated by 5 min. During these trials, mice were placed in the bright chamber for 30 s after which the sliding door was removed and the latency to enter the dark chamber was measured. In the training trial, mice were conditioned to avoid stepping through the dark compartment by delivering two consecutive 3-s footshocks (0.6 mA) 10 s after stepping into the dark compartment. Retention of an avoidance response was tested 5 min and 24 h later by placing the mice in the light chamber for 30 s before the sliding door was removed and the time taken to enter the dark chamber was measured for up to a maximum of 6 min. The footshocks were omitted during testing. Learning was assessed by comparing the step-through latencies during training and testing trials.

2.6.2. Contextual fear conditioning

Naive mice were placed in a conditioning chamber equipped with a house light and a stainless steel grid floor and allowed to explore it for 2 min, then received two consecutive 3-s footshocks (0.7 mA) and allowed to recover for 1 min before being returned to their home cages. To assess contextual fear memory, mice were tested in the same chamber without shock 5 min and 24 h later and scored for conditioned fear expressed as freezing behavior (cessation of all movements except those related to breathing) every 10 s for 2 min. Freezing was quantified and presented as a percentage of the 13 intervals over 2 min.

2.7. Data analysis

Data from each of the experiments were recorded for each mouse and averaged for each group. Data from all experiments are expressed as mean ± S.E.M. Genotype differences in the swimming speed, spatial bias, annulus crossings, sensorimotor reflexes and contextual conditioning were assessed by one-way analysis of variance (ANOVA). Escape latencies and locomotor activity were analyzed by repeated measures ANOVA using genotype and trials/days as factors. All analyses were followed by post-hoc Duncan's Range test ($\alpha=0.05$). Other variables were analyzed by unpaired *t*-tests.

3. Results

3.1. Water maze

During the first acquisition phase, $D_1-/-$ mice demonstrated a learning deficit compared to $D_1+/+$ and $D_1+/-$ mice, as indicated by longer escape latencies to

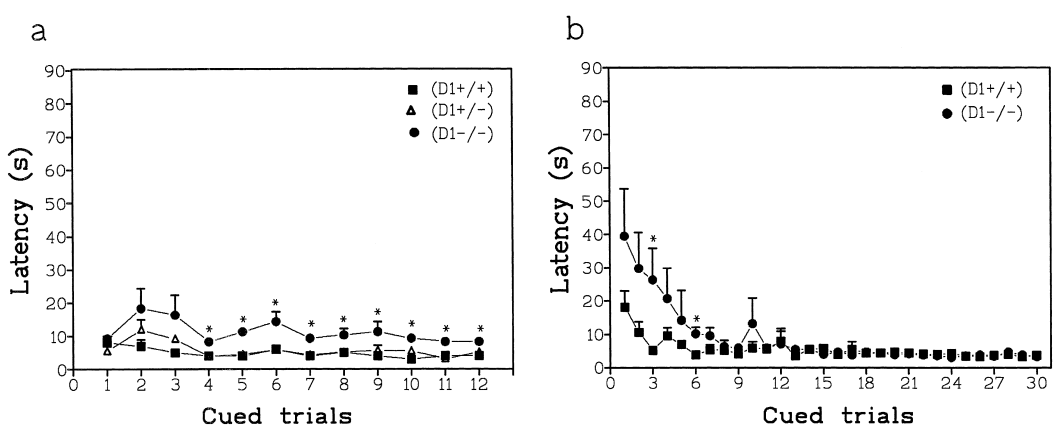

Fig. 5. Escape latencies to find a submerged platform marked with a visual cue over 90-s training trials. (a) Mice were previously trained for the acquisition of a spatial task and its reversal in the water maze. $D_1-/-$ mice took slightly but significantly more time than $D_1+/+$ mice to find the cued platform. (b) Mice were naive to the water maze. $D_1-/-$ mice initially displayed longer latencies to locate the cued platform and showed a steep decline over trials and finally acquired identical escape latencies as $D_1+/+$ mice. Data shown are mean values ± S.E.M. *, Significantly different from $D_1+/+$ and $D_1+/-$ mice ($P<0.05$).

locate a hidden platform (Fig. 1). Analysis of escape latencies across all trials revealed a significant main effect of genotype, $F(2,35) = 22.49$, $P < 0.00001$, and trial, $F(15,495) = 15.06$, $P < 0.00001$, but no significant interaction between these two factors. These results indicate that $D_1 -/-$ mice took a significantly longer time to find the hidden platform than control mice on all trials, however, the escape latencies for all groups were improved over subsequent trials. The initial escape latencies for $D_1 +/+$ (47 ± 9 s), $D_1 +/-$ (52 ± 16 s) and $D_1 -/-$ (76 ± 7 s) mice were significantly higher for all groups ($P < 0.001$) than on the last trial for $D_1 +/+$, $D_1 +/-$ and $D_1 -/-$ mice, respectively (7 ± 1 s, 5.3 ± 1.3 s and 17 ± 6 s). Despite the 50% reduction in dopamine D_1 receptor density in $D_1 +/-$ mice, their performance was identical to that of the wild-type mice. Data obtained are the average of two replicate experiments.

Results of the Probe Trial 1 indicated that $D_1 -/-$ mice failed to develop a spatial bias to the previous platform quadrant (Fig. 2). ANOVA indicated a significant main effect of genotype on time spent in the target quadrant, $F(2,35) = 11.20$, $P < 0.0002$, as well as in the number of annulus crossings through the previous platform location, $F(2,35) = 7.94$, $P < 0.002$. Post-hoc comparisons indicated that $D_1 -/-$ mice displayed less selective searching behavior for the absent platform and spent significantly less time ($P < 0.015$) in the target quadrant compared to $D_1 +/+$ and $D_1 +/-$ mice. The percentage of time spent in the target quadrant was $40 \pm 3\%$ for $D_1 +/+$ mice, $47 \pm 7\%$ for $D_1 +/-$ mice and $27 \pm 1\%$ for $D_1 -/-$ mice, indicating that $D_1 +/+$ and $D_1 +/-$ mice spent a greater proportion of the cutoff time (60 s), whereas $D_1 -/-$ mice spent about one quarter of the time (chance level) in the target quadrant that previously contained the platform. Moreover, $D_1 -/-$ mice made significantly fewer direct crosses over the previous platform position than did the $D_1 +/-$ and $D_1 +/+$ mice ($P < 0.0015$) (Fig. 2). Analysis of the swimming speed for $D_1 +/+$ (285.63 ± 16.43 cm/min), $D_1 +/-$ (273.38 ± 13.73 cm/min) and $D_1 -/-$ (340.48 ± 16.98 cm/min) mice indicated that the mutant mice had significantly ($P < 0.02$) higher swimming speed than the other groups. On the first reversal trial, all mice took a longer time to find the newly located hidden platform compared to the last trial with the previous platform location. In addition, there was no significant difference in initial escape latencies between the mutant and control mice (Fig. 3). However, on the second and remaining trials, only the $D_1 +/+$ and $D_1 +/-$ mice showed a decline in escape latencies. Analysis of escape latencies revealed a significant main effect of genotype, $F(2,35) = 4.51$, $P < 0.02$, and trial, $F(11,363) = 6.09$, $P < 0.00001$. Post-hoc comparisons indicated that $D_1 -/-$ mice had significantly ($P < 0.02$) longer escape latencies than control mice. Over the 12 trials, the $D_1 -/-$ mice continued to have difficulty with no major improvement in escape latencies noted.

Probe Trial 2 revealed lack of spatial bias to the new target quadrant in $D_1 -/-$ mice. ANOVA indicated a significant main effect of genotype on time spent in the target quadrant, $F(2,35) = 21.16$, $P < 0.00001$, as well as in the number of annulus crossings through the previous location that contained the new platform, $F(2,35) = 13.18$, $P < 0.0001$. Post-hoc comparisons indicated that $D_1 -/-$ mice spent less time ($P < 0.0001$) in the target quadrant ($52 \pm 3\%$, $62 \pm 7\%$ and $31 \pm 2\%$ for $D_1 +/+$, $D_1 +/-$ and $D_1 -/-$ mice, respectively) and had a significantly reduced number of annulus crossings ($P < 0.0001$) compared to the $D_1 +/+$ and $D_1 +/-$ mice (Fig.4). These results suggest that the mutant mice failed to use spatial information to remember the new location of the hidden platform.

When the same mice were subjected to visible cue training after the Probe Trial 2, $D_1 -/-$ mice exhibited significantly higher escape latencies than the $D_1 +/+$ and $D_1 +/-$ mice across Trials 4–12 (Fig. 5a), although $D_1 -/-$ mice performed considerably better on this visual task than during the acquisition and reversal trials as

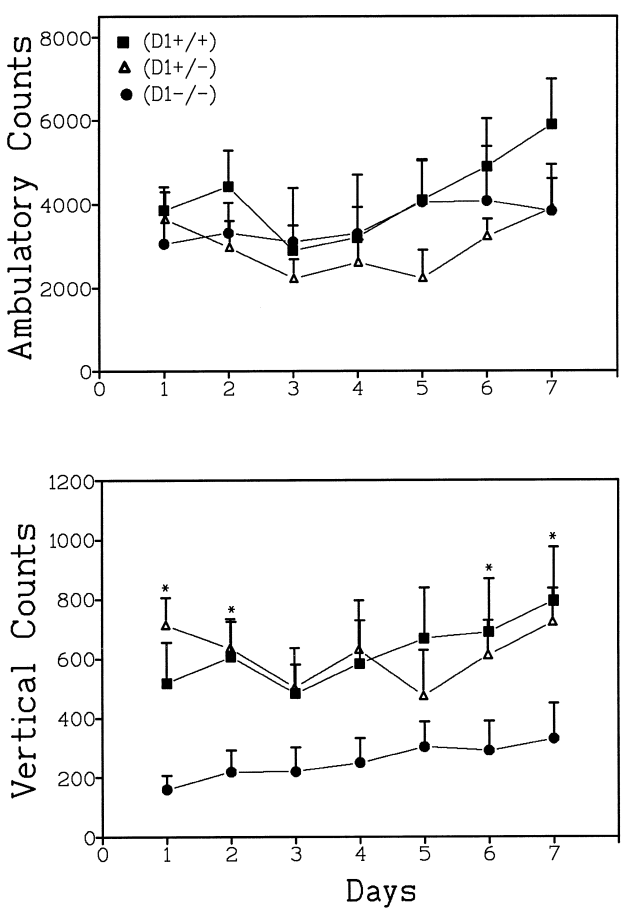

Fig. 6. Spontaneous locomotor activity test. All mice were placed individually in activity boxes and their ambulatory activity and rearing as measured by photocell beam breaks were scored over 60-min trial sessions. Data shown are mean values ± S.E.M. *, Significantly different from $D_1 +/+$ and $D_1 +/-$ mice ($P < 0.05$).

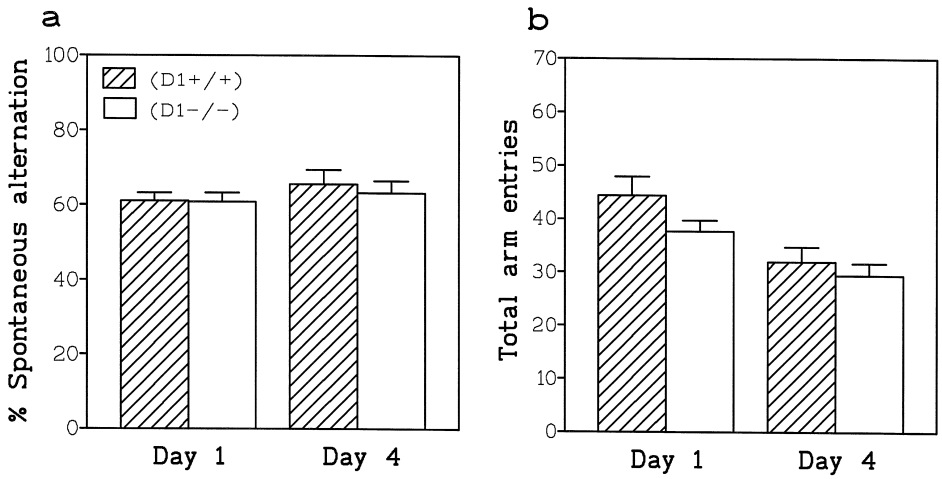

Fig. 7. Performance in the Y maze showing (a) spontaneous alternation (b) total arm entries. Data shown are mean values ± S.E.M. No significant differences were detected between genotypes.

indicated by significantly shorter escape latencies ($P < 0.001$). ANOVA detected significant effect of genotype, $F(2,32) = 16.01$, $P < 0.0001$, and trial, $F(11,352) = 3.18$, $P < 0.0004$, but no significant genotype × trial interaction. It is important to note that the difference in escape latencies between the $D_1-/-$ and $D_1+/+$ or $D_1+/-$ mice was very small with measures in the range of 5–7 s, however, owing to the very short escape latencies of the $D_1+/+$ and $D_1+/-$ mice, this difference was significant. When naive groups of $D_1-/-$ and $D_1+/+$ mice were given only cued training trials, $D_1-/-$ mice initially took a longer time to find the platform. ANOVA on Trials 1–6 detected a significant effect of genotype, $F(1,18) = 8.2$, $P < 0.01$, and trial, $F(5,90) = 2.95$, $P < 0.02$, although no significant interaction between genotype and trials was detected. These results indicate that $D_1-/-$ mice had significantly longer escape latencies than $D_1+/+$ mice on Trial 3 ($P < 0.03$) and 6 ($P < 0.004$) only, however, both groups showed significant improvement over trials. No significant effect of genotype was observed over Trials 7–30 (Fig. 5b), indicating that with extended training in a visually cued paradigm, $D_1-/-$ mice finally acquired identical latencies as control mice.

3.2. Spontaneous locomotor activity

The $D_1-/-$ mice exhibited normal locomotor activity that was indistinguishable from that of the $D_1+/+$ mice (Fig. 6a); however, rearing was significantly reduced ($P < 0.001$) in $D_1-/-$ mice compared to the wild-type and heterozygous siblings (Fig. 6b).

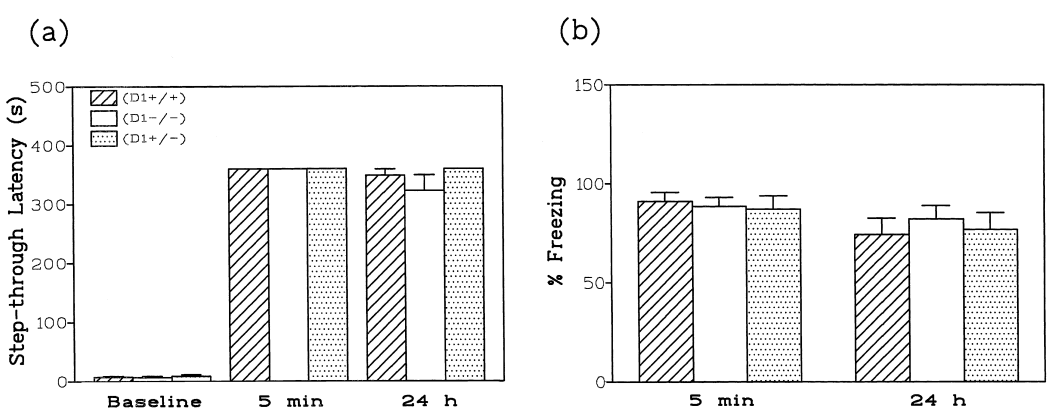

Fig. 8. Contextual conditioning showing (a) passive avoidance learning. Mean step-through latencies (± S.E.M.) during the training trials and 6 min testing trials. All mice entered the dark chamber within 10 s during training (baseline), whereas during the testing trials given 5 min and 24 h after shock exposure, all mice had significantly high avoidance latencies. No significant differences were detected among the genotypes. (b) fear response expressed as freezing behavior. Mean percentage freezing (± S.E.M.) during 2-min testing trials given 5 min and 24 h after shock exposure. No significant differences were detected among the genotypes.

3.3. Sensorimotor tasks

All scores of sensorimotor functions measured were normal and comparable among all genotypes, indicating no significant differences in visual acuity, muscle strength, coordination and equilibrium.

3.4. Spontaneous alternation

There were no significant differences between $D_1-/-$ and $D_1+/+$ mice in any of the measures for the spontaneous alternation testing with either single or multiple trial(s) (Fig. 7a and b).

3.5. Passive avoidance conditioning

No differences among any of the genotypes in the avoidance latencies were found ($P = 0.8$). All mice exhibited increased latencies to enter the dark chamber (previously paired with footshocks) when tested at 5 min and 24 h after training (Fig. 8a).

3.6. Contextual fear conditioning

No differences ($P = 0.8$) among any of the genotypes in the acquisition and expression of conditioned fear responses were observed. None of the genotypes demonstrated any freezing behavior before conditioning, however, after conditioning, all mice learned to associate the conditioning chamber with the shock as manifested by similar levels of freezing responses in this context 5 min and 24 h after training (Fig. 8b).

4. Discussion

We demonstrated that $D_1-/-$ mice have a deficit in processing spatial information in the water maze, as indicated by significantly longer initial escape latencies compared to $D_1+/+$ and $D_1+/-$ mice during the acquisition trials. Overall, all mice were able to perform competently in the swim task. $D_1-/-$ mice swam in longer, less directed paths before they reached the platform, with some mice constantly swimming around the maze wall (thigmotaxis) or climbing onto the platform and remaining on it for few seconds before reentering the water, whereas the $D_1+/+$ and $D_1+/-$ mice had shorter and more direct paths. $D_1-/-$ mice took a longer time than $D_1+/+$ and $D_1+/-$ mice to locate the platform. They, however, showed a decline in escape latencies across trials, indicating that some learning had taken place by the end of the acquisition trials. Lesions of the dopamine-rich nucleus accumbens in rats have been attributed to result in disrupted search strategies (Annett et al., 1989), since spatial information utilized by the intact hippocampal formation is normally used by motor structures to actually guide spatial navigational behavior in the water maze. In particular, dopamine D_1 receptors have been implicated in incentive learning (Beninger, 1983; Beninger and Miller, 1998). Therefore, it is possible that the $D_1-/-$ mice exhibited longer latencies because they failed to learn the location of the platform and/or that the platform provided an escape from the water. Reentering the water or swimming around the wall could be interpreted as deficits in attention to the more salient cues combined possibly with stress and anxiety encountered from being in the water, which may perturb learning capabilities; however, habituation to these events with prolonged trials could have taken place and may explain the improvement over trials exhibited by the $D_1-/-$ mice.

The results of the Probe Trial 1 suggest a specific memory deficit in $D_1-/-$ mice for the correct platform location. In the absence of the platform, these mutant mice spent less time in the previous training quadrant and showed fewer direct annulus crossings than the control mice. These observations might seem contradictory to the conclusion that the mutant mice were able to learn to locate the hidden platform by the end of the acquisition phase. However, the fact that these mutant mice spent $\sim 25\%$ (chance level) of the scoring time in the training quadrant during Probe Trial 1, supports the hypothesis that the mutant mice may have reached the hidden platform by using nonspatial (random) strategies, as reflected by approximately equal time spent in all four quadrants during the probe trial.

When mice were required to find the hidden platform at a different location, the $D_1+/+$ and $D_1+/-$ mice were quick to learn the new location. In contrast, $D_1-/-$ mice had consistently longer escape latencies that did not decline across trials. The difference between performance on the acquisition versus the reversal trials may indicate that these mice were unable to develop a new search strategy to learn the new platform location. This pattern of results suggests that $D_1-/-$ mice may have a behavioral ''inflexibility'' that results in difficulties in changing a previously learned behavior when a shift in task demand occurs. The deficit in spatial reversal seen in $D_1-/-$ mice was confirmed by lack of spatial bias in Probe Trial 2.

During the cued training trials, when the platform was still submerged but marked with a visible cue, the $D_1-/-$ mice took slightly more time than the $D_1+/+$ and $D_1+/-$ mice to locate the platform (Fig. 5a). One interpretation of this result could be that $D_1-/-$ mice are visually impaired. However, this possibility was ruled out since tests of sensorimotor function revealed normal visual acuity in the $D_1-/-$ mice. In addition, naive $D_1-/-$ mice exposed only to the cued task for the first time, showed significant improvement in escape latencies over trials and eventually acquired identical performance as the $D_1+/+$ mice (Fig. 5b) showing that motivation, motoric ability to swim or ability to perceive proximal

cues were not altered. The difference seen on the first visual task could be due to the fact that the $D_1 -/-$ mice swam in longer and less directed paths before they reached the platform compared to control mice, which may be due to a deficit in general attentional processes. However, it could also be argued that if the mice have difficulty in learning a new task they would be impaired on the cued task also.

Additional tests of spontaneous locomotor activity showed that forward locomotion was not altered in $D_1 -/-$ mice, although rearing was reduced. Taken together, these results indicate that the observed longer escape latencies in $D_1 -/-$ mice cannot be attributed to obvious motor or visual impairment.

Compared to the deficit seen in the water maze, there was no significant difference between the $D_1 -/-$ and $D_1 +/+$ mice in spontaneous alternation in a Y-maze. In so far as performance in this test represents a basic measure of working memory, this finding suggests that lack of the dopamine D_1 receptor, specifically in the prefrontal cortex, does not impair this form of memory and that the deficit seen in the water maze task might be specific to spatial cognitive processes. The difference in performance between the two tests raises an interesting point. While performance of the spontaneous alternation task may, to a lesser extent, require utilization of some spatial information, there are important differences, e.g., in the water maze, mice must escape from a stressful situation (aversive motivation), whereas in the Y maze, there were no noxious events encountered in exploring the arms. Therefore, it is possible that $D_1 -/-$ mice experienced a stress-induced disruption in acquisition of spatial information in the water maze, a phenomenon which did not occur in the nonstressful Y maze task.

We investigated hippocampus-dependent associative learning further in $D_1 -/-$ mice using contextual fear conditioning and one trial step-through passive avoidance tasks. No differences in fear responses among any of the genotypes was observed in either task. These results indicate that $D_1 -/-$ mice were capable of acquiring contextual learning, providing evidence for the specificity of the water maze spatial deficit and suggesting that even though both spatial and contextual learning and memory are hippocampus-dependent, they may be mediated by different neuronal pathways responsible for induction of neuronal plasticity, learning and memory consolidation. Indeed evidence indicates that α-amino-3-hydroxy-5-methylisosoxazole-4-propionic acid (AMPA) glutamate receptors, dopamine D_5, β-adrenergic, and serotonin $5HT_{1A}$ receptors in the hippocampus and other dopaminergic, noradrenergic and serotonergic pathways modulate memory consolidation of an aversively-motivated learning in rats (Izquierdo and Medina, 1997; Izquierdo et al., 1998). In addition, many types of long-term potentiation have been suggested to be differentially modulated by different neurotransmitter systems (Bliss and Collingridge, 1993). It is possible, therefore, that the hippocampal mechanism will activate one or another type of long-term potentiation depending on the task and on the anatomic region that plays a primary role.

The fact that in the water maze $D_1 -/-$, $D_1 +/+$ and $D_1 +/-$ mice improved with successive trials suggests that $D_1 -/-$ mice are capable of learning. It is likely that dopamine D_1 receptor deletion may not impair learning per se but may rather modulate other mechanisms directly mediating learning and memory. Indeed, dopamine, via D_1 receptors, stimulates cortical and hippocampal acetylcholine release (Day and Fibiger, 1992; Imperato et al., 1993; Acquas et al., 1994; Hersi et al., 1995a,b) and modulates NMDA receptor-mediated responses (Levine et al., 1996) as well as induction of long-term potentiation in rats (Huang and Kandel, 1995; Otmakhova and Lisman, 1996; Kusuki et al., 1997). Considerable evidence indicated that both the cholinergic and glutamatergic systems have roles in spatial learning and memory (Hagan and Morris, 1987; Lamberty and Gower, 1991; Riedel and Reymann, 1996), hippocampal long-term potentiation and other mnemonic process (Morris et al., 1986; Bliss and Collingridge, 1993; Tsien et al., 1996). It is, therefore, possible that deletion of the dopamine D_1 receptor may have affected the hippocampal cholinergic/glutamatergic synaptic activity, resulting in impaired induction of long-term potentiation. Consistent with this hypothesis, recent studies have indicated that, in dopamine D_1 receptor-deficient mice, dopamine D_1 receptor agonists did not potentiate responses mediated by activation of NMDA receptors (Levine et al., 1996) and prevented the late phase of hippocampal long-term potentiation, suggesting that the synergistic activation of the dopaminergic synapses is necessary for long-term potentiation maintenance (Matthies et al., 1997). Moreover, dopamine D_1/D_5 receptors stimulate adenylyl cyclase activity in various brain regions including the hippocampus and neocortex, leading to an increase in cyclic adenosine monophosphate (cAMP) and activation of certain protein kinases proposed to play a role in long-term potentiation, spatial learning or memory consolidation of an aversively motivated learning (Wehner et al., 1990; Huang and Kandel, 1995; Wu et al., 1995; Tan and Liang, 1996; Abel et al., 1997; Bernabeu et al., 1997; Bach et al., 1999; Schafe et al., 1999). This notion is supported by the finding that dopamine D_1 receptor-mediated production of cAMP is completely absent in membranes of dopamine D_1 receptor-deficient mice (Friedman et al., 1997). These findings provide clear evidence that lack of the dopamine D_1 receptor might possibly implicate reduced hippocampal/cortical signal transduction and hence altered dopamine D_1 receptor-mediated synaptic plasticity, which may contribute to the spatial learning deficit seen in these mice.

In summary, $D_1 -/-$ mice exhibited deficits in spatial learning and memory consisting of slower learning and poor memory of the platform location, and a deficit in

learning a new task. This deficit was seen only in the water maze task which requires organization of complex navigational behaviors, however, unconditioned behaviors, such as locomotion and spontaneous alternation, as well as nonspatial and associative learning abilities appear to be preserved in $D_1 -/-$ mice. On the basis of these findings, we conclude that the dopamine D_1 receptor is part of a neural network that plays an important role in mediating at least one aspect of the cognitive processes, namely spatial learning and memory. However, it is as yet unclear whether the deficit in spatial learning seen in $D_1 -/-$ mice could be due to an impairment in spatial memory, or is coupled to an incentive learning deficit as well. Additional studies are underway to investigate the mechanism(s) by which dopamine D_1 receptor modulates spatial learning and memory.

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