ELSEVIER

European Journal of Pharmacology 399 (2000) 171–181

ejp

www.elsevier.nl/locate/ejphar

Modification of dopamine D_1 receptor knockout phenotype in mice lacking both dopamine D_1 and D_3 receptors

Joanna M. Karasinska [a], Susan R. George [a,b,c], Mufida El-Ghundi [a], Paul J. Fletcher [c], Brian F. O'Dowd [a,c,*]

[a] *Department of Pharmacology, University of Toronto, Medical Science Building, 1 King's College Circle, Toronto, Ontario, Canada M5S 1A8*
[b] *Department of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8*
[c] *Centre for Addiction and Mental Health, 33 Russell Street, Toronto, Ontario, Canada M5S 2S1*

Received 17 January 2000; received in revised form 25 April 2000; accepted 28 April 2000

Abstract

Experimental evidence suggests that dopamine D_1 and D_3 receptors may interact in an opposing or synergistic fashion. To investigate interactions between both receptors in behaviour, we have used dopamine D_1 and D_3 receptor knockout mice to generate mice lacking both receptors. $D_1^{-/-}D_3^{-/-}$ mice were viable, fertile and showed no gross morphological abnormalities. In an open field, they exhibited lower activity than wild-type, $D_1^{-/-}$ and $D_3^{-/-}$ mice. $D_1^{-/-}D_3^{-/-}$ mice performed equally poorly in the rotarod and Morris water maze tasks as their $D_1^{-/-}$ littermates. Basal locomotor activity and anxiety-like behaviour were normal in $D_1^{-/-}D_3^{-/-}$ mice. Combined deletion of both receptors abolished the exploratory hyperactivity and anxiolytic-like behaviour of dopamine D_3 receptor mutant phenotype and further attenuated the low exploratory phenotype of $D_1^{-/-}$ mice. These results imply an interaction of both receptors in the expression of exploratory behaviour in a novel environment, and the need for the presence of intact dopamine D_1 receptor for the expression of certain behaviours manifested in dopamine D_3 receptor mutant phenotype. In addition, dopamine D_1 receptor, but not dopamine D_3 receptor, is involved in the ability to perform on the rotarod and spatial learning.

Keywords: Dopamine D_1 receptor; Dopamine D_3 receptor; Knockout mice; Locomotor activity; Exploratory behaviour; Elevated plus maze; Rotarod; Spatial learning

1. Introduction

Brain dopamine is involved in the control of locomotor activity, learning and memory, motivation, and reward through the nigrostriatal and mesocorticolimbic pathways. Its effects are mediated by five G protein-coupled receptors, divided into two subclasses: D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, D_4) receptors based on sequence homology, signal transduction and pharmacology (for review see Missale et al., 1998).

Dopamine D_1 receptor is found at the highest levels in caudate–putamen, nucleus accumbens and olfactory tubercle and at lower levels in cortex, hippocampus and hypothalamus (Fremeau et al., 1991; Weiner et al., 1991). Pharmacological data has implicated dopamine D_1 receptor in the regulation of locomotor activity (Dreher and Jackson, 1989; Essman et al., 1993) and cognitive processes, including learning and memory (Ichihara et al., 1989; Sawaguchi and Goldman-Rakic, 1991; Williams and Goldman-Rakic, 1995; Muller et al., 1998), as well as the reinforcing effects of drugs of abuse, including cocaine (Caine and Koob, 1994; Self et al., 1996; Epping-Jordan et al., 1998). Dopamine D_3 receptor is expressed predominantly in nucleus accumbens, olfactory tubercle and islands of Calleja (Bouthenet et al., 1991; Landwehrmeyer et al., 1993). Based on its anatomical distribution and pharmacological studies, dopamine D_3 receptor is thought to be involved in reward and cognitive behaviour (for review see Shafer and Levant, 1998). This receptor plays an inhibitory role on locomotion (Daly and Waddington, 1993; Waters

* Corresponding author. Department of Pharmacology, University of Toronto, Medical Science Building, Room 4353, 1 King's College Circle, Toronto, Ontario, Canada M5S 1A8. Tel.: +1-416-978-7579; fax: +1-416-971-2868.

E-mail address: brian.odowd@utoronto.ca (B.F. O'Dowd).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00347-2

et al., 1993; Svensson et al., 1994), and may also be involved in the reinforcing properties of cocaine (Parsons et al., 1996; Caine et al., 1997; Pilla et al., 1999).

Identification of specific functions of dopamine D_1 and D_3 receptors and their interactions in vivo has been difficult because of the lack of ligands acting exclusively on each receptor. Dopamine D_1 and D_3 receptor ligands also have some selectivity for other dopamine D_1-like and D_2-like receptors. In the past decade, gene-targeting techniques have been successfully used to generate mice lacking various G protein-coupled receptors, including dopamine receptors (Rohrer and Kobilka, 1998). Indeed, there are reports of over 70 single and three double G protein-coupled receptor knockouts, including a dopamine D_2/D_3 receptor double knockout (Jung et al., 1999). Various aspects of dopamine-mediated behaviour were characterised in dopamine D_1 and D_3 receptor mutants. Reports indicate that dopamine D_1 receptor mutants displayed lower activity in a novel open field (Drago et al., 1994; Smith et al., 1998), but their locomotor activity, measured over longer period of time, was either unchanged (El-Ghundi et al., 1999) or increased (Xu et al., 1994; Clifford et al., 1998). In addition, dopamine D_1 receptor knockout mice exhibited impairments in sequence coordination and duration of grooming movements (Cromwell et al., 1998) and a spatial learning deficit (Smith et al., 1998; El-Ghundi et al., 1999). With respect to reward, dopamine D_1 receptor mutants displayed retained cocaine-conditioned place preference (Miner et al., 1995) and lower preference for alcohol (El-Ghundi et al., 1998). Mice lacking dopamine D_3 receptor exhibited locomotor hyperactivity (Accili et al., 1996) and lower anxiety levels (Steiner et al., 1997). Several putative dopamine D_3 receptor selective ligands were found to have the same effects on dopamine D_3 receptor knockout and wild-type mice (Boulay et al., 1999; Xu et al., 1999), emphasizing the need for caution when assigning specific functions to individual dopamine receptors based on pharmacological studies.

Dopamine D_1 and D_3 receptors are co-expressed in a large number of neurons in nucleus accumbens and in granule cells of the islands of Calleja (Le Moine and Bloch, 1996; Ridray et al., 1998; Schwartz et al., 1998), raising the possibility of interactions at a single neuronal level. Evidence has shown that dopamine D_1 and D_3 receptors exert opposite effects on c-*fos* expression in islands of Calleja (Ridray et al., 1998; Schwartz et al., 1998) and a synergistic effect on body temperature decrease (Barik and de Beaurepaire, 1998). In hemiparkinsonian rats, the levodopa-induced dopamine D_3 receptor expression was shown to be mediated by dopamine D_1 receptor (Bordet et al., 1997). In a human medulloblastoma cell line, the stimulation of dopamine D_1 receptor led to mRNA upregulation of both dopamine D_1 and D_3 receptors (Levavi-Sivan et al., 1998). A study using knockout mice reported that dopamine D_1 receptor-mediated c-*fos* induction was reduced in dopamine D_3 receptor deficient mice (Jung and Schmauss, 1999).

Interactions between dopamine D_1-like and D_2-like receptors in behaviour were reported on locomotor activity, learning and reinforcement (Dreher and Jackson, 1989; Ichihara et al., 1992; Essman et al., 1993; Koshikawa et al., 1996; Ikemoto et al., 1997). However, the individual roles of dopamine D_1 and D_3 receptors in these interactions are unknown. To investigate possible interactions of dopamine D_1 and D_3 receptors in behavioural processes, we have generated mice lacking both receptors. Here, for the first time, we report the effects of deletion of both receptors on locomotor activity, exploration, anxiety, motor control and spatial learning.

2. Materials and methods

2.1. Animals

The generation of dopamine D_1 receptor knockout ($D_1^{-/-}$) and dopamine D_3 receptor knockout ($D_3^{-/-}$) mice has been described previously (Drago et al., 1994; Accili et al., 1996). The $D_1^{-/-}$ mice were a gift from Dr. J. Drago (Monash University, Clayton, Australia) and the $D_3^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). Dopamine D_1/D_3 receptor double knockout ($D_1^{-/-}D_3^{-/-}$) mice were generated by crossing $D_1^{-/-}$ and $D_3^{-/-}$ mice. The resulting heterozygotes ($D_1^{+/-}D_3^{+/-}$) of the F1 generation were bred to produce the F2 generation. F2 $D_1^{-/-}D_3^{+/-}$ mice were intercrossed to obtain $D_1^{-/-}D_3^{-/-}$ and $D_1^{-/-}$ offspring with predicted frequencies of 25% in F3. The $D_3^{-/-}$ and wild-type controls were generated by breeding $D_3^{+/-}$ mice, derived from the same original dopamine D_3 receptor knockout mice as the F2 $D_1^{-/-}D_3^{+/-}$ mice. Mice were genotyped for dopamine D_1 and D_3 receptor mutations by polymerase chain reaction (PCR) on genomic DNA derived from tail biopsies (Puregene DNA Isolation Kit, Gentra Systems, Minneapolis, MN). The primer sequences and conditions required for genotyping dopamine D_1 receptor mutation are available on the Jackson Laboratory web page (http://www.jax.org). The PCR protocol for genotyping dopamine D_3 receptor mutants was developed by Dr. J. Drago. The forward primer, 5′TGGATGTGGAATGTGTGCGAG3′, recognising the sequence within the neomycin resistance gene, and the backward primer, 5′GAAACCAAAGAGGAGAGGGCAGGAC3′, recognising a DNA sequence of dopamine D_3 receptor gene, were used to detect the mutated gene. The final amplified fragment was about 200 bp. The wild-type allele was detected about the SalI site, which represents the site into which the neomycin cassette was cloned (Accili et al., 1996). The forward and reverse primers used were 5′GCAGTGGTCATGCCAGTTCACTATCAG3′ and 5′CCTGTTGTGCTGAAACC-

AAAGAGGAGAGG3′, respectively. The amplified fragment was about 137 bp.

The animals were group housed and kept on a 12 h light–12 h dark cycle (lights on at 0700 h) under standard conditions in accordance with the guidelines of the Canadian Council on Animal Care. Male wild-type, $D_1^{-/-}$, $D_3^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice were used for all tests. The same subjects were used in all experiments. Behavioural testing began when mice were between 12 and 19 weeks old and lasted for 6 months. All testing took place during the light phase of the light–dark cycle.

2.2. Locomotor activity

The assessment of basal horizontal locomotion was conducted in clear, rectangular plastic cages of 27×15 cm and 17 cm high. The cages were identical to the home cages, except that they did not contain floor bedding. The test cages were placed in frames equipped with six photocell beams on each of the opposite longer sides. Before the test session, mice were habituated to the cages in 15-min sessions on 3 consecutive days. One week after the habituation sessions, the test session was conducted and photobeam breaks were recorded for 60 min as a measure of basal locomotor activity.

2.3. Open field

The apparatus consisted of a square enclosed Plexiglas box of 60×60 cm and 30 cm high. The floor was white and divided into nine squares of 20×20 cm each. To begin testing, each mouse was placed in the central square of the field and allowed 15-min exploration. A video camera was used to record the sessions and the peripheral and central square crossings, as well as the number of rearing events were counted from the videotape.

2.4. Elevated plus maze

The maze consisted of two opposite open arms, 30 cm long and 5 cm wide, and two opposite closed arms of same dimensions, surrounded by 15 cm high walls. The four arms were attached to a central square platform of 5×5 cm. The plus maze was elevated 70 cm above the floor. At the beginning of the test session, each mouse was placed on the central platform facing one of the closed arms and allowed 15-min exploration. The sessions were videotaped and the number of closed arm entries with four paws, as well as the number of open arm entries with four paws and two forepaws, and the time spent in the open arms, were recorded.

2.5. Rotarod

The rotarod was a 3-cm diameter cylinder rotating at 10 rpm. Testing on the rotarod took place on 2 consecutive days. On day 1, each mouse was given a 10-min training session. The mouse was placed on the immobile rod and the speed was turned on to 10 rpm. If the mouse fell from the rotarod, it was placed back on. Two hours after the training session, performance was tested in a 3-min session. The latency to fall off the rotating rod was measured. On day 2, mice that performed poorly on the first probe trial (latency < 30 s) were given another 10 min training session. Two hours later, a 3-min test session was conducted for all mice.

2.6. Water maze

The mice were tested in the hidden and cued versions of the Morris water maze task. The apparatus consisted of a white circular tank of 120 cm diameter and 30 cm high, filled with water to a depth of 23 cm. The water was rendered opaque by the addition of a non-toxic white paint, and maintained at room temperature. In the hidden version of the task, a platform 12 cm in diameter was submerged 1 cm below the water surface. In the cued version, a 15 cm high and 2.5 cm diameter red cylinder was attached to the centre of the platform.

2.6.1. Hidden platform

During place training, the platform was located in the middle of one of the four quadrants, labelled NE, NW, SE and SW. The location of the platform was chosen randomly and kept constant for each mouse. Training took place on 3 consecutive days and consisted of four trials in three blocks per day for a total of 36 trials. On day 1, before the first trial, each mouse was allowed a 30-s free swim without the platform to allow acclimation to the tank. At the beginning of each trial, the mouse was placed in the tank facing the wall, at one of four start locations labelled N, S, E and W. The order of the start location was random within a block of trials. The mouse was allowed to swim for 60 s and the latency to find the platform was recorded. If the platform was not located during 60 s, the animal was manually guided towards it and given a score of 61 s. Each mouse was allowed to stay on the platform for 60 s in-between trials. The time interval between blocks was 2.5 h. On day 3, 1 h after the last trial, the platform was removed and a 60 s probe trial was conducted. Time spent in each quadrant and annulus crossings were recorded from a videotape of the probe trial.

2.6.2. Cued version

The procedure was identical to the hidden version, except that platform location was changed between the NE, NW, SE and SW quadrants within a block, and no probe trial was conducted.

2.7. Statistical analysis

In all experiments, data was recorded for each mouse and expressed as mean of each group $\pm$ S.E.M. For most

comparisons, one-way analysis of variance (ANOVA), followed by Newman–Keuls post hoc test ($\alpha = 0.05$), was used to assess genotype differences. Time course horizontal activity in the open field, rotarod fall latencies, water maze escape latencies and quadrant preference were analysed by two-way ANOVA using genotype as the between-subjects factor and time block/day/trial block/quadrant as the repeated measures factors. The remaining data was analysed by unpaired *t*-test.

3. Results

3.1. Generation of $D_1^{-/-}D_3^{-/-}$ mice

The $D_1^{-/-}D_3^{-/-}$ mutants were healthy, and had no gross physical abnormalities. They were fertile, producing litters of expected size and sex distribution. $D_1^{-/-}D_3^{-/-}$ mice were smaller in size, which is consistent with previous reports of the smaller size of $D_1^{-/-}$ mice (Drago et al., 1994; Xu et al., 1994). Since $D_1^{-/-}$ mice require a diet of hydrated food to thrive after weaning (Drago et al., 1994; El-Ghundi et al., 1998), the $D_1^{-/-}D_3^{-/-}$ mice received mashed pellets upon weaning. Weight measurements during the experiments indicated that $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice had body weights 10–20% lower than wild-type and $D_3^{-/-}$ mice.

3.2. Locomotor activity

To investigate the effect of deletion of both dopamine D_1 and D_3 receptors on spontaneous locomotor activity, we measured forward locomotion in the double mutants and compared it to $D_1^{-/-}$, $D_3^{-/-}$ and wild-type controls, using automated photocell beam boxes. No significant differences were observed among the four genotypes in horizontal activity in the 60-min session ($F(3,35) = 2.65$) (Fig. 1).

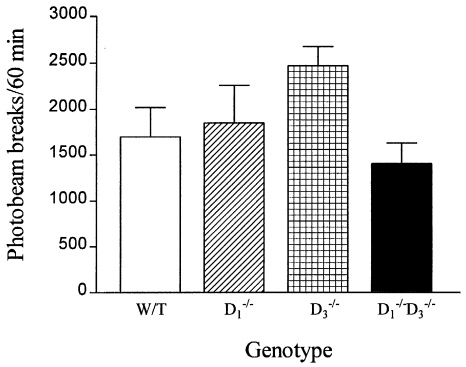

Fig. 1. Basal locomotor activity of wild-type ($n = 10$), $D_1^{-/-}$ ($n = 8$), $D_3^{-/-}$ ($n = 10$) and $D_1^{-/-}D_3^{-/-}$ ($n = 11$) mice. Mice were placed individually into activity boxes and horizontal locomotor activity was measured by photobeam breaks for 60 min. Values represent means ± S.E.M. One-way ANOVA did not reveal significant differences among the genotypes.

3.3. Open field

In order to find out how the deficiency of both dopamine D_1 and D_3 receptors affected exploratory behaviour in a novel environment, horizontal (square crossings) and vertical (rearing) activities were measured in a large open field. There was a significant difference among the groups in total horizontal crossings ($F(3,34) = 12.66$; $P < 0.0001$) (Fig. 2a). Further analysis indicated that $D_1^{-/-}D_3^{-/-}$ mice exhibited a decreased number of crossings compared to wild-type ($P < 0.001$), $D_1^{-/-}$ ($P < 0.05$) and $D_3^{-/-}$ mice ($P < 0.001$). $D_1^{-/-}$ mice were significantly less active than either wild-type or $D_3^{-/-}$ mice ($P < 0.05$). There was no significant difference in the total number of square crossings between wild-type and $D_3^{-/-}$ mice over the 15-min session. A detailed time course analysis demonstrated a significant main effect of genotype ($F(3,34) = 27.13$) and time block ($F(2,35) = 25.45$), but no interaction (genotype × time block). ANOVA of each 5-min time block revealed significant differences in square crossings (1–5 min: $F(3,34) = 13.59$; $P < 0.0001$, 6–10 min: $F(3,34) = 10.85$; $P < 0.0001$, 11–15 min: $F(3,34) = 6.04$; $P < 0.01$). Subsequent analysis indicated that $D_1^{-/-}D_3^{-/-}$ mice exhibited lower activity than wild-type ($P < 0.001$), $D_1^{-/-}$ ($P < 0.05$) and $D_3^{-/-}$ mice ($P < 0.001$), in the first 5-min block of the test session (Fig. 2b). In the 6–10 and 11–15 min blocks, the activity levels of $D_1^{-/-}D_3^{-/-}$ mice were not significantly different from their $D_1^{-/-}$ littermates, but were significantly lower than the levels of wild-type and $D_3^{-/-}$ mice ($P < 0.01$). The decrease in horizontal activity of $D_1^{-/-}$ mice was observed only in the 1–5 min time block. Interestingly, in the 6–10 min block, $D_3^{-/-}$ mice showed an increase in square crossings when compared to wild-type mice ($P < 0.05$). This hyperactivity was not observed in the 1–5 and 11–15 min blocks. The distribution of horizontal activity between the peripheral and central squares of the open field did not differ significantly among the genotypes. Normalised centre square entries (centre entries/total crossings) indicated that although $D_1^{-/-}$ and $D_3^{-/-}$ mice tended to enter the centre square more often than wild-type mice, this difference was not significant. Moreover, a time course analysis of centre entries did not reveal any differences among the groups in any of the time blocks (data not shown).

Analysis of vertical activity indicated significant differences among the genotypes ($F(3,34) = 55.58$; $P < 0.0001$). $D_1^{-/-}$ mice reared less, and $D_3^{-/-}$ mice reared more than the wild-type mice ($P < 0.001$; Fig. 2c). $D_1^{-/-}D_3^{-/-}$ mice reared significantly less than either wild-type or $D_3^{-/-}$ mice ($P < 0.001$). Although $D_1^{-/-}D_3^{-/-}$ mice appeared to rear less than $D_1^{-/-}$ mice, ANOVA did not reveal a statistically significant difference. Nevertheless, a simple comparison of the two groups by an unpaired *t*-test yielded a significant difference ($t = 2.48$; $P = 0.022$), indicating a tendency towards decreased rearing in $D_1^{-/-}D_3^{-/-}$ mice.

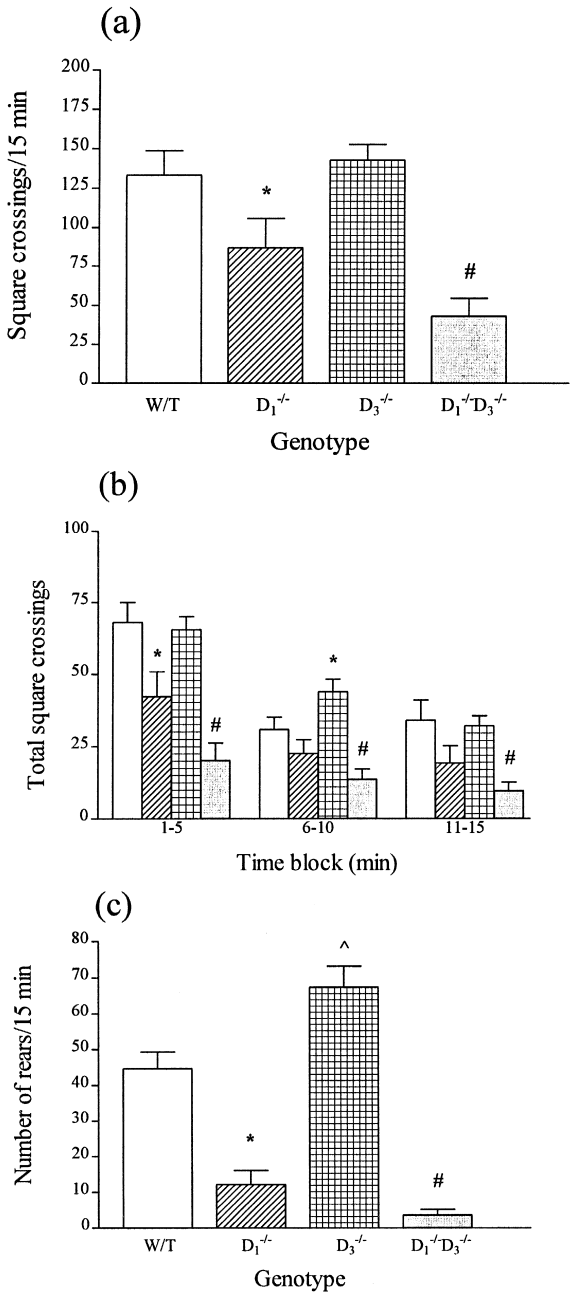

Fig. 2. Exploratory behaviour in a novel open field of wild-type ($n = 10$, white bars), $D_1^{-/-}$ ($n = 7$, diagonal bars), $D_3^{-/-}$ ($n = 10$, checkered bars) and $D_1^{-/-}D_3^{-/-}$ ($n = 11$, shaded bars) mice. Each mouse was placed in the centre square of the open field and (a) total horizontal distance travelled in 15 min was measured. Data are mean square crossings ± S.E.M. $^{*}P < 0.05$, compared to wild-type and $D_3^{-/-}$ mice. $^{\#}P < 0.001$–0.05, compared to wild-type, $D_1^{-/-}$ and $D_3^{-/-}$ mice. (b) Time course analysis of total horizontal activity. Values represent mean square crossings ± S.E.M. in each 5 min block. 1–5 min: $^{*}P < 0.01$–0.05, compared to wild-type and $D_3^{-/-}$ mice. $^{\#}P < 0.001$–0.05, compared to wild-type, $D_1^{-/-}$ and $D_3^{-/-}$ mice. 6–10 min: $^{*}P < 0.01$–0.05, compared to wild-type and $D_1^{-/-}$ mice. $^{\#}P < 0.001$–0.01, compared to wild-type and $D_3^{-/-}$ mice. 11–15 min: $^{\#}P < 0.01$, compared to wild-type and $D_3^{-/-}$ mice. (c) Number of rears in 15 min. Data are means ± S.E.M. $^{*}P < 0.001$, compared to wild-type and $D_3^{-/-}$ mice. $^{\wedge}P < 0.001$ compared to wild-type mice. $^{\#}P < 0.001$, compared to wild-type and $D_3^{-/-}$ mice, and $P = 0.022$ compared to $D_1^{-/-}$ mice (unpaired t-test).

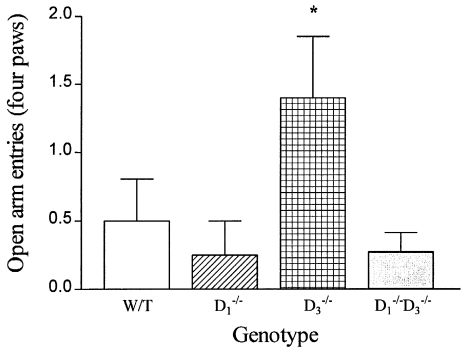

Fig. 3. Behaviour of wild-type ($n = 10$), $D_1^{-/-}$ ($n = 8$), $D_3^{-/-}$ ($n = 10$) and $D_1^{-/-}D_3^{-/-}$ ($n = 11$) mice in the elevated plus maze. Each mouse was placed on the centre platform and the number of arm entries was counted for 15 min. Data are means ± S.E.M. $D_3^{-/-}$ mice entered the open arms with four paws more often than other genotypes. $^{*}P < 0.05$, compared to wild-type, $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice.

3.4. Elevated plus maze

In order to study the effects of combined lack of dopamine D_1 and D_3 receptors on anxiety-related behaviour, we examined the performance of the mice on the elevated plus maze task. The entries into open arms with two forepaws vs. all four paws were recorded to observe if mice that entered open arms with all paws, a measure of decreased anxiety, also tended to cross more often into the open arms with the two forepaws. No differences in closed arm entries ($F(3,35) = 2.12$) and open arm entries with two forepaws ($F(3,35) = 1.55$) were observed among the genotypes (data not shown). $D_1^{-/-}D_3^{-/-}$ mice did not differ in their entries into the open arms with four paws from either wild-type or $D_1^{-/-}$ mice (Fig. 3). As expected, $D_3^{-/-}$ mice entered the open arms with four paws more often than any other genotype, when expressed as either the number of open arm entries (Fig. 3) or as a percentage of all entries (data not shown) ($P < 0.05$). The increased exploration of the open arms with four paws observed in $D_3^{-/-}$ mice was therefore abolished by the concurrent deletion of dopamine D_1 receptor, as observed in $D_1^{-/-}D_3^{-/-}$ mice. No difference in time spent in the open arms with either four or two paws was observed among the genotypes. Although $D_3^{-/-}$ mice appeared to spend more time in open arms with four paws than any other genotype (data not shown), this difference failed to show statistical significance. Moreover, when the variable was expressed as time spent per each entry, no difference was observed among the groups (data not shown).

3.5. Rotarod

Motor control of $D_1^{-/-}D_3^{-/-}$ mice was investigated using the rotarod. All mice were able to stay on the immobile cylinder for 3 min. A significant main effect of genotype ($F(3,33) = 67.56$; $P < 0.0001$), but no significant main effect of day and no interaction (genotype ×

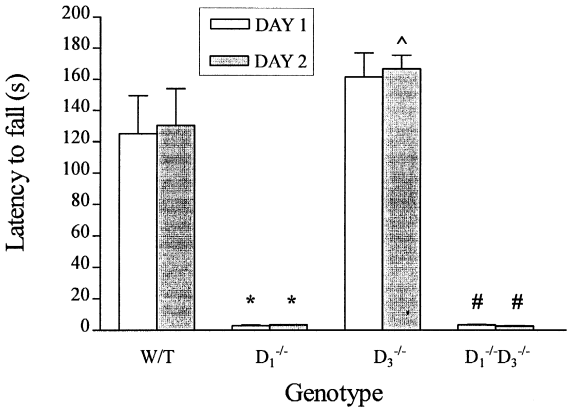

Fig. 4. Performance of wild-type ($n=10$), $D_1^{-/-}$ ($n=8$), $D_3^{-/-}$ ($n=9$) and $D_1^{-/-}D_3^{-/-}$ ($n=10$) mice on the rotarod. Each mouse was placed on the rotarod and the speed was turned on to 10 rpm. The latency to fall was measured, maximum time allowed was 3 min. Values represent group mean latencies ± S.E.M. during probe trials on day 1 and day 2. $^{*}P<0.001$, and $^{\#}P<0.001$, compared to wild-type and $D_3^{-/-}$ mice. $^{\wedge}P<0.05$, compared to wild-type mice.

day), was observed. $D_1^{-/-}D_3^{-/-}$ mice performed very poorly on the task on both days of testing (Fig. 4). The latency to fall off the rotating cylinder in the double mutants was significantly lower than in either the wild-type or the $D_3^{-/-}$ mice on each day ($P<0.001$). The performance of $D_1^{-/-}D_3^{-/-}$ mice, however, did not differ from $D_1^{-/-}$ mice. $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice fell off the cylinder immediately after it started rotating, during training and trial sessions. Their performance did not improve on day 2 despite another 10-min training session. The latency to fall was the same in wild-type and $D_3^{-/-}$ mice on day 1. On day 2, however, $D_3^{-/-}$ mice performed better than wild-type mice, staying longer on the rotarod ($P<0.05$).

3.6. Water maze

We tested the ability of $D_1^{-/-}D_3^{-/-}$ mice to locate an escape platform submerged in water using distal (outside of tank) and proximal (marked platform) cues, to investigate if deficiency of both receptors affects the performance in the task. No differences in swim speeds were found among wild-type, $D_1^{-/-}$ and $D_3^{-/-}$ mice (data not shown). $D_1^{-/-}D_3^{-/-}$ mice, however, demonstrated significantly

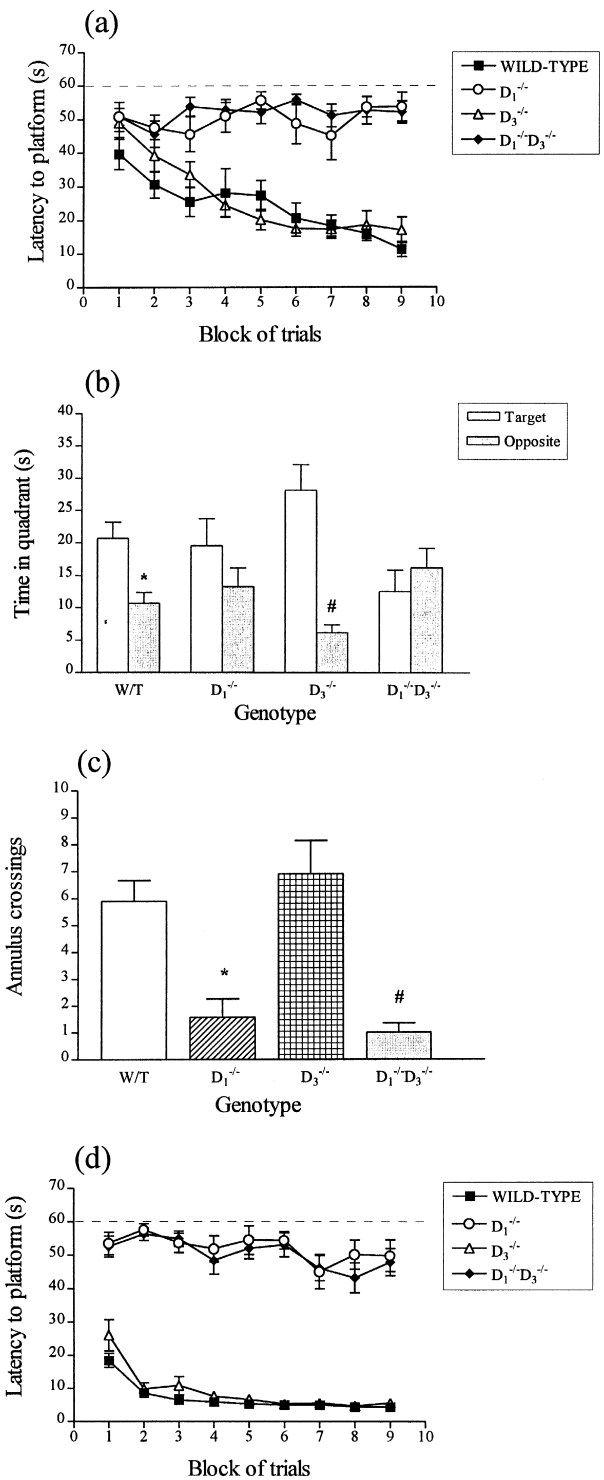

Fig. 5. Performance of wild-type ($n=10$), $D_1^{-/-}$ ($n=7$), $D_3^{-/-}$ ($n=10$) and $D_1^{-/-}D_3^{-/-}$ ($n=11$) mice in the water maze task. Each mouse was placed into the tank and the latency to find (a) the hidden platform was measured. The maximum time allowed was 60 s. Each block represents the mean latencies in four trials ± S.E.M. Both $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice had higher latencies to platform than wild-type and $D_3^{-/-}$ mice. During the probe trial, the platform was removed and (b) development of spatial bias for target versus opposite quadrant was measured. Values represent the means of time spent in each quadrant ± S.E.M. Wild-type mice: $^{*}P<0.05$, compared to target quadrant. $D_3^{-/-}$ mice: $^{\#}P<0.0001$, compared to target quadrant. $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice did not develop a significant preference for the target quadrant. (c) Annulus crossings during the probe trial. Data are expressed as mean crossings through platform location ± S.E.M. Wild-type and $D_3^{-/-}$ mice crossed the platform location more often than $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice. $^{*}P<0.001$ and $^{\#}P<0.001$, compared to wild-type and $D_3^{-/-}$ mice. (d) Escape latencies to visible platform in cued version of the task. Values represent mean latencies in four trials ± S.E.M. Wild-type and $D_3^{-/-}$ mice performed better than $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice.

lower swim speeds than the other three groups ($F(3,34) = 7.83$; $P < 0.01$). In addition, observation of the swim paths revealed that $D_1^{-/-}D_3^{-/-}$ and $D_1^{-/-}$ mice often floated in the water and tended to swim around the walls of the tank (thigmotaxis).

In the hidden version of the water maze, a significant main effect of genotype ($F(3,34) = 132.2$; $P < 0.0001$) and trial block ($F(8,315) = 5.22$; $P < 0.0001$) were observed, as well as an interaction (genotype × trial block, $F(24,315) = 3.24$; $P < 0.0001$). Both $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice exhibited longer latencies to platform than wild-type mice by trial block 2, and $D_3^{-/-}$ mice by trial block 3 and all blocks thereafter (Fig. 5a). The escape performance of wild-type and $D_3^{-/-}$ mice improved over training as indicated by the decreasing latencies to platform. The analysis of the data from the probe trial revealed no main effect of genotype, but a significant main effect of quadrant ($F(1,34) = 16.78$; $P < 0.001$) and an interaction (genotype × quadrant, $F(3,68) = 7.16$; $P < 0.001$). A simple analysis of the swim paths indicated that only wild-type and $D_3^{-/-}$ mice spent more time in the target quadrant vs. the opposite quadrant ($t = 2.73$; $P = 0.0231$ and $t = 4.37$; $P = 0.0018$, respectively, paired t-test) (Fig. 5b). $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice spent similar amounts of time in the target and opposite quadrants, indicating lack of learning of the escape platform location. A significant difference among the genotypes was observed in annulus crossings ($F(3,34) = 15.26$; $P < 0.0001$). Wild-type and $D_3^{-/-}$ mice crossed the location of the escape platform more often than either $D_1^{-/-}$ or $D_1^{-/-}D_3^{-/-}$ mice ($P < 0.001$), indicating retention of memory of the platform location (Fig. 5c). No differences in annulus crossings were observed between wild-type and $D_3^{-/-}$ mice, and between $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice.

In the cued version of the task, significant main effects of genotype ($F(3,34) = 742.7$; $P < 0.0001$) and trial block ($F(8,315) = 7.86$; $P < 0.0001$) and an interaction (genotype × trial block, $F(24,315) = 1.55$, $P < 0.05$) were observed. The escape latencies of wild-type and $D_3^{-/-}$ mice decreased rapidly over the trials (Fig. 5d). Both $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice, however, showed no improvement in locating the visible platform over the trials, as their latencies remained higher in all trial blocks. No difference in latencies was observed between wild-type and $D_3^{-/-}$ mice, as well as between $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice, in all trial blocks.

4. Discussion

We have previously characterised the dopamine D_1 receptor both pharmacologically and using knockout mice (Ng et al., 1995; El-Ghundi et al., 1998; George et al., 1998; El-Ghundi et al., 1999; Jin et al., 1999). To further investigate dopamine D_1 receptor function and its interactions with the dopamine D_3 receptor in dopamine-mediated behaviours, we generated $D_1^{-/-}D_3^{-/-}$ mice and studied their performance in various behavioural tests. The major findings of these tests suggest that: (1) the combined lack of dopamine D_1 and D_3 receptors further attenuates the low exploratory phenotype resulting from dopamine D_1 receptor mutation, (2) the presence of intact dopamine D_1 receptor is necessary for the expression of some aspects of the dopamine D_3 receptor knockout phenotype, including increases in rearing in the open field and open arm entries in the plus maze, and (3) impairments in behavioural tests observed in $D_1^{-/-}$ mice, such as the rotarod and water maze, are not affected by concurrent deletion of dopamine D_3 receptor.

$D_1^{-/-}D_3^{-/-}$ mice exhibited normal spontaneous locomotor activity in a familiar environment, when compared to mice with functional dopamine D_1 and D_3 receptors. These findings suggest that the presence of either, or both receptors, is not required for the expression of normal basal forward locomotion. Lack of significant change in basal locomotor activity in either $D_1^{-/-}$ mice, as demonstrated in the present and past studies (El-Ghundi et al., 1999), or $D_3^{-/-}$ mutants, further supports these findings. However, $D_1^{-/-}D_3^{-/-}$ mice exhibited significantly lower locomotor activity than wild-type, $D_1^{-/-}$ and $D_3^{-/-}$ mice in the open field, a test for exploratory behaviour and anxiety. Dopamine D_1 receptor knockout mice have been reported to show lower locomotor activity and rearing in an open field (Drago et al., 1994; Smith et al., 1998), and dopamine D_3 receptor knockout mice were found to be hyperactive in the same test (Accili et al., 1996). Our findings that deletion of dopamine D_1 receptor attenuates, while deletion of dopamine D_3 receptor stimulates exploratory behaviour are, therefore, in agreement with these reports. However, an increase in locomotor activity in dopamine D_1 receptor mutants was reported by other groups (Xu et al., 1994; Clifford et al., 1998). The differences in results could be attributed to factors, such as environmental differences and variations in test protocols. As indicated by our results, a distinction should be made between basal locomotion tests which involve long sessions and allow habituation, and tests of exploratory activity which are of shorter duration and are affected by the novelty of the environment. Thus, the difference in activity in the locomotion and open field tests of $D_1^{-/-}D_3^{-/-}$ mice and their $D_1^{-/-}$ littermates may be attributed to the different types of behaviour measured by these tests. In the locomotion test, mice were habituated to the activity cages, prior to the test session, in order to reduce the element of novelty, and were thus familiar with the test environment. In addition, the test cages were small and they resembled the home cages. The open field, however, was a large bright novel space to which the mice were exposed for the first time during the test session, allowing us to study exploratory behaviour. Therefore, our results suggest that the concurrent deletion of dopamine D_1 and D_3 receptors further attenuates the already low exploratory activity observed in $D_1^{-/-}$ mice. In fact, $D_1^{-/-}D_3^{-/-}$ mice showed

impairment in response initiation, as indicated by the lowest activity in the initial 5 min of the open field test, and the fact that some $D_1^{-/-}D_3^{-/-}$ mutants stayed in the central square for a few minutes before initiating movement. The low locomotor activity of $D_1^{-/-}D_3^{-/-}$, as well as $D_1^{-/-}$ mice in the open field test, could reflect not only the attenuation of exploratory activity, but also changes in the anxiety state of these mice. However, results obtained from the plus maze test suggest that $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice exhibit normal anxiety levels when compared to wild-type mice. There were also no changes in the centre square entries in the open field, a measure of anxiety level, between $D_1^{-/-}$, $D_1^{-/-}D_3^{-/-}$ and wild-type mice. According to our findings, the exploratory hyperactivity (i.e. the increase in both rearing, and horizontal activity in the 6–10 min block) and anxiolytic-like effect of dopamine D_3 receptor mutation were eliminated with concurrent deletion of dopamine D_1 receptor. This suggests that dopamine D_1 receptor, in the absence of dopamine D_3 receptor, may facilitate the stimulation of these behaviours. This implies a possible inhibitory effect of dopamine D_3 receptor in these processes.

The inability of $D_1^{-/-}D_3^{-/-}$ mice to perform on the rotarod indicates a possible motor control deficit, which could be involved in the observed attenuated rearing behaviour. In order for mice to remain on the rotating rod, normal coordination between the frontal and hind paws, as well as the ability to learn the task, are required. However, $D_1^{-/-}D_3^{-/-}$ mice and their $D_1^{-/-}$ littermates fell off the rod immediately after it started rotating, suggesting that they failed to respond to the stimulus, i.e. the rotating cylinder. This indicates that an impairment in movement initiation, and not a motor control deficit, may be responsible for the inability of $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice to walk on the rotarod. Previous studies reporting that $D_1^{-/-}$ mice did not exhibit changes in motor coordination (Drago et al., 1994; El-Ghundi et al., 1999) support these findings. Although $D_1^{-/-}D_3^{-/-}$ mice performed poorly on both the rotarod and water maze tasks, their impairment was identical to that of $D_1^{-/-}$ mice. These findings suggest the involvement of dopamine D_1 but not D_3 receptors in the ability to perform on the rotarod, and to locate the escape platform in the water maze, since $D_3^{-/-}$ mice performed normally in both tasks. It should be noted, however, that the impairments demonstrated by $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice resulted in very low fall latencies in the rotarod test and very high latencies in the water maze, possibly creating a floor and ceiling effect, respectively. Therefore, a potential further decrease in performance due to dopamine D_3 receptor deletion may have been impossible to measure.

In the present study $D_1^{-/-}$ mice were impaired in the water maze, an effect consistent with that reported by Smith et al. (1998). However, this deficit was more severe than that reported previously by our laboratory (El-Ghundi et al., 1999). In this latter study, $D_1^{-/-}$ mice were clearly impaired in the water maze but did show a decline in escape latencies in both the hidden and cued versions of the task. Procedural differences between the present study and the study of El-Ghundi et al. (1999) could explain these different patterns of results. In comparison to the 60 s trial maximum used in this report, El-Ghundi et al. (1999) used a 90 s trial maximum. Thus, the shorter trial length used in the present study would be likely to reduce the chances of the impaired $D_1^{-/-}$ mice to locate the platform. Additionally, while El-Ghundi et al. (1999) used an inter-trial interval of approximately 6 min, during which mice were returned to their home cages, mice in the present study were subjected to consecutive trials with a 60 s inter-trial interval on the platform. It is possible that this massed trial procedure may have been more stressful than the one in which long intervals intervened between successive trials. Given that dopamine in prefrontal cortex is thought to be involved in mediating responses to stress (Horger and Roth, 1996), it is possible that any stress engendered by exposing $D_1^{-/-}$ mice to massed swim trials may have affected performance and/or learning in these animals. The inability of $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice to locate the escape platform in both the hidden and cued versions of the task supports the role of dopamine D_1 receptor in cognitive processes. In addition to a spatial learning deficit, i.e. the inability to learn to associate external cues with the location of the escape platform, the impairment in the water maze may be a result of other factors. The impairment could have resulted from increased thigmotaxis, as well as periods of floating instead of swimming observed in both groups. Although $D_1^{-/-}D_3^{-/-}$ mice exhibited lower swim speeds, this unlikely caused their poor performance, since the impaired $D_1^{-/-}$ mutants showed normal swim speeds. The rotarod results may again suggest motor deficits, which may have affected the swimming performance and, thus, the ability of $D_1^{-/-}D_3^{-/-}$ mice to locate the escape platform. We believe, however, that swimming disability as a result of motor incoordination was not the reason for impairments in the water maze, since $D_1^{-/-}$ mice developed normal swim speeds compared to wild-type mice, and both $D_1^{-/-}$ and $D_1^{-/-}D_3^{-/-}$ mice were able to climb onto the platform. Visual impairments were unlikely to have caused the poor performance of $D_1^{-/-}D_3^{-/-}$ mice, since naïve $D_1^{-/-}$ mice were able to locate the cued platform (El-Ghundi et al., 1999). Moreover, according to studies by another group, initial processing of visual information is unchanged in dopamine D_1 receptor knockout mice (Smith et al., 1998). It is possible that $D_1^{-/-}D_3^{-/-}$ mutants failed to learn the association between the platform and escape from water. One of the limitations of the water maze task, is its restriction to aversively motivated behaviour (Morris, 1981), which is thought to be mediated in part by dopamine D_1 receptor. Therefore, impairments in motivational processes, in addition to impaired spatial learning and response initiation (floating) as well as thigmotaxis, may all

be variables contributing to the poor performance of $D_1^{-/-}D_3^{-/-}$ mice in the water maze. Future studies are needed to further investigate the role of both dopamine D_1 and D_3 receptors in specific aspects of motivation and learning.

There may be several possible mechanisms via which the two receptors interact. Co-localisation of dopamine D_1 and D_3 receptors on single neurons suggests that they may interact at the cellular membrane or the second messenger level. Several G protein-coupled receptors, including dopamine receptors, have been shown to form heterodimers (Jordan and Devi, 1999; Ng et al., 1999; Xie et al., 1999; Rocheville et al., 2000). Therefore, it is conceivable that dopamine D_1 and D_3 receptors physically interact on the cell surface forming heterodimers, which in turn may affect their agonist binding and G protein coupling. Alternatively, dopamine D_1/D_3 receptor interactions may also occur as a result of dopamine D_1 and D_3 receptor coupling to the same second messenger signalling pathways such the adenylyl cyclase and the mitogen-activated protein kinase pathways (Schwartz et al., 1998; Obadiah et al., 1999). The mechanism(s) behind the interaction between dopamine D_1 and D_3 receptors remains to be revealed.

The possibility of compensatory changes by other proteins, in this case, other dopamine receptors, in $D_1^{-/-}D_3^{-/-}$ mutants has to be considered. It has been reported that dopamine D_2 receptor mutants exhibit increased expression of dopamine D_3 receptor which may in fact compensate for the lacking dopamine D_2 receptor functions (Jung et al., 1999). The same study, however, reports no changes in dopamine D_2 receptor expression in dopamine D_3 receptor mutants, which is consistent with previous reports (Accili et al., 1996; Xu et al., 1997). Dopaminergic neurons and D_1-like binding also appeared normal in dopamine D_3 receptor knockout mice (Xu et al., 1997). Similarly, dopamine containing systems and D_2-like binding sites were preserved in $D_1^{-/-}$ mutants (Xu et al., 1994; El-Ghundi et al., 1998).

In conclusion, $D_1^{-/-}D_3^{-/-}$ mice have demonstrated a significant additive role of dopamine D_1 and D_3 receptors in at least one aspect of behaviour, the expression of normal exploratory activity. We have shown that the exploratory $D_1^{-/-}$ phenotype was potentiated in dopamine D_1/D_3 receptor double mutants, and that the concurrent deletion of dopamine D_1 receptor prevented the expression of the phenotype observed in $D_3^{-/-}$ mutants. Our findings imply a dopamine D_1/D_3 receptor interaction in the regulation of exploratory activity in mice. Future studies will further investigate dopamine D_1/D_3 receptor interactions in learning and memory, motivation and reward.

Acknowledgements

The authors would like to thank Dr. John Drago, Dr. David Sibley and Dr. Heiner Westphal, for the generation and gift of the dopamine D_1 receptor knockout mice. This study was supported by the Medical Research Council of Canada, the National Institute on Drug Abuse and the Smokeless Tobacco Research Council.

References

Accili, D., Fishburn, C.S., Drago, J., Steiner, H., Lachowicz, J.E., Park, B.H., Gauda, E.B., Lee, E.J., Cool, M.H., Sibley, D.R., Gerfen, C.R., Westphal, H., Fuchs, S., 1996. A targeted mutation of the D_3 dopamine receptor gene is associated with hyperactivity in mice. Proc. Natl. Acad. Sci. U. S. A. 93, 1945–1949.

Barik, S., de Beaurepaire, R., 1998. Hypothermic effects of dopamine D_3 receptor agonists in the island of Calleja Magna. Potentiation by D_1 activation. Pharmacol. Biochem. Behav. 60, 313–319.

Bordet, R., Ridray, S., Carboni, S., Diaz, J., Sokoloff, P., Schwartz, J.C., 1997. Induction of dopamine D_3 receptor expression as a mechanism of behavioral sensitization to levodopa. Proc. Natl. Acad. Sci. U. S. A. 94, 3363–3367.

Boulay, D., Depoortere, R., Rostene, W., Perrault, G., Sanger, D.J., 1999. Dopamine D_3 receptor agonists produce similar decreases in body temperature and locomotor activity in D_3 knock-out and wild-type mice. Neuropharmacology 38, 555–565.

Bouthenet, M.L., Souil, E., Martres, M.P., Sokoloff, P., Giros, B., Schwartz, J.C., 1991. Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D_2 receptor mRNA. Brain Res. 564, 203–219.

Caine, S.B., Koob, G.F., 1994. Effects of dopamine D_1 and D_2 antagonists on cocaine self-administration under different schedules of reinforcement in the rat. J. Pharmacol. Exp. Ther. 270, 209–218.

Caine, S.B., Koob, G.F., Parsons, L.H., Everitt, B.J., Schwartz, J.C., Sokoloff, P., 1997. D_3 receptor test in vitro predicts decreased cocaine self-administration in rats. NeuroReport 8, 2373–2377.

Clifford, J.J., Tighe, O., Croke, D.T., Sibley, D.R., Drago, J., Waddington, J.L., 1998. Topographical evaluation of the phenotype of spontaneous behaviour in mice with targeted gene deletion of the D_{1A} dopamine receptor: paradoxical elevation of grooming syntax. Neuropharmacology 37, 1595–1602.

Cromwell, H.C., Berridge, K.C., Drago, J., Levine, M.S., 1998. Action sequencing is impaired in D_{1A}-deficient mutant mice. Eur. J. Neurosci. 10, 2426–2432.

Daly, S.A., Waddington, J.L., 1993. Behavioural effects of the putative D_3 dopamine receptor agonist 7-OH-DPAT in relation to other ''D_2-like'' agonists. Neuropharmacology 32, 509–510.

Drago, J., Gerfen, C.R., Lachowicz, J.E., Steiner, H., Hollon, T.R., Love, P.E., Ooi, G.T., Grinberg, A., Lee, E.J., Huang, S.P. et al., 1994. Altered striatal function in a mutant mouse lacking D_{1A} dopamine receptors. Proc. Natl. Acad. Sci. U. S. A. 91, 12564–12568.

Dreher, J.K., Jackson, D.M., 1989. Role of D_1 and D_2 dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats. Brain Res. 487, 267–277.

El-Ghundi, M., George, S.R., Drago, J., Fletcher, P.J., Fan, T., Nguyen, T., Liu, C., Sibley, D.R., Westphal, H., O'Dowd, B.F., 1998. Disruption of dopamine D_1 receptor gene expression attenuates alcohol-seeking behavior. Eur. J. Pharmacol. 353, 149–158.

El-Ghundi, M., Fletcher, P.J., Drago, J., Sibley, D.R., O'Dowd, B.F., George, S.R., 1999. Spatial learning deficit in dopamine D_1 receptor knockout mice. Eur. J. Pharmacol. 383, 95–106.

Epping-Jordan, M.P., Markou, A., Koob, G.F., 1998. The dopamine D_1 receptor antagonist SCH 23390 injected into the dorsolateral bed nucleus of the stria terminalis decreased cocaine reinforcement in the rat. Brain Res. 784, 105–115.

Essman, W.D., McGonigle, P., Lucki, I., 1993. Anatomical differentiation within the nucleus accumbens of the locomotor stimulatory actions of selective dopamine agonists and d-amphetamine. Psychopharmacology (Berlin) 112, 233–241.

Fremeau, R.T. Jr., Duncan, G.E., Fornaretto, M.G., Dearry, A., Gingrich, J.A., Breese, G.R., Caron, M.G., 1991. Localization of D_1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. Proc. Natl. Acad. Sci. U. S. A. 88, 3772–3776.

George, S.R., Lee, S.P., Varghese, G., Zeman, P.R., Seeman, P., Ng, G.Y., O'Dowd, B.F., 1998. A transmembrane domain-derived peptide inhibits D_1 dopamine receptor function without affecting receptor oligomerization. J. Biol. Chem. 273, 30244–30248.

Horger, B.A., Roth, R.H., 1996. The role of mesoprefrontal dopamine neurons in stress. Crit. Rev. Neurobiol. 10, 395–418.

Ichihara, K., Nabeshima, T., Kameyama, T., 1989. Differential effects of pimozide and SCH 23390 on acquisition of learning in mice. Eur. J. Pharmacol. 164, 189–195.

Ichihara, K., Nabeshima, T., Kameyama, T., 1992. Effects of dopamine receptor agonists on passive avoidance learning in mice: interaction of dopamine D_1 and D_2 receptors. Eur. J. Pharmacol. 213, 243–249.

Ikemoto, S., Glazier, B.S., Murphy, J.M., McBride, W.J., 1997. Role of dopamine D_1 and D_2 receptors in the nucleus accumbens in mediating reward. J. Neurosci. 17, 8580–8587.

Jin, H., Xie, Z., George, S.R., O'Dowd, B.F., 1999. Palmitoylation occurs at cysteine 347 and cysteine 351 of the dopamine D_1 receptor. Eur. J. Pharmacol. 386, 305–312.

Jordan, B.A., Devi, L.A., 1999. G-protein-coupled receptor heterodimerization modulates receptor function. Nature 399, 697–700.

Jung, M.Y., Schmauss, C., 1999. Decreased c-*fos* responses to dopamine D_1 receptor agonist stimulation in mice deficient for D_3 receptors. J. Biol. Chem. 274, 29406–29412.

Jung, M.Y., Skryabin, B.V., Arai, M., Abbondanzo, S., Fu, D., Brosius, J., Robakis, N.K., Polites, H.G., Pintar, J.E., Schmauss, C., 1999. Potentiation of the D_2 mutant motor phenotype in mice lacking dopamine D_2 and D_3 receptors. Neuroscience 91, 911–924.

Koshikawa, N., Kitamura, M., Kobayashi, M., Cools, A.R., 1996. Contralateral turning elicited by unilateral stimulation of dopamine D_2 and D_1 receptors in the nucleus accumbens of rats is due to stimulation of these receptors in the shell, but not the core, of this nucleus. Psychopharmacology (Berlin) 126, 185–190.

Landwehrmeyer, B., Mengod, G., Palacios, J.M., 1993. Differential visualization of dopamine D_2 and D_3 receptor sites in rat brain. A comparative study using in situ hybridization histochemistry and ligand binding autoradiography. Eur. J. Neurosci. 5, 145–153.

Le Moine, C., Bloch, B., 1996. Expression of the D_3 dopamine receptor in peptidergic neurons of the nucleus accumbens: comparison with the D_1 and D_2 dopamine receptors. Neuroscience 73, 131–143.

Levavi-Sivan, B., Park, B.H., Fuchs, S., Fishburn, C.S., 1998. Human D_3 dopamine receptor in the medulloblastoma TE671 cell line: cross-talk between D_1 and D_3 receptors. FEBS Lett. 439, 138–142.

Miner, L.L., Drago, J., Chamberlain, P.M., Donovan, D., Uhl, G.R., 1995. Retained cocaine conditioned place preference in D_1 receptor deficient mice. NeuroReport 6, 2314–2316.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., Caron, M.G., 1998. Dopamine receptors: from structure to function. Physiol. Rev. 78, 189–225.

Morris, R.G.M., 1981. Spatial localization does not require the presence of local cues. Learn. Motiv. 12, 239–260.

Muller, U., von Cramon, D.Y., Pollmann, S., 1998. D_1- versus D_2-receptor modulation of visuospatial working memory in humans. J. Neurosci. 18, 2720–2728.

Ng, G.Y., Trogadis, J., Stevens, J., Bouvier, M., O'Dowd, B.F., George, S.R., 1995. Agonist-induced desensitization of dopamine D_1 receptor-stimulated adenylyl cyclase activity is temporally and biochemically separated from D_1 receptor internalization. Proc. Natl. Acad. Sci. U. S. A. 92, 10157–10161.

Ng, G.Y., Clark, J., Coulombe, N., Ethier, N., Hebert, T.E., Sullivan, R., Kargman, S., Chateauneuf, A., Tsukamoto, N., McDonald, T., Whiting, P., Mezey, E., Johnson, M.P., Liu, Q., Kolakowski, L.F. Jr., Evans, J.F., Bonner, T.I., O'Neill, G.P., 1999. Identification of a $GABA_B$ receptor subunit, gb2, required for functional $GABA_B$ receptor activity. J. Biol. Chem. 274, 7607–7610.

Obadiah, J., Avidor-Reiss, T., Fishburn, C.S., Carmon, S., Bayewitch, M., Vogel, Z., Fuchs, S., Levavi-Sivan, B., 1999. Adenylyl cyclase interaction with the D_2 dopamine receptor family; differential coupling to Gi, Gz, and Gs. Cell. Mol. Neurobiol. 19, 653–664.

Parsons, L.H., Caine, S.B., Sokoloff, P., Schwartz, J.C., Koob, G.F., Weiss, F., 1996. Neurochemical evidence that postsynaptic nucleus accumbens D_3 receptor stimulation enhances cocaine reinforcement. J. Neurochem. 67, 1078–1089.

Pilla, M., Perachon, S., Sautel, F., Garrido, F., Mann, A., Wermuth, C.G., Schwartz, J.C., Everitt, B.J., Sokoloff, P., 1999. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D_3 receptor agonist. Nature 400, 371–375.

Ridray, S., Griffon, N., Mignon, V., Souil, E., Carboni, S., Diaz, J., Schwartz, J.C., Sokoloff, P., 1998. Coexpression of dopamine D_1 and D_3 receptors in islands of Calleja and shell of nucleus accumbens of the rat: opposite and synergistic functional interactions. Eur. J. Neurosci. 10, 1676–1686.

Rocheville, M., Lange, D.C., Kumar, U., Patel, S.C., Patel, R.C., Patel, Y.C., 2000. Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 288, 154–157, 2000 Apr.

Rohrer, D.K., Kobilka, B.K., 1998. G protein-coupled receptors: functional and mechanistic insights through altered gene expression. Physiol. Rev. 78, 35–52.

Sawaguchi, T., Goldman-Rakic, P.S., 1991. D_1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251, 947–950.

Schwartz, J.C., Diaz, J., Bordet, R., Griffon, N., Perachon, S., Pilon, C., Ridray, S., Sokoloff, P., 1998. Functional implications of multiple dopamine receptor subtypes: the D_1/D_3 receptor coexistence. Brain Res. Rev. 26, 236–242.

Self, D.W., Barnhart, W.J., Lehman, D.A., Nestler, E.J., 1996. Opposite modulation of cocaine-seeking behavior by D_1- and D_2-like dopamine receptor agonists. Science 271, 1586–1589.

Shafer, R.A., Levant, B., 1998. The D_3 dopamine receptor in cellular and organismal function. Psychopharmacology (Berlin) 135, 1–16.

Smith, D.R., Striplin, C.D., Geller, A.M., Mailman, R.B., Drago, J., Lawler, C.P., Gallagher, M., 1998. Behavioural assessment of mice lacking D_{1A} dopamine receptors. Neuroscience 86, 135–146.

Steiner, H., Fuchs, S., Accili, D., 1997. D_3 dopamine receptor-deficient mouse: evidence for reduced anxiety. Physiol. Behav. 63, 137–141.

Svensson, K., Carlsson, A., Waters, N., 1994. Locomotor inhibition by the D_3 ligand *R*-(+)-7-OH-DPAT is independent of changes in dopamine release. J. Neural. Transm.: Gen. Sect. 95, 71–74.

Waters, N., Svensson, K., Haadsma-Svensson, S.R., Smith, M.W., Carlsson, A., 1993. The dopamine D_3 receptor: a postsynaptic receptor inhibitory on rat locomotor activity. J. Neural Transm.: Gen. Sect. 94, 11–19.

Weiner, D.M., Levey, A.I., Sunahara, R.K., Niznik, H.B., O'Dowd, B.F., Seeman, P., Brann, M.R., 1991. D_1 and D_2 dopamine receptor mRNA in rat brain. Proc. Natl. Acad. Sci. U. S. A. 88, 1859–1863.

Williams, G.V., Goldman-Rakic, P.S., 1995. Modulation of memory fields by dopamine D_1 receptors in prefrontal cortex. Nature 376, 572–575.

Xie, Z., Lee, S.P., O'Dowd, B.F., George, S.R., 1999. Serotonin 5-HT_{1B} and 5-HT_{1D} receptors form homodimers when expressed alone and heterodimers when co-expressed. FEBS Lett. 456, 63–67.

Xu, M., Moratalla, R., Gold, L.H., Hiroi, N., Koob, G.F., Graybiel, A.M., Tonegawa, S., 1994. Dopamine D_1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell 79, 729–742.

Xu, M., Koeltzow, T.E., Santiago, G.T., Moratalla, R., Cooper, D.C., Hu, X.T., White, N.M., Graybiel, A.M., White, F.J., Tonegawa, S., 1997. Dopamine D_3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D_1 and D_2 receptors. Neuron 19, 837–848.

Xu, M., Koeltzow, T.E., Cooper, D.C., Tonegawa, S., White, F.J., 1999. Dopamine D_3 receptor mutant and wild-type mice exhibit identical responses to putative D_3 receptor-selective agonists and antagonists. Synapse 31, 210–215.