



Pergamon

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

NEURO  
PHARMACOLOGY

Neuropharmacology 44 (2003) 473–481

[www.elsevier.com/locate/neuropharm](http://www.elsevier.com/locate/neuropharm)

# RO-10-5824 is a selective dopamine D4 receptor agonist that increases novel object exploration in C57 mice

Susan B. Powell <sup>a</sup>, Martin P. Paulus <sup>a</sup>, Deborah S. Hartman <sup>b,1</sup>, Thierry Godel <sup>b</sup>,  
Mark A. Geyer <sup>a,\*</sup>

<sup>a</sup> Dept. of Psychiatry, University of California, San Diego, La Jolla, CA 92093, USA

<sup>b</sup> F. Hoffmann-La Roche, CH-4070 Basel, Switzerland

Received 6 June 2002; received in revised form 2 December 2002; accepted 4 December 2002

## Abstract

Novelty seeking as a behavioral phenomenon emerges as a compromise between approach and avoidance behavior. Although novelty seeking is thought to play a role in drug abuse and in cognition, the biological basis for this construct is poorly understood. At a genetic level, dopamine D4 receptors (D4R) appear to be critical for the behavioral expression of novelty seeking. In humans, polymorphisms of D4R have been associated with novelty-seeking traits in general and attention deficit-hyperactivity disorder in particular. Similarly, D4R (–/–) mice exhibit less novel object exploration than D4R (+/+) mice. Due to the paucity of selective D4R ligands for use in behavioral pharmacology studies, few studies have examined the behavioral effects of D4R compounds in animals. The present experiments characterized RO-10-5824, a new, selective D4R partial agonist with minimal affinity for dopamine D2 and D3 receptors, and tested the hypothesis that activation of D4R increases the investigation by mice of a novel object placed in the center of a familiar open field. C57BL/6J and DBA/1J male mice were used in a dose response study of the selective D4R partial agonist RO-10-5824 (0, 1.0, 3.0, or 10.0 mg/kg). While having no effect on the amount of locomotor activity in novel or familiar environments, RO-10-5824 (10.0 mg/kg) increased time spent in the center of the enclosure in the presence of a novel object in C57 but not DBA mice. These results support the hypothesis that stimulation of D4R can enhance novelty seeking in mice and that this effect may be dependent on subtle genetic differences.

© 2003 Elsevier Science Ltd. All rights reserved.

**Keywords:** Approach-avoidance; Novelty seeking; Strain differences; D4 agonist; Open field; Locomotion

## 1. Introduction

Explorations of novel environments and novel stimuli are critical to an organism's survival. Exploratory drives have been well documented in several species (see Berlyne, 1960). In a Y-maze, rats will explore the novel arm preferentially over the two familiar arms (Dellu et al., 2000), and hungry rats will choose a new runway in the presence of runways in which they have repeatedly obtained food (Berlyne, 1960). In addition to showing preferences for novel environments, rats also show an increased preference for discrete novel objects (Berlyne,

1960; Beesheer and Bevins, 2000; Ennaceur and Delacour, 1988; Sheldon, 1969). The constant balance for an organism to weigh the relative risks and benefits of a particular environmental situation has been conceptualized as an approach-avoidance conflict (e.g., approach rewarding stimuli vs avoid predators; Berlyne, 1960). The aversive properties of a novel environment have been demonstrated in rodents through paradigms such as a modified open field test that measures “novelty-induced suppression of feeding” in food-deprived rats (Rex et al., 1998). Novelty seeking is an important construct related to this approach-avoidance conflict. Specifically, novel stimuli evoke a conflict in animals by eliciting both approach and avoidance behaviors concurrently (Berlyne, 1960; Montgomery, 1955; Welker, 1957). The degree of novelty seeking has been implicated as a variable that is predictive of drug-taking tend-

\* Corresponding author. Tel.: 619-543-3582; fax: 619-543-2493.

E-mail address: [mark@mag.ucsd.edu](mailto:mark@mag.ucsd.edu) (M.A. Geyer).

<sup>1</sup> Present address: AstraZeneca Pharmaceuticals, Wilmington, DE 19850, USA.

encies in animals (Piazza et al., 1989; Kabbaj et al., 2000) and of the risk of developing substance abuse in humans (Pedersen et al., 1989; Teichman et al., 1989; Kosten et al., 1994). Hence, novelty seeking as a personality dimension (Cloninger, 1987) has received increasing attention in psychiatric and genetic research.

Dopamine D4 receptors (D4R) have been implicated in novelty seeking in both human and animal studies. In humans, specific tandem repeat polymorphisms (e.g., 7 allele variant) of the dopamine D4R have been related to novelty-seeking dimensions of personality scales (Benjamin et al., 1996; Ebstein et al., 1996). The link between D4R and novelty seeking has been debated, with subsequent investigations either supporting (Noble et al., 1998; Kuhn et al., 1999; Tomitaka et al., 1999) or questioning the association between the D4R gene and novelty seeking (Jonsson et al., 2002; Mitsuyasu et al., 2001). The D4R gene has also been linked to attention deficit hyperactivity disorder [ADHD] (Faraone et al., 2001) as well as substance abuse (Kotler et al., 1997). Using a battery of paradigms assessing the exploration of novel and familiar environments to examine novelty seeking in mice, a previous study found that D4R (–/–) mice exhibited less novel object exploration, decreased entries into the center of an open field, and increased latency to emerge from a (safe) cylinder (Dulawa et al., 1999).

Thus, if the lack of D4 receptors is associated with a reduced exploration of a novel object and D4 receptors are critical for the balance between approach and avoidance behaviors, one would hypothesize that a D4 receptor agonist increases exploration of a novel object. Therefore, the present investigation tested the hypothesis that acute administration of a D4R agonist increases exploration of a novel object. Support for this hypothesis would provide further evidence for the role of D4 receptors in modulating novelty-seeking responses. Mice of different strains differ dramatically, however, in their responses to novelty (Elias et al., 1975; Crabbe and Belknap, 1992). Therefore, we sought to clarify the influence of the genetic background by selecting two different strains, C57BL/6J and DBA/1J mice, that differ dramatically in their locomotor behavior and responses to novelty. Support for this hypothesis would emphasize that the modulatory role of D4R in novelty seeking depends critically on the trait of the animal and may provide some explanation for divergent findings in both human and animal studies for the role of the D4 receptor in novelty seeking.

## 2. Methods

### 2.1. *In vitro* studies

#### 2.1.1. *Materials*

Haloperidol, clozapine, dopamine, quinpirole, and (-)apomorphine were purchased from Research Bio-

chemicals International (RBI). <sup>3</sup>H-spiperone (89 Ci/mmol), 3H-SCH23390 and 35S-GTPγS (1000 Ci/mmol) were purchased from Amersham. RO-10-5824 (Godel et al., 1997) was tested as both a free base and as a hydrochloric salt.

#### 2.1.2. *Cell lines*

CHO-K1 cell lines stably expressing the human D4.4 dopamine receptor isoform (Asghari et al., 1994), as well as untransfected control cells, were obtained from H. H. M. Van Tol, Toronto. CHO-D2 and GH4-D1 cell lines are described in (Lanau et al., 1997b), and CHO-D3 and CHO-D5 cells are described in (Lanau et al., 1997a).

#### 2.1.3. *Radioligand binding assays*

CHO-D4.4 and CHO-D2 cells were adapted to suspension culture in DHI-TIPP media (Schlaeger and Schumpp, 1992) and grown in 23.5-liter airlift fermentors. Cell membranes were prepared and binding assays performed as described in (Lanau et al., 1997b). Briefly, competition binding was carried out in binding buffer (50 mM Tris-HCl pH 7.4, 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl<sub>2</sub>, 4 mM MgCl<sub>2</sub>) in a 0.5 ml final volume in 96 well-plates with 23.1 μg and 4.5 μg of membrane protein per well for CHO-D4 and CHO-D2 assays, respectively, in the presence of 200 pM <sup>3</sup>H-spiperone and increasing concentrations of competitor ligand for 90 min at room temperature. Competition binding was performed in the presence of 500 pM 3H-SCH23390 for D1 and D5 assays, and 200 pM of 3H-7-OH-DPAT for D3R assays. 5.7, 68, and 37.6 μg of membrane protein per well were used for D1, D3, and D5 receptor assays, respectively. Data were analyzed using EBDA and LIGAND for the Apple Macintosh.

#### 2.1.4. *<sup>35</sup>S-GTPγS binding assay*

Preparation of cell membranes and performance of <sup>35</sup>S-GTPγS assays are described in Lanau et al., 1997b.

## 2.2. *Behavioral studies*

### 2.2.1. *Subjects*

64 C57BL/6J (C57) and 40 DBA/1J (DBA) male mice were obtained from Jackson Laboratories (Bar Harbor, ME). Behavioral testing occurred between 8–9 weeks of age when the mice weighed approximately 20–40 g. Mice were housed four to a cage and kept on a 12 h light/dark cycle (lights off at 9:00 am) with food (Harlan Teklad, Madison, WI) and water available ad libitum, except during behavioral testing. Testing occurred during the dark phase between 11:00 am and 6:00 pm. Experiments were approved by the local animal care and use committee and were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. All efforts were made to minimize the

number of animals used and the suffering of animals in these experiments.

### 2.2.2. Drugs

For behavioral experiments, RO-10-5824 was dissolved in 0.6% Tween 80 in saline at the following doses (0, 1.0, 3.0, and 10.0 mg/kg). For locomotor activity and pattern assessments, injections of RO-10-5824 or saline were administered intraperitoneally (IP) 10 min before behavioral testing. All injections were given at a volume of 5 ml/kg body weight.

### 2.2.3. Apparatus

The video-tracker (VT) consisted of four adjacent white plastic enclosures (41 × 41 × 34 cm) surrounded by a white plastic curtain. Each mouse was tested individually in a separate enclosure. A video camera, mounted 158 cm above the enclosures, provided the signal for the Polytrack digitizer (San Diego Instruments; San Diego, CA). The signal was processed to obtain the left-uppermost coordinate for each of the four animals simultaneously. The signal was stored in a PC computer for further off-line processing. For this investigation, the (*x,y*) position (in pixels) of each animal was sampled at a rate of 18.18 Hz and used to generate a (*x,y,t*) coordinate file consisting of the *x*-location, the *y*-location, and the duration of time (*t*) spent at that location. The spatio-temporal resolution of each event recorded was 0.32 cm, 0.32 cm, and 55 msec, which corresponded to a maximum speed of 25 cm/sec.

### 2.2.4. Locomotor—novel object testing

Previous dose response studies in our laboratory in F2 mice (C57 × 129 hybrid) indicated that RO-10-5824 (at 3.0 and 10.0 mg/kg) did not increase overall locomotor activity (unpublished observations). In order to determine whether the D4 agonist would have appreciable effects on the amount or patterns of locomotion or time spent in the center of the enclosure, we conducted an initial experiment in male C57 mice with 10 mg/kg RO-10-5824. In this experiment, mice were administered RO-10-5824 (10.0 mg/kg, IP; *n* = 12) or saline (*n* = 12) 10 min prior to being placed in the VT chamber for a 60-min period.

In a second experiment, 39 C57 and 40 DBA mice were tested over a three-day period. On days 1 and 2, mice were placed into the VT for 60 min and locomotor behavior was recorded. On the third day, mice were placed into the VT chamber for 60 min. Following the 60-min period, mice were removed from the chambers and administered 0, 1.0, 3.0, or 10.0 mg/kg of RO-10-5824 (*n* = 9–11 per group). Mice were assigned to drug group pseudo-randomly, with each dose being represented in a cage of four mice. A novel paper cup measuring 9.5 cm in height and 7.5 cm in diameter at the rim was placed upside down in the center of each open field

and secured to the floor with tape. Mice were returned to the VT 10 min following injection and tested for an additional 30 min.

Each mouse was placed in the bottom left hand corner of each enclosure at the start of the test session. The movements of the mice were tracked for either 30 or 60 min, with data being stored in 6 or 12, 5-min blocks, respectively. The amount of locomotor activity was measured by the distance traveled, i.e. tracing the consecutive locations of the animal using the highest resolution of the video-tracker and calculating the distance between them. The locomotor activity data were analyzed based on a strain-by-time or a strain-by-drug-by-time analysis of variance (ANOVA).

### 2.2.5. Data analysis

For analyses, the VT is subdivided into nine regions (center [1], walls [4], and corners [4]). Transitions were defined as the total number of entries into the nine regions of the VT chamber. Center duration is defined as the amount of time (min) spent in the center square of the arena (20.5 × 20.5 cm). To compare similar levels of habituation to a novel environment, data from the first 30 min of the three sessions without the cup were analyzed. Three factor ANOVAs with strain as a between subject factor and day and block (3, 10 min blocks) as within subject factors were applied to two different measures: transitions and time spent in the center. Separate three-factor ANOVAs with strain and drug as between subject factors and block as a within subject factor were performed on transitions and time spent in the center for the 30 min period in which the cup was in the center. For significant interactions with strain, subsequent post-hoc ANOVAs for each strain were conducted to determine the nature of the interaction (with alpha level set at 0.025). In the separate experiment of RO-10-5824 in C57 mice, two-way ANOVAs with drug as a between subjects factor and time as a within subject factor were performed on transitions and time spent in the center.

## 3. Results

### 3.1. Identification of RO-10-5824

To identify novel compounds with selectivity for the dopamine D4 receptor, CHO cells expressing recombinant human D4.4R (CHO-D4.4 cells) were grown in 23-liter airlift fermentors (> 10<sup>10</sup> cells per batch), and crude membranes were prepared for binding assays. The D4.4 receptor isoform was selected because it is the most commonly found in the human population (Lichter et al., 1993; Van Tol et al., 1992; Asghari et al., 1994). A total of 8540 compound mixtures (ten compounds each) were screened in <sup>3</sup>H-spiperone competition assays, and after deconvolution of mixtures, 24 discrete active structures

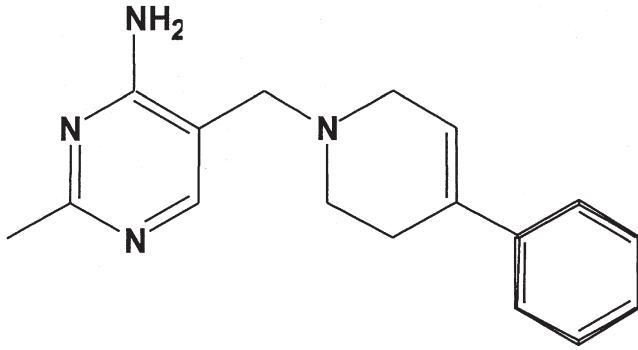


Fig. 1. Chemical structure of RO-10-5824 (Godel et al., 1997).

were identified. One of these actives, RO-10-5824, showed high affinity binding with a  $K_i = 5.2 \pm 0.9$  nM ( $n = 3$ ), 250-fold selectivity vs human D3R, and > 1000 fold selectivity for D4 vs human D2, D1, and D5 receptors. The structure of RO-10-5824, which has a calculated LogP value of 2.431, is shown in Fig. 1.

Functional activity was evaluated in GTP $\gamma$ S activation binding assays using CHO-D4.4 cell membranes prepared specifically for these assays according to Lanau et al., 1997b. RO-10-5824 stimulated  $^{35}$ S-GTP $\gamma$ S binding with an EC<sub>50</sub> value of  $205 \pm 67$  nM ( $n = 7$ ) and maximal induction at  $36 \pm 4\%$  above basal level. Comparisons of EC<sub>50</sub> values and activation levels for RO-10-5824 with known dopaminergic agonists are shown in Table 1. No stimulation of  $^{35}$ S-GTP $\gamma$ S binding was induced by RO-10-5824 in untransfected control CHO cells lacking the D4.4R. Stimulation of RO-10-5824-induced  $^{35}$ S-GTP $\gamma$ S binding in CHO-D4.4 membranes was blocked by the dopaminergic antagonists haloperidol and clozapine with IC<sub>50</sub> values of  $209 \pm 66$  nM ( $n = 3$ ) and  $643 \pm 244$  nM ( $n = 3$ ), respectively. These values are consistent with reported IC<sub>50</sub>s for haloperidol and clozapine antagonism of dopamine in similarly prepared D4.4R membranes (Lanau et al., 1997b). Based on its high binding affinity, functional potency, and selectivity for the D4R, RO-10-5824 was selected for behavioral studies.

### 3.2. Strain comparisons of response to novel environment

The locomotor activity profile across Day 1–3 differed significantly between C57 and DBA mice. As can be

Table 1  
Profile of dopaminergic agonists in  $^{35}$ S-GTP $\gamma$ S binding assays at the human D4.4R

Agonist	EC <sub>50</sub> (nM)	Relative activation level (percent over basal)
Dopamine	$189 \pm 14$ ( $n = 41$ )	$88.2 \pm 4.6\%$
RO-10-5824	$205 \pm 67$ ( $n = 7$ )	$36.3 \pm 3.7\%$
(-)apomorphine	$146 \pm 75$ ( $n = 4$ )	$77.5 \pm 8.9\%$
Quinpirole	$101 \pm 25$ ( $n = 4$ )	$91.0 \pm 12.5\%$

seen in Fig. 2 (panel A), C57 mice showed an increased number of transitions compared to DBA mice [main effect of strain;  $F(1,77) = 90.43$ ,  $p < 0.01$ ]. There was a significant effect of day [ $F(2,154) = 4.22$ ,  $p < 0.05$ ] and time [ $F(2,154) = 18.13$ ,  $p < 0.01$ ] on transitions, with the number of transitions decreasing across the blocks within a session. The changes in transitions across days were strain-dependent, as confirmed by a significant strain by day interaction [ $F(2,154) = 12.86$ ,  $p < 0.01$ ]. Although C57 mice showed a greater number of transitions on each day compared to DBA mice, transitions decreased in C57 mice across the three days (main effect of day  $F(2,78) = 30.68$ ,  $p < 0.01$ ; post-hoc ANOVA), but remained the same in DBA mice across the three days (effect of day not significant  $F(2,76) = 2.12$ , n.s.; post-hoc ANOVA).

In addition to displaying greater amounts of overall locomotion than DBA mice, C57 mice also spent more time in the center of the enclosure (Fig. 2B) [main effect of strain;  $F(1,77) = 141.23$ ,  $p < 0.01$ ]. There was also a significant effect of day [ $F(2,154) = 43.23$ ,  $p < 0.01$ ] and time [ $F(2,154) = 13.63$ ,  $p < 0.01$ ] on center duration. There were significant strain by time [ $F(2,154) = 7.89$ ,  $p < 0.01$ ] and strain by day interactions [ $F(2,154) = 29.2$ ,  $p < 0.01$ ]. During the 60 min exposure to the testing environment, C57 mice spent increasingly more time in the center (main effect of time,  $F(2,78) = 14.78$ ,  $p < 0.01$ ; post-hoc ANOVA). In comparison, DBA mice neither increased nor decreased their time spent in the center over the course of the session (effect of time,  $F(2,76) = 1.0$ , n.s.; post-hoc ANOVA). C57 mice decreased the time spent in the center over the course of the three days (main effect of day,  $F(2,78) = 51.18$ ,  $p < 0.01$ ; post-hoc ANOVA); whereas DBA mice spent the same amount of time in the center during Days 1–3 (effect of day,  $F(2,76) = 2.98$ , n.s.; post-hoc ANOVA).

### 3.3. Effect of RO-10-5824 on novel object exploration

In the initial experiment with C57 mice, the high dose (10.0 mg/kg) of RO-10-5824 did not increase center entries in the open field in a single 60-min session without the novel object present (drug [ $F(1,22) = 0.35$ , n.s.]; Fig. 3A), nor did it increase overall transitions (drug [ $F(1,22) = 0.13$ , n.s.]; Fig. 3B).

During the novel object testing, C57 mice showed higher levels of overall locomotor activity (significant effect of strain [ $F(1,71) = 40.59$ ,  $p < 0.01$ ] on transitions; Fig. 4A). There was no main effect of drug [ $F(3,71) = 2.45$ , n.s.], but there was a significant effect of time [ $F(2,142) = 108.35$ ,  $p < 0.01$ ] on transitions. There were no strain by drug [ $F(3,71) = 0.51$ , n.s.], drug by time [ $F(6,142) = 0.77$ , n.s.], or strain by time [ $F(2,142) = 0.17$ , n.s.] interactions.

There were no significant effects of strain [ $F(1,71)$

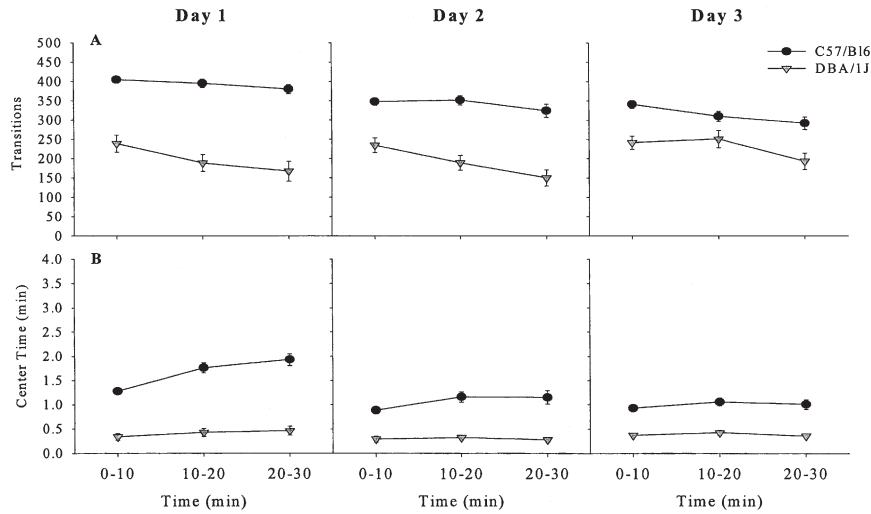


Fig. 2. Comparison of C57BL/6J and DBA/1J mice on measures of transitions (A) and time spent in center (B) during the first 30 min of each locomotor session across the three days.

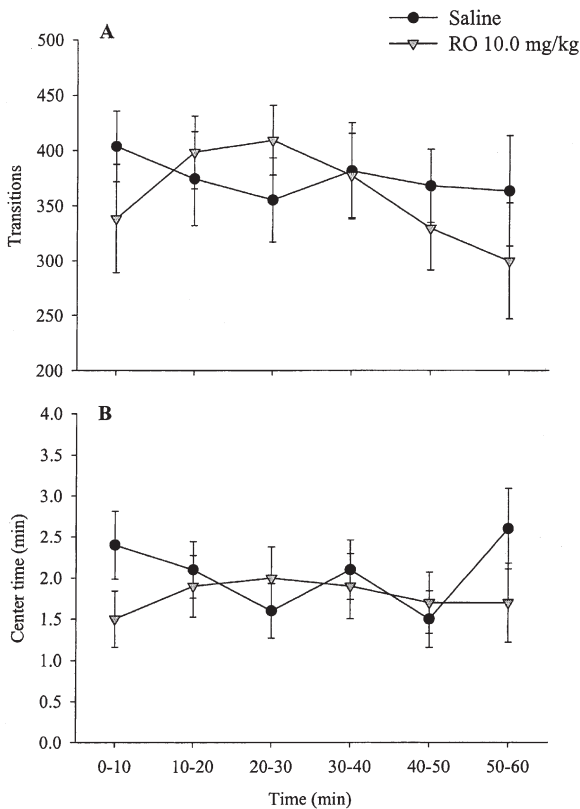


Fig. 3. Transitions (A) and time spent in center (B) in response to RO-10-5824 (10.0 mg/kg) in C57BL/6J mice.

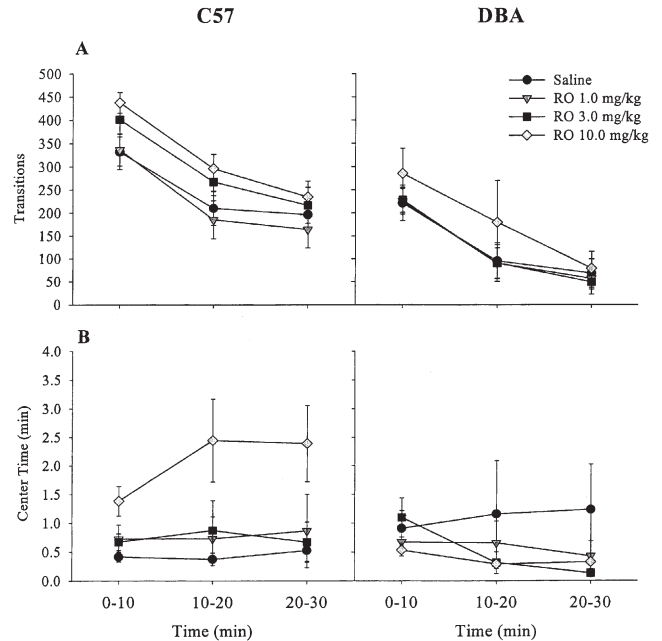


Fig. 4. Effects of RO-10-5824 (0, 1.0, 3.0, 10.0 mg/kg) on transitions (A) and time spent in center (B), during novel object exposure in C57BL/6J and DBA/1J mice.

= 2.27,  $p > 0.05$ ] or drug [ $F(3,71) = 1.29$ , n.s.] on the amount of time spent in the center (Fig. 4B). Similarly, there was no effect of time [ $F(2,142) = 0.09$ , n.s.] nor was there a time by drug interaction [ $F(6,142) = 1.32$ , n.s.]. As predicted by the second hypothesis, however, there was a strain by drug interaction [ $F(3,71) = 4.12$ ,  $p < 0.01$ ]. Subsequent post-hoc ANOVAs for each strain

were conducted to determine the nature of the strain by drug interaction (with alpha level set at 0.025). In C57 mice, there was a significant effect of drug [ $F(3,35) = 4.46$ ,  $p < 0.01$ ], and in DBA mice, there was no effect of drug [ $F(3,36) = 0.92$ , n.s.], confirming that RO-10-5824 increased the amount of time spent in the center by C57 but not by DBA mice. There was also a strain by time interaction [ $F(2,142) = 3.18$ ,  $p < 0.04$ ], with C57 mice either increasing center duration or remaining the same over the 30-min session and DBA mice tending to decrease time spent in the center over the session. In C57 mice, there was no effect of time [ $F(2,70) = 2.34$ ,

n.s.] and no time by drug interaction [ $F(6,70) = 1.57$ , n.s.]. Similarly, in DBA mice, there was no effect of time [ $F(2,70) = 1.11$ , n.s.] or a time by drug interaction [ $F(6,70) = 1.23$ , n.s.].

#### 4. Discussion

The D4 agonist RO-10-5824 by itself had no effect on activity level or time spent in the center of the enclosure but increased exploration of a novel object in the center by C57 but not by DBA mice. The increased exploration of a novel object by a D4 agonist is consistent with the previous observation that the lack of D4 receptors is associated with decreased novel object exploration in mice (Dulawa et al., 1999). In combination, these results support the general hypothesis that D4 receptors are important for modulating novelty seeking. Whereas the D4R agonist increased the center time in the presence of a novel object for C57 mice, which showed high levels of locomotor activity, this drug had no effect on DBA mice, which showed low levels of locomotor activity and exploration. This finding suggests that baseline activity may be a critical factor in the ability of D4 receptor agonists to increase novel object exploration.

Based on *in vitro* binding data, RO-10-5824 appears to be selective for D4R and act as a partial agonist at the D4R in functional assays. RO-10-5824 showed high affinity binding with 250-fold selectivity for D4R vs human D3R and > 1000 fold selectivity for D4R vs human D2, D1, and D5 receptors. Based on  $^{35}\text{S}$ -GTP $\gamma$ S binding assays, RO-10-5824 appears to be a partial agonist at D4R. This assay reflects the activation of G proteins and may be better at detecting partial agonism than other functional assays such as cAMP accumulation (Gazi et al., 2000). Although the behavioral effects of RO-10-5824 on novel object exploration would be strengthened by complementary studies examining the blockade of these effects with D4R antagonists, unfortunately, there are relatively few selective D4R antagonists commercially available for pharmacological studies. From reports in the literature it is difficult to determine whether D4R compounds act as full agonists, partial agonists, or antagonists. For example, L-745,870 has been reported as an antagonist at human D4 receptors (Patel et al., 1997) and as a partial agonist at rat D4 receptors in some assays (Gazi et al., 2000). Perhaps a better way to address this question would be to examine the effects of the D4 agonist in D4 KO mice. It may also be interesting to examine the effects of dopamine D2 receptor agonists on novel object exploration and whether D2 receptor antagonists will block the effects of RO-10-5824. D2 compounds, however, are also not pharmacologically selective for subtypes of dopamine receptors. For example, the putative D2 agonist quinpir-

ole also acts as an agonist at D4 receptors in the GTP $\gamma$ S activation assays (see Table 1). These initial studies were designed to characterize the effects of stimulation of D4 receptors on novel object exploration, using a selective D4R agonist. Subsequent studies should examine the effects of other D2-family ligands in this paradigm and in conjunction with RO-10-5824.

In the current study, RO-10-5824 did not produce overall increases in locomotor activity in the context of the novel object test in either mouse strain. Additionally, the initial examination of the 10.0 mg/kg dose of RO-10-5824 in C57 mice did not produce an increase in transitions or center time in a 60-min locomotor activity session. Although these data indicate that RO-10-5824 (10.0 mg/kg) does not produce increased locomotor activity and time spent in the center in a *novel* environment, we cannot conclude that RO-10-5824 would not produce increased center time if tested in well-habituated mice using similar procedures to those used for novel object testing. Consistent with the opposite effects observed in D4R knockout mice on a C57BL/6J background (Dulawa et al., 1999), we hypothesize that D4R activation augments approach behavior as opposed to reducing avoidance. The important distinction to be made is whether the mouse is spending more time in the center because the aversiveness of the center has been decreased (i.e., reducing avoidance) or because the exploratory drive has been increased (i.e., increasing approach). This increased exploration is unlikely to reflect a reduction in the avoidance of the center for several reasons. First, the novelty paradigm used in this experiment attempted to decrease avoidance tendencies by first habituating the mice to the environment (see Dulawa et al., 1999). Prior to the introduction of the novel cup, the mice had been exposed to the testing arena for one hour/day over three days. Thus, the novelty associated with the arena was decreased relative to the novelty associated with the cup. Second, in C57 mice, we found that the dose of RO-10-5824 (10.0 mg/kg) that increased exploration of the novel object did not alter time spent in the center in a novel environment when no novel object was present.

It is important to point out that the assessment of novelty in the current study is an indirect measure of novel object exploration. We did not directly assess object interaction in these experiments. From our anecdotal viewing of the videotapes, however, it appears that a significant portion of center time is spent in close proximity to the object. The area that was denoted as the 'center' was a 20 cm<sup>2</sup> area in the middle of the VT chamber. When no object is present, mice may enter this area, but traverse through it very quickly. Conversely, when a novel object is present, the mice spend more time exploring the object, sometimes even crawling on the object. From these experiments we can conclude that in the presence of a novel object, mice given 10.0 mg/kg

of RO-10-5824 spent more time in the center of an open field, which is otherwise aversive, compared to vehicle-treated mice. Thus, the combination of the object and the drug resulted in a shift in the approach-avoidance conflict of the mice.

Several investigators have examined 'novelty detection' by showing a preference for a novel object over a familiar one (Beesheer and Bevins, 2000; Beesheer et al., 2001). This novelty detection can be blocked by muscarinic antagonists, but not by dopamine antagonists (Beesheer et al., 2001). A failure to show a preference for the novel object after a delay is interpreted as an impairment in the retrieval or neural storage of the memory of the previously presented object (Ennaceur and Delacour, 1988). The increased time spent exploring the novel object with RO-10-5824 in C57 mice is most likely not due to a decrease in retrieval or neural storage capacity. These novelty detection paradigms measure preference for novel objects over familiar ones, rather than habituation to a novel object. In our paradigm, we cannot directly assess neural storage, but are able to examine habituation to the novel object. Determining whether RO-10-5824 alters habituation processes is difficult in view of the fact that vehicle-treated mice did not show significant increases in center time with object exposure and did not show a decrease in center time over the 30 min session (i.e., habituation). If the mice are habituated to the novel object, we would predict that the pattern would be an increase in the time spent in proximity to the object initially and then a decrease over the course of the session. This indeed may be the pattern that we would observe if we tested the mice for longer periods of time. There was no evidence that C57 mice on 10.0 mg/kg RO-10-5824 habituated to the object, indeed the time spent in the center *increased* over the 30 min session. Along the same lines, if the difference in the effects of RO-10-5824 in the two strains can be attributed to a decreased neural storage capacity in C57 mice (or decreased habituation to the novel object), sampling of the object in DBA mice would be high initially and then decrease throughout the session. In contrast, DBA mice given RO-10-5824 showed very little increase in center time and that which was observed was evident later in the session. At their peak, C57 mice given 10.0 mg/kg of RO-10-5824 are only spending 1/4 of their time in the center with the novel object (Fig. 4). Perhaps a better examination of habituation to the novel object would be to place the object in an area that is less aversive (i.e., in the corner or near the walls), which is possible with this paradigm.

Previous investigations have not shown a specific behavioral 'ethogram' for D4 compounds in general (Clifford and Waddington, 2000). While the D4 agonist CP-226,269 failed to show any significant effects on behavior, PD 168077 dose dependently increased locomotor behavior without inducing stereotypy (Clifford

and Waddington, 2000). These studies did not, however, include explicit assessments of responses to novel objects. A link between D4 receptors and the locomotor hyperactivity associated with neonatal 6-OHDA lesions in rats has been suggested (Zhang et al., 2001; Zhang et al., 2002a,2002b). Specifically, three of four D4R antagonists decreased hyperactivity in neonatal 6-OHDA-lesioned rats without having an effect on locomotor activity in controls. Conversely, the D4R agonist CP-226, 269 exacerbated hyperactivity in 6-OHDA lesion rats but had no effect in controls (Zhang et al., 2001). Consistent with an attenuation of responses to novelty similar to that seen in the D4R knockout mice (Dulawa et al., 1999), the D4R antagonist effects were interpreted to reflect a facilitation of habituation to the novel environment (Zhang et al., 2002a). It will be of interest to determine whether these D4R ligands alter behavioral responses to specific novel stimuli in neonatal 6-OHDA-lesioned rats.

D4 dopamine receptors are widely distributed in the frontal cortex and hippocampus, two brain regions that have long been implicated in subserving behavioral habituation and responses to novel stimuli (Ariano et al., 1997; Wedzony et al., 2000; Mrzljak et al., 1996). In addition to dopaminergic modulation via the D4R in frontal cortex, noradrenergic systems may also critically influence similar targets since D4R have high affinity for norepinephrine (Newman-Tancredi et al., 1997; Lanau et al., 1997b). The potential importance of interactions between norepinephrine and D4R stimulation should also be investigated in this paradigm, given that norepinephrine is implicated in responses to novelty (Flicker and Geyer, 1982; Pudovkina et al., 2001).

Considering the diffuse distribution of D4R in the cortex, it is plausible that occupancy of the D4R has a modulatory role on a number of different behaviors, with approach-avoidance behavior being a particularly sensitive target for D4 receptor modulation. The D4R has been implicated in the genetics of ADHD and in novelty seeking, both of which may involve common processes, such as alterations in habituation to novel stimuli. Thus, considering the primarily cortical distribution patterns of the D4 receptor and preclinical findings such as the present results, there is ample reason to explore further the role of D4 receptors in novelty seeking and attentional problems associated with ADHD.

Increased novelty seeking has been implicated in an increased susceptibility to drug-taking behavior in rodents (Piazza et al., 1989; Kabbaj et al., 2000). These findings in rodents are similar to those in human studies, which have shown an increased risk of substance use disorders in subjects with high sensation-seeking measures on rating scales (Pedersen et al., 1989; Teichman et al., 1989; Kosten et al., 1994). The development of sensitive and reliable measures of 'novelty seeking' in addition to assessing the response to a novel environ-

ment may increase the ability to predict susceptibility to drug-taking behavior in rodents. For example, the response to novelty in the playground maze (Nicholls et al., 1992) was shown to predict amphetamine conditioned place preference (Klebaur and Bardo, 1999). Thus, the novel object paradigm used in the current studies may provide a sensitive measure related to 'drug seeking' behavior.

The overall findings of the current experiments support a role for D4R in the modulation of novelty seeking. Ro-10-5824 was shown to be a highly selective, partial agonist at D4R. The D4R agonist increased novel object exploration in C57 mice, suggesting a direct link between stimulation of these receptors and increased approach behavior.

### Acknowledgements

The authors would like gratefully to acknowledge Dr. E-J Schlaeger for CHO-D4.4 cell fermentation for large-scale membrane preparations, and Corine Goepfert and Marie-Therese Zenner for expert technical assistance in competition &  $^{35}\text{S}$ -GTP $\gamma\text{S}$  binding studies. We would also like to acknowledge Arturo Solis for his help in conducting the behavioral studies and Virginia Lehman-Masten for help with data analysis. Mark Geyer holds an equity interest in San Diego Instruments, Inc. This work was supported by grants from the National Institute of Mental Health (MH61326) and the National Institute on Drug Abuse (DA02925; DA14200).

### References

- Ariano, M.A., Wang, J., Noblett, K.L., Larson, E.R., Sibley, D.R., 1997. Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Research* 752, 26–34.
- Asghari, V., Schoots, O., van Kats, S., Ohara, K., Jovanovic, V., Guan, H.C., Bunzow, J.R., Petronis, A., Van Tol, H.H., 1994. Dopamine D4 receptor repeat: analysis of different native and mutant forms of the human and rat genes. *Mol Pharmacol* 46, 364–373.
- Beesheer, J., Bevins, R.A., 2000. The role of environmental familiarization in novel-object preference. *Behav Process* 50, 19–29.
- Beesher, J., Short, K.R., Bevins, R.A., 2001. Dopaminergic and cholinergic antagonism in a novel-object detection task with rats. *Behav Brain Res* 126, 211–217.
- Benjamin, J., Li, L., Patterson, C., Greenberg, B.D., Murphy, D.L., Hamer, D.H., 1996. Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. *Nat Genet* 12, 81–84.
- Berlyne, D.E., 1960. *Conflict, Arousal, and Curiosity*. McGraw-Hill Book Co, New York.
- Clifford, J.J., Waddington, J.L., 2000. Topographically based search for an 'Ethogram' among a series of novel D(4) dopamine receptor agonists and antagonists. *Neuropsychopharmacology* 22, 538–544.
- Cloninger, C.R., 1987. A systematic method for clinical description and classification of personality variants. A proposal. *Arch Gen Psychiatry* 44, 573–588.
- Crabbe, J.C., Belknap, J.K., 1992. Genetic approaches to drug dependence. *Trends Pharmacol Sci* 13, 212–219.
- Dellu, F., Contarino, A., Simon, H., Koob, G.F., Gold, L.H., 2000. Genetic differences in response to novelty and spatial memory using a two-trial recognition task in mice. *Neurobiol Learn Mem* 73, 31–48.
- Dulawa, S.C., Grandy, D.K., Low, M.J., Paulus, M.P., Geyer, M.A., 1999. Dopamine D4 receptor knock-out mice exhibit reduced exploration of novel stimuli. *J Neurosci* 19, 9550–9556.
- Ebstein, R.P., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., Bennett, E.R., Nemanov, L., Katz, M., Belmaker, R.H., 1996. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. *Nat Genet* 12, 78–80.
- Elias, P.K., Elias, M.F., Eleftheriou, B.E., 1975. Emotionality, exploratory behavior, and locomotion in aging inbred strains of mice. *Gerontologia* 21, 46–55.
- Ennaceur, A., Delacour, J., 1988. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* 31, 47–59.
- Faraone, S.V., Doyle, A.E., Mick, E., Biederman, J., 2001. Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. *Am J Psychiatry* 158, 1052–1057.
- Flicker, C., Geyer, M.A., 1982. Behavior during hippocampal microinfusions. I. Norepinephrine and diversive exploration. *Brain Res* 257, 79–103.
- Gazi, L., Schoeffer, P., Nunn, C., Croskery, K., Hoyer, D., Feuerbach, D., 2000. Cloning, expression, functional coupling and pharmacological characterization of the rat dopamine D4 receptor. *Naunyn-Schmiedeberg's Arch Pharmacol* 361, 555–564.
- Godel, T., Reimer, C., Edenhofer, A., 1997. PCT Int. Appl. WO 9713759.
- Jonsson, E.G., Ivo, R., Gustavsson, J.P., Geijer, T., Forslund, K., Mattila-Evenden, M., Rylander, G., Cichon, S., Propping, P., Bergman, H., Sberg, M., Nothen, M.M., 2002. No association between dopamine D4 receptor gene variants and novelty seeking. *Mol Psychiatry* 7, 18–20.
- Kabbaj, M., Devine, D.P., Savage, V.R., Akil, H., 2000. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. *J Neurosci* 20, 6983–6988.
- Klebaur, J.E., Bardo, M.T., 1999. Individual differences in novelty seeking on the playground maze predict amphetamine conditioned place preference. *Pharmacol Biochem Behav* 63, 131–136.
- Kosten, T.A., Ball, S.A., Rounsaville, B.J., 1994. A sibling study of sensation seeking and opiate addiction. *J Nerv Ment Dis* 182, 284–289.
- Kotler, M., Cohen, H., Segman, R., Gritsenko, I., Nemanov, L., Lerer, B., Kramer, I., Zer-Zion, M., Kletz, I., Ebstein, R.P., 1997. Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. *Mol Psychiatry* 2, 251–254.
- Kuhn, K.U., Meyer, K., Nothen, M.M., Gansicke, M., Papassotiropoulos, A., Maier, W., 1999. Allelic variants of dopamine receptor D4 (DRD4) and serotonin receptor 5HT2c (HTR2c) and temperament factors: replication tests. *Am J Med Genet* 88, 168–172.
- Lanau, F., Brockhaus, M., Pink, J.R., Franchet, C., Wildt-Perinic, D., Goepfert, C., Probst, A., Hartman, D.S., 1997a. Development and characterization of antibodies against the N terminus of the human dopamine D4 receptor. *J Neurochem* 69, 2169–2178.
- Lanau, F., Zenner, M.T., Civelli, O., Hartman, D.S., 1997b. Epinephrine and norepinephrine act as potent agonists at the recombinant human dopamine D4 receptor. *J Neurochem* 68, 804–812.
- Lichter, J.B., Barr, C.L., Kennedy, J.L., Van Tol, H.H., Kidd, K.K., Livak, K.J., 1993. A hypervariable segment in the human dopamine receptor D4 (DRD4) gene. *Hum Mol Genet* 2, 767–773.
- Mitsuyasu, H., Hirata, N., Sakai, Y., Shibata, H., Takeda, Y., Nino-

- miya, H., Kawasaki, H., Tashiro, N., Fukumaki, Y., 2001. Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene (DRD4) with schizophrenia and personality traits. *J Hum Genet* 46, 26–31.
- Montgomery, K.C., 1955. The relation between fear induced by novel stimulation and exploratory drive. *J Comp Phys Psychol* 48, 254–260.
- Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R., Goldman-Rakic, P.S., 1996. Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. *Nature* 381, 245–248.
- Newman-Tancredi, A., Audinot-Bouchez, V., Gobert, A., Millan, M.J., 1997. Noradrenaline and adrenaline are high affinity agonists at dopamine D4 receptors. *Eur J Pharmacol* 319, 379–383.
- Nicholls, B., Springham, A., Mellanby, J., 1992. The playground maze: a new method for measuring directed exploration in the rat. *J Neurosci Methods* 43, 171–180.
- Noble, E.P., Ozkaragoz, T.Z., Ritchie, T.L., Zhang, X., Belin, T.R., Sparkes, R.S., 1998. D2 and D4 dopamine receptor polymorphisms and personality. *Am J Med Genet* 81, 257–267.
- Patel, S., Freedman, S., Chapman, K.L., Emms, F., Fletcher, A.E., Knowles, M., Marwood, R., McAllister, G., Myers, J., Patel, S., Curtis, N., Kulagowski, J.J., Leeson, P.D., Ridgill, M., Graham, M., Matheson, S., Rathbone, D., Watt, A.P., Bristow, L.J., Rupniak, N.M.J., Baskin, E., Lynch, J.J., Ragan, C.I., 1997. Biological profile of L-745,870, a selective antagonist with high affinity for the dopamine D4 receptor. *J Pharmacol Exp Ther* 283, 636–647.
- Pedersen, W., Clausen, S.E., Lavik, N.J., 1989. Patterns of drug use and sensation-seeking among adolescents in Norway. *Acta Psychiatr Scand* 79, 386–390.
- Piazza, P.V., Deminiere, J.M., Le Moal, M., Simon, H., 1989. Factors that predict individual vulnerability to amphetamine self-administration. *Science* 245, 1511–1513.
- Pudovkina, O.L., Kawahara, Y., de Vries, J., Westerink, B.H., 2001. The release of noradrenaline in the locus coeruleus and prefrontal cortex studied with dual-probe microdialysis. *Brain Res* 906, 38–45.
- Rex, A., Voigt, J.P., Voits, M., Fink, H., 1998. Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs. *Pharmacol Biochem Behav* 59, 677–683.
- Schlaeger, E.J., Schumpp, B., 1992. Propagation of a mouse myeloma cell line J558L producing human CD4 immunoglobulin G1. *J Immunol Methods* 146, 111–120.
- Sheldon, A.B., 1969. Preference for familiar versus novel stimuli as a function of the familiarity of the environment. *J Comp Physiol Psychol* 67, 516–521.
- Teichman, M., Barnea, Z., Ravav, G., 1989. Personality and substance use among adolescents: a longitudinal study. *Br J Addict* 84, 181–190.
- Tomitaka, M., Tomitaka, S., Otuka, Y., Kim, K., Matuki, H., Sakamoto, K., Tanaka, A., 1999. Association between novelty seeking and dopamine receptor D4 (DRD4) exon III polymorphism in Japanese subjects. *Am J Med Genet* 88, 469–471.
- Van Tol, H.H., Wu, C.M., Guan, H.C., Ohara, K., Bunzow, J.R., Civelli, O., Kennedy, J., Seeman, P., Niznik, H.B., Jovanovic, V., 1992. Multiple dopamine D4 receptor variants in the human population. *Nature* 358, 149–152.
- Wedzony, K., Chocyk, A., Mackowiak, M., Fijal, K., Czyrak, A., 2000. Cortical localization of dopamine D4 receptors in the rat brain-immunocytochemical study. *J Physiol Pharmacol* 51, 205–221.
- Welker, W.I., 1957. 'Free' versus 'forced' exploration of a novel situation by rats. *Psychol Rep* 3, 95–108.
- Zhang, K., Tarazi, F.I., Baldessarini, R.J., 2001. Role of dopamine D(4) receptors in motor hyperactivity induced by neonatal 6-hydroxydopamine lesions in rats. *Neuropsychopharmacology* 25, 624–632.
- Zhang, K., Davids, E., Tarazi, F.I., Baldessarini, R.J., 2002a. Effects of dopamine D4 receptor-selective antagonists on motor hyperactivity in rats with neonatal 6-hydroxydopamine lesions. *Psychopharmacology (Berl)* 161, 100–106.
- Zhang, K., Tarazi, F.I., Davids, E., Baldessarini, R.J., 2002b. Plasticity of dopamine D4 receptors in rat forebrain: temporal association with motor hyperactivity following neonatal 6-hydroxydopamine lesioning. *Neuropsychopharmacology* 26, 625–633.