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7-OH-DPAT and PD 128907 Selectively Activate the D3 Dopamine Receptor in a Novel Environment

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The D3 dopamine receptor is expressed primarily in limbic brain areas, and appears to play an inhibitory role in rodent locomotor behavior. Evidence suggests a potential role for the D3 receptor in the pathology of neuropsychiatric disease. Progress in elucidating D3 receptor function has been hampered, however, by a lack of well-characterized, selective ligands and by conflicting information regarding the behavioral phenotype of D3 receptor knockout mice. Here, we describe studies evaluating the behavioral effects of (±)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) and PD 128907, two D3 receptor agonists whose *in vivo* selectivity has been a topic of considerable controversy. We demonstrate that both compounds inhibit locomotion under novel environmental conditions in wild-type (WT) mice, but are without measurable behavioral effect under identical conditions in D3 receptor knockout mice. Additionally, we demonstrate that at low, D3 selective doses, these compounds are without behavioral effect in both WT and D3 receptor knockout mice that have acclimated to the testing environment. These findings suggest that D3 receptor stimulation inhibits novelty-stimulated locomotion, and establish conditions for the use of 7-OH-DPAT and PD 128907 as D3 receptor agonists *in vivo*. Potential implications of these observations are discussed.

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INTRODUCTION

Since it was cloned in 1990 (Sokoloff *et al*, 1990), numerous studies have investigated D3 dopamine (DA) receptor pharmacology and function. D3 receptor mRNA and protein are expressed primarily in limbic brain areas associated with motivated, appetitive, and emotional behaviors (Bouthenet *et al*, 1991; Levesque *et al*, 1992; Landwehrmeyer *et al*, 1993a, b; Richtand *et al*, 1995; Levant, 1998; Khan *et al*, 1998; Gurevich and Joyce, 1999; Diaz *et al*, 2000). The earliest reports describing the highly restricted localization of D3 receptor expression suggested a potential role in psychosis (Sokoloff *et al*, 1990, 1992). Additionally, D3 receptor function is of particular interest because evidence suggests its effects are primarily inhibitory (Accili *et al*, 1996; Flores *et al*, 1996; Xu *et al*, 1997; Ekman *et al*, 1998; Menalled *et al*, 1999; Betancur *et al*, 2001), and that loss of this inhibitory function might contribute pathologically to neuropsychiatric disease (Flores *et al*, 1996; Richtand *et al*, 2001a, b).

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Progress in understanding D3 receptor function has long been hindered, however, by two major barriers. First, lack of availability of suitably well-characterized selective ligands has hampered pharmacological studies. *In vitro* selectivity of previously available agonists and antagonists has been limited (Pugsley *et al*, 1995; Levant, 1997), and conflicting data regarding *in vivo* selectivity of these compounds has further hampered their usefulness (Daly and Waddington, 1993; Ahlenius and Salmi, 1994; Levant *et al*, 1996; Bancroft *et al*, 1998; Xu *et al*, 1999; Boulay *et al*, 1999a, b). Secondly, descriptions of the behavioral phenotype of D3 receptor knockout mice have been variable, confounding clear determination of the behavioral role mediated by the D3 receptor (Depoortere, 1999; Boulay *et al*, 1999b; Waddington *et al*, 2001). Two separate labs have observed increased exploratory locomotion in D3 knockout mice measured during the first 5 (Xu *et al*, 1997) or 15 min (Accili *et al*, 1996) of exposure to a novel environment, while two other groups failed to observe a difference in locomotor response to a novel environment between D3 knockout and wild-type (WT) mice (Betancur *et al*, 2001; Waddington *et al*, 2001). This implies that additional, as yet uncharacterized, factors, such as specific environmental conditions, contributions of genetic background, competing effects of D3 receptor loss on different neuronal pathways, and/or developmental accommodations in response to the lack of D3 receptor,

are likely to make important contributions to modulating the measured behaviors. In combination, these impediments have prevented a clear understanding of the behavioral role(s) mediated by the D3 receptor.

Two DA receptor agonists with limited *in vitro* selectivity for D3 over D2 receptor are commercially available and have been widely studied. ± -7-Hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) preferential D3/D2 binding is between 5.2- and 78-fold, depending upon assay conditions (Levant, 1997), while (+)-PD 128907 has an approximately 18-fold greater affinity for cloned D3 *vs* D2L receptors, with weaker affinity for cloned D4 and no significant affinity for other receptors tested (Pugsley *et al*, 1995). Selectivity of these compounds for D3 *vs* D2 receptor *in vivo*, however, has been a topic of much controversy (Daly and Waddington, 1993; Ahlenius and Salmi, 1994; Pugsley *et al*, 1995; Levant *et al*, 1996; Bancroft *et al*, 1998; Xu *et al*, 1999; Boulay *et al*, 1999a, b). Here we describe studies evaluating the behavioral effects of 7-OH-DPAT and PD 128907 in WT mice under both novel and acclimated conditions. Parallel studies in D3 receptor knockout mice serve as a control to demonstrate D3 selectivity of the observed behavioral effect. We demonstrate that both 7-OH-DPAT and PD 128907 inhibit locomotion through D3 receptor activation under novel environmental conditions, but, at D3-selective doses, are without measurable behavioral effect under acclimated conditions. These data establish conditions for use of these compounds as D3 receptor agonists *in vivo* and support an inhibitory role for the D3 DA receptor in regulating novelty-stimulated rodent locomotion.

MATERIALS AND METHODS

Subjects

Homozygous WT and D3 receptor mutant breeding pairs (+/+ × +/+ or −/− × −/−) used to generate the mice used in this study were offspring of mice used in previous D3 agonist studies (Xu *et al*, 1997). Genetic backgrounds of both mutant and WT mice were C57BL/6 × 129Sv, and had been subsequently bred for three generations with C57BL/6 mice. Mutant and WT mice were housed in separate cages, with no more than five animals per cage, under controlled temperature and humidity on a 12-h light/dark cycle (0500 on; 1700 off). Food and water were available *ad libitum*. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

Genotypes of breeder pairs and representative offspring were confirmed by Southern blotting as described (Xu *et al*, 1997) or by polymerase chain reaction (PCR). For PCR, a tail clip was collected from each mouse and combined with 500 µl digestion buffer (50 mM Tris-HCl pH 8.0, 100 mM EDTA, 0.5% SDS) and 25 µl (5.0 U) Proteinase K (Life Technologies, Carlsbad, CA). Tail preparations were incubated overnight at 55°C on a rocking platform. DNA was resalted in 5 M NaCl, precipitated in 100% ethanol, and resuspended in 200 µl H_2O. Each PCR reaction contained the following: 1.0 µl mouse DNA (approximately 50 ng), 2.5 µl 10 × PCR buffer, 0.2 mM dNTP mix, 1.5 or 2.5 mM $MgCl_2$ (1.5 mM for D3 primers, 2.5 mM for Neo cassette primers), 0.25 U Platinum Taq Polymerase (all reagents from Life Technologies, Carlsbad, CA), 25 pmol each primer (Midland Certified Reagent Company, Midland, TX), and H_2O to a final volume of 25 µl. Reactions were carried out separately for each primer pair: one set complementary to exon 1 of the mouse D3 receptor (upstream-5′-GCTCAC-CACTAGGTAGTTG-3′, downstream-5′-ACCTCTGAGCCA-GATAAGC-3′) and one complementary to the Neomycin cassette, which replaced exon 1 in mutant mice (upstream-5′-CAAGATGGATTGCACGCAGG-3′, downstream-5′-AG-CAAGGCGAGATGACAGGA-3′). Cycling parameters were as follows: 94°C for 30 s, 59°C for 30 s, 72°C for 30 s, 40 cycles for D3 primers; 94°C for 30 s, 62°C for 30 s, 72°C for 30 s, 35 cycles for Neo cassette primers. PCR products were visualized on ethidium-bromide-stained 2% agarose gels. Reactions lacking template DNA and reactions containing DNA from animals whose genotypes had been confirmed by Southern blot served as negative and positive controls, respectively.

Drugs

7-OH-DPAT (Sigma, St Louis, MO) and (+)-PD 128907 (Tocris, Ballwin, MO) were dissolved in 0.9% saline. Drug concentrations are described as hydrobromide or hydrochloride salts, respectively.

Behavioral Testing Equipment

Behavioral testing was performed in 30 custom-designed residential activity chambers (RACs), modeled after chambers designed by Segal and Kuczenski (1987). Each chamber consists of a lighted, ventilated, sound-attenuated cabinet (Cline Builders, Covington, KY) housing a $40 \times 40 \times 38\ cm^3$ Plexiglas enclosure. A fan in each enclosure provides air circulation and constant background noise. Lights inside the chambers were coordinated with the vivarium light cycle, and behavioral testing was performed during the 'lights-on' portion of the cycle (between 0900 and 1700). Locomotion was monitored with a 16 × 16 photo beam array (San Diego Instruments, San Diego, CA) located 1.25 cm above the floor of the enclosure. Locomotion was expressed as crossovers, defined as entry into any of the active zones of the chamber, as shown in Figure 1. Shading in this figure is used solely to indicate which areas of the chamber are 'active' for purposes of data collection. There are no variations in the color of the floors inside the chambers.

Procedures

Male mice, 9–16 weeks old, were removed from the vivarium and transported to the testing facility (located in an adjacent building) on the day of testing. In an effort to minimize the effects of stress due to transfer to the testing facility, a 30-min period was allowed prior to testing. For the novel environmental condition, mice without prior RAC exposure were injected with saline, 7-OH-DPAT (10.0, 50.0, or 100.0 µg/kg), or PD 128907 (10.0, 25.0, or 50.0 µg/kg) and immediately placed in the RAC. Locomotion was then measured for 30 min following injection, except in the case of the experiment comparing saline response in acclimated and nonacclimated mice, for which locomotion was

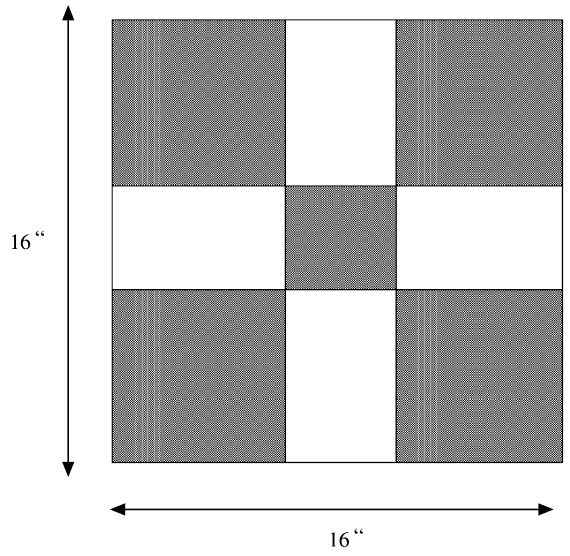

Figure 1 Diagram of zone map used in RACs. A crossover was recorded each time the test subject entered one of the shaded areas.

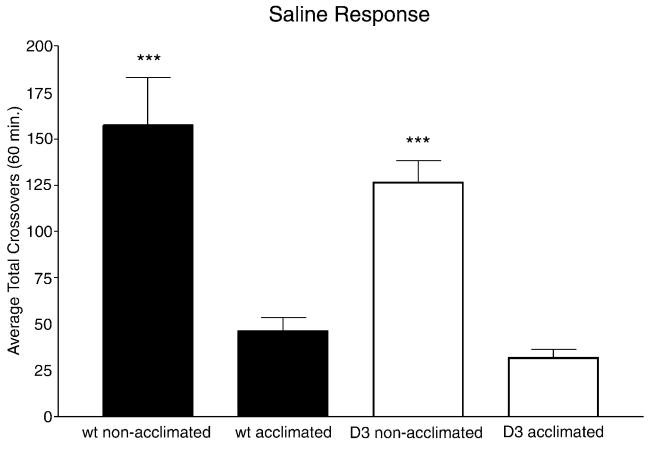

Figure 2 Locomotor response to saline injection in acclimated and nonacclimated animals. ($n = 29$ for mutant nonacclimated and WT acclimated; 24 for WT nonacclimated; 28 for mutant acclimated. ***$p < 0.001$ compared to the same genotype acclimated (two-tailed *t*-test). Bars represent mean ± SEM of total crossovers for each group during the first 60 min after saline injection.

recorded for 60 min (see Figure 2). For the acclimated environmental condition, mice were placed in the RAC for 1 h. Following this 1-h acclimation period, mice were injected with saline, 7-OH-DPAT, or PD 128907, immediately returned to the RAC, and locomotion measured for 30 min (unless otherwise specified) following injection. All injections were given subcutaneously in a volume of 1 ml/kg.

Statistical Analysis

Data were collected in 3-min bins following drug or vehicle injection. Results are expressed as group mean ± SEM of total crossovers. Within-genotype comparisons were made by one-way ANOVA, followed by Dunnett's *post hoc* test with correction for multiple comparisons. Significance was set at $p < 0.05$.

RESULTS

Novel Environment Increases Locomotor Response to Saline Injection

The locomotor response following saline injection was determined in WT and D3 receptor knockout mice in both novel and acclimated environments. Locomotor activity was significantly reduced in both WT and D3 receptor mutant mice following a 1-h acclimation period ($t_{51} = 4.443$, $p < 0.0001$ for WT; $t_{55} = 7.494$, $p < 0.0001$ for mutant; see Figure 2).

At Low Dose, 7-OH-DPAT Inhibits Novelty-induced Locomotor Activity in WT but not D3 Receptor Mutant Mice

The effect of D3 agonist 7-OH-DPAT on novelty-stimulated locomotion was determined in D3 receptor knockout and WT mice. As shown in Figure 3, 7-OH DPAT suppressed locomotion in WT animals at all doses tested (10.0 and 50.0 μg/kg, $p < 0.001$; 100.0 μg/kg, $p < 0.01$). In contrast to the response of WT mice, locomotor responses to saline and 10 μg/kg 7-OH-DPAT did not differ significantly in mutant mice (Dunnett's test $p > 0.05$), suggesting that this low 7-OH-DPAT dose selectively activates D3 receptors. As in WT mice, 7-OH-DPAT significantly suppressed locomotion at 50.0 ($p < 0.05$) and 100.0 μg/kg ($p < 0.001$) in D3 mutant mice. These data suggest that higher 7-OH-DPAT doses activate both D2 and D3 receptors.

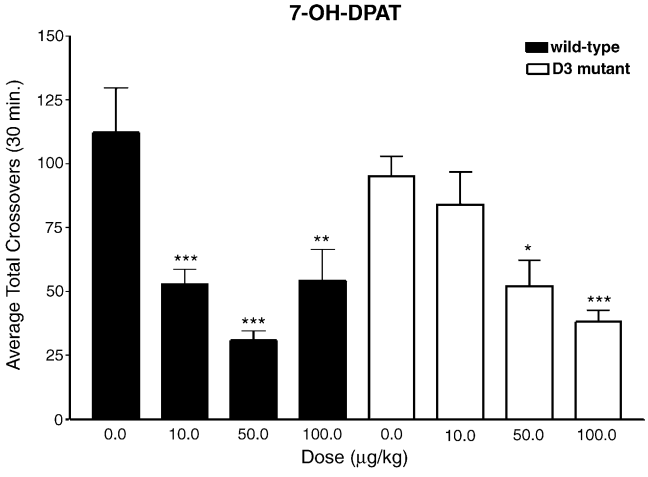

Figure 3 Effect of 7-OH-DPAT on locomotor activity in nonacclimated, mice. ($n = 29$ for mutant saline and mutant 50 μg/kg, 28 for mutant 10 μg/kg and mutant 100 μg/kg, 27 for WT 50 μg/kg, 25 for WT 100 μg/kg, and 24 for WT 10 μg/kg and WT saline). *$p < 0.05$ compared to the same genotype saline; **$p < 0.01$ compared to the same genotype saline; ***$p < 0.001$ compared to the same genotype saline (Dunnett's test). Bars represent mean ± SEM of total crossovers for each treatment group during the first 30 min after drug administration.

At Low Dose, PD 128907 Inhibits Novelty-induced Locomotor Activity in WT but not D3 Receptor Mutant Mice

The effect of D3 agonist PD 128907 on novelty-induced locomotor activity was similarly determined in D3 knockout and WT mice. Mice were injected subcutaneously with vehicle (saline) or PD 128907 (10–50 μg/kg) and immediately placed in the RAC. Locomotion was measured for 30 min following injection. As shown in Figure 4, PD 128907 suppressed locomotion at all doses tested in WT mice (10.0 μg/kg, $p<0.01$; 25.0 and 50.0 μg/kg, $p<0.001$). In contrast, 10 μg/kg PD 128907 had no inhibitory effect on locomotion in mice lacking D3 receptor ($p>0.05$). Similar to WT mice, locomotion was significantly suppressed by PD 128907 doses of 25 and 50 μg/kg in D3 receptor mutant mice ($p<0.001$ for both doses).

At Low Dose, 7-OH-DPAT and PD 128907 are Without Inhibitory Effect in Acclimated WT and D3 Knockout Mice

In order to test the hypothesis that D3 DA receptor activation does not inhibit locomotion in acclimated mice, we determined the locomotor response of acclimated mice to D3-selective doses (determined by studies described above) of 7-OH-DPAT and PD 128907. WT and D3 receptor mutant mice were acclimated to the testing chamber for 1 h prior to injection with saline, 7-OH-DPAT (10.0 μg/kg), or PD 128907 (10.0 μg/kg). Locomotion was recorded for 30 min following injection. The overall effect of agonist treatment in acclimated D3 receptor mutant mice was significant (ANOVA, $F_{2,84}=3.675$, $p=0.0296$), but *post hoc* analyses revealed no significant locomotor suppression or activation for either drug in WT or D3 receptor mutant mice ($p>0.05$, Dunnett's test) (Figure 5). These findings demonstrate that D3 receptor stimulation does not significantly inhibit locomotion in acclimated mice.

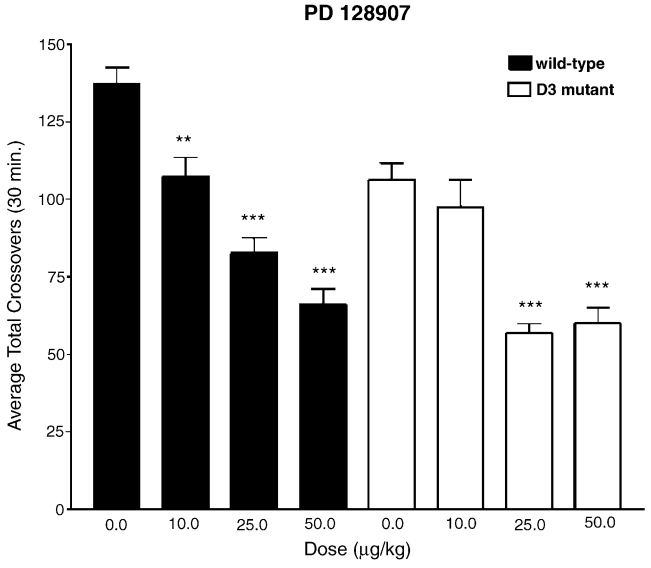

Figure 4 Effect of PD 128907 on locomotor activity in nonacclimated, WT mice. ($n=24$ for WT saline and WT 10 μg/kg; 23 for WT 25 μg/kg, mutant saline, mutant 25 μg/kg and mutant 50 μg/kg; 22 for WT 50 μg/kg). $^{**}p<0.01$ compared to the same genotype saline; $^{***}p<0.001$ compared to the same genotype saline (Dunnett's test). Bars represent mean ± SEM of total crossovers for each treatment group during the first 30 min after drug administration.

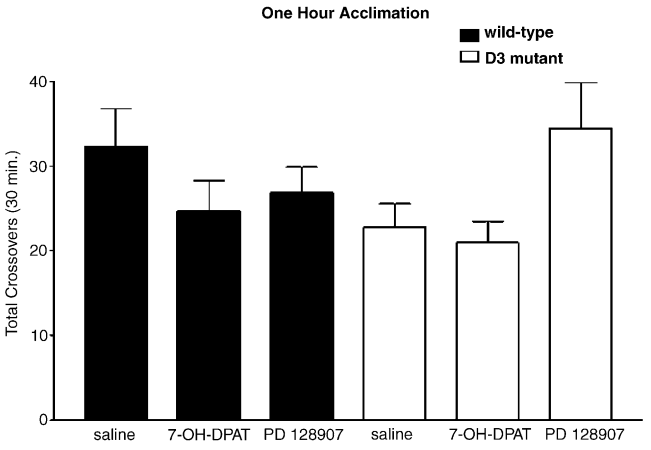

Figure 5 Effect of D3 receptor-selective agonist doses on locomotor activity in acclimated animals. Response of WT and D3 receptor mutant mice to saline, 10.0 μg/kg 7-OH-DPAT, or 10.0 μg/kg PD 128907 ($n=29$ for WT saline, WT 7-OH-DPAT, WT PD 128907 and mutant PD 128907; $n=28$ for mutant saline and mutant 7-OH-DPAT) (single factor ANOVA $F_{2,85}=1.076$, $p=0.3455$ for WT, $F_{2,84}=3.675$, $p=0.0296$ for mutants). *Post hoc* analyses revealed no significant locomotor suppression or activation for either drug in WT or D3 receptor mutant mice ($p>0.05$, Dunnett's test). Bars represent mean ± SEM of total crossovers for each treatment group during the first 30 min after drug administration.

DISCUSSION

D3 Agonist Selectivity *in vivo* is Controversial

Progress in understanding D3 DA receptor function has long been hindered by the lack of suitably well-characterized D3 receptor agonists and antagonists. The selectivity of available D3 agonists is limited, and the usefulness of available compounds has been further hindered by questions surrounding the selectivity of these compounds *in vivo*. Early behavioral characterizations of 7-OH-DPAT and PD 128907, examining doses up to 10 mg/kg, demonstrated U-shaped dose–response curves for both compounds (Daly and Waddington, 1993; Ahlenius and Salmi, 1994; Pugsley *et al*, 1995), suggesting D3 receptor activation at low doses and increasing D2 receptor occupancy at higher doses. Estimates of *in vivo* D2 DA receptor occupancy across a range of 7-OH-DPAT doses, based upon D2 receptor protection from *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydro-quinilone (EEDQ) alkylation, suggest that 7-OH-DPAT doses below 0.3 mg/kg are devoid of significant D2 receptor occupancy (Levant *et al*, 1996). Similarly, recent studies demonstrated effects of low PD 128907 doses on DA release in WT, but not D3 receptor knockout mice, suggesting selective effects of PD 128907 through D3 receptor activation when given at sufficiently low dose, in the range of 0.03–0.1 mg/kg i.p. (Zapata *et al*, 2001). In contrast, evidence from several labs has suggested a lack of selectivity *in vivo,* at any dose,for both 7-OH-DPAT (Starr and Starr, 1995; Gonzalez and Sibley, 1995) and PD 128907 (Bristow *et al*, 1998). In

particular, studies in two different laboratories using DA receptor knockout mice suggested that neither 7-OH-DPAT nor PD 128907 inhibit locomotion through selective D3 receptor stimulation (Xu *et al*, 1999; Boulay *et al*, 1999b). Both laboratories observed a similar hypolocomotor response to PD 128907 or 7-OH-DPAT in WT and D3 receptor mutant mice , while the locomotor inhibitory effect was absent in D2 receptor mutant mice (Boulay *et al*, 1999a). Although all but one of these studies (Xu *et al*, 1999) employed 7-OH-DPAT doses at least 10-fold higher than the 10 μg/kg dose inhibitory to rodent locomotion in other investigations (Daly and Waddington, 1993), these results were interpreted by both laboratories as suggesting that locomotor inhibitory effects of 7-OH-DPAT, previously thought to result from D3 activation (Svensson *et al*, 1994), are mediated through D2 autoreceptors or other receptors.

Novelty-stimulated Locomotion may Account for Divergent Findings

The influence of novelty on rodent locomotion (Figure 2) might account for the diverse observations in the literature regarding the effect of D3 receptor stimulation on locomotion. Recent studies in which D3 agonists produced similar hypolocomotor responses in WT and D3 mutant mice evaluated locomotion in mice habituated to the testing cage by either repeated injection (Boulay *et al*, 1999b) or habituation period (Xu *et al*, 1999). In contrast, earlier studies describing the inhibitory influence of D3 receptor on locomotion (Xu *et al*, 1997), as well as studies describing the effects of D3 antisense oligonucleotides on locomotion reaching a similar conclusion (Menalled *et al*, 1999; Ekman *et al*, 1998), evaluated D3 modulation of exploratory behavior in non-habituated animals. Unique mechanisms modulating novelty-induced exploratory locomotion have previously been described (Hooks and Kalivas, 1995). As we have previously suggested (Richtand *et al*, 2001b), these observations suggest that locomotor inhibitory effects of selective D3 agonists are most readily detected in a novel, nonacclimated environment.

Data Suggest a Novelty-D3 DA Receptor Interaction

Our findings demonstrate the environmental dependence of discernible behavioral effects resulting from D3 receptor stimulation. We observe locomotor inhibitory effects of PD 128907 and 7-OH-DPAT mediated via D3 DA receptor activation in animals that are naïve to the behavioral testing chamber. This effect was not detectable in acclimated mice in our study. While there is a trend toward increased locomotion in acclimated D3 knockout mice receiving PD 128907, *post hoc* analysis revealed that this effect was not significant. PD 128907 agonist effects at postsynaptic D2 receptors, in mice lacking functional D3 receptor, could account for the observed result.

Similar to our findings, studies of mice acclimated to the testing environment either by repeated injection (Boulay *et al*, 1999b) or habituation period (Xu *et al*, 1999) observed no locomotor inhibitory effect of selective D3 receptor stimulation. The failure to detect D3-mediated locomotor inhibition in acclimated mice might result from the difficulty in detecting a decrease in an already low value. This possibility is unlikely, however, in light of the fact that we routinely observe a 30–40% inhibition of locomotion in both acclimated D3 knockout and acclimated WT mice with high 7-OH-DPAT and PD 128907 doses (data not shown), comparable to the robust locomotor inhibition previously reported by others testing acclimated mice with similarly high-dose 7-OH-DPAT and PD 128907 administration (Xu *et al*, 1999; Boulay *et al*, 1999b). Thus, other mechanisms, likely reflecting modulation of dopaminergic function through cortical input, could account for these findings.

The environmental context of D3 receptor-mediated behavioral effects may have particular relevance to DA-mediated influence of drug-dependent behaviors. Rodent locomotor response to a novel environment predicts the propensity to self-administer a variety of drugs of abuse (Piazza *et al*, 1990; Grimm and See, 1997; Klebaur *et al*, 2001; Suto *et al*, 2001). Sensitization of the locomotor response to amphetamine, cocaine, and morphine is more robust when drug is administered in a novel environment than when it is administered in the home cage (Badiani *et al*, 1995, 2000; Fraioli *et al*, 1999). Response to novelty also predicts sensitivity to the locomotor effects of, and behavioral sensitization to, psychostimulants (Hooks *et al*, 1991, 1992). It has been theorized that behavioral sensitization may underlie the development of drug craving, and thus initiate the addictive behaviors seen in drug dependence (Robinson and Berridge, 1993). Evidence suggests that downregulation of D3 receptor function may contribute to sensitization to stimulant drugs of abuse (Richtand *et al*, 2000, 2001a, b).

A possible role for the D3 receptor in the reinforcing properties of drugs of abuse is further suggested by the finding that 7-OH-DPAT, given in nonreinforcing doses, decreases cocaine self-administration (Caine and Koob, 1993; Parsons *et al*, 1996) and prevents expression of morphine (Rodriguez *et al*, 1995) and amphetamine (Khroyan *et al*, 1998) conditioned place preference. This link is further enhanced by studies demonstrating attenuation of the discriminative stimulus properties of cocaine and amphetamine by the partial D3 receptor agonist BP 897 (Beardsley *et al*, 2001). Of particular interest, BP 897 inhibits cocaine-seeking behavior, which is specifically dependent upon the presentation of environmental drug-associated stimuli (Pilla *et al*, 1999). These observations suggest a possible unmasking of a D3 receptor population in a novel environment, allowing recruitment for activation by reward-related pathways. Further study is needed to test this speculative hypothesis.

As described in Figure 2, we did not observe a significant locomotor increase in non-acclimated D3 knockout mice relative to WT mice over the 60-min observation period. Two separate labs have observed increased exploratory locomotion in D3 knockout mice measured during the first 5 (Xu *et al*, 1997) or 15 min (Accili *et al*, 1996) of exposure to a novel environment. It has been suggested that loss of D3 receptors in the islands of Calleja and olfactory tubercle in D3 knockout mice may affect exploratory behaviors (Xu *et al*, 1997). Two other groups, however, failed to observe a difference in locomotor response to a novel environment between D3 knockout and WT mice (Betancur *et al*, 2001; Waddington *et al*, 2001). The variability in D3 knockout mouse locomotor response to novel environments suggests

that additional, as yet uncharacterized, factors, such as interaction with specific environmental conditions, contributions of genetic background, competing effects of D3 receptor loss on different neural pathways, and/or developmental accommodation(s) to loss of D3 receptor function, are likely to exert important interactions in modulating the measured behaviors. Since these studies utilized lines of D3 receptor knockout mice generated by different strategies in two different labs, differences in gene targeting strategies and interlaboratory variability in behavioral testing procedures may also contribute significantly to these discrepancies. This variability of the D3 knockout behavioral response to novelty does not in any way, however, argue against our conclusions from the observed behavioral response to pharmacological treatment. The data presented demonstrate inhibition of locomotion by a D3 agonist in mice with a functional D3 receptor. This same treatment is without measurable effect, even comparing large group sizes ($n = 24$–29 mice/group), in mice lacking a functional D3 receptor. Further study is needed to more completely characterize additional specific factors influencing locomotor behavioral response in D3 receptor knockout mice.

We used mice with a mixed genetic background in our study. Future work with congenic strains of mice will help to clarify the contributions of genetic background to novelty-induced D3 receptor activation. Nevertheless, both the large number of mice studied and the clear differences between D3 mutant mice and WT mice in locomotor responses to low doses of both 7-OH-DPAT and PD 128907 suggest that the D3 receptor is activated by these drugs in a novel environment, and that this finding is unlikely to be due to an effect of the genetic background.

Another possible explanation for our findings may be through the action of D3 agonists at D2/D3 DA receptor heterodimers, which likely have a higher affinity for 7-OH-DPAT than D2 receptor homodimers (Scarselli *et al*, 2001). While evidence suggests that in most rodent brain regions D3 and D2 receptors are not colocalized within the same neuronal populations, a limited subset of structures in which colocalization could occur has been described (Bouthenet *et al*, 1991; Larson and Ariano, 1995; Le Moine and Bloch, 1996; Khan *et al*, 1998).

Novelty and DA Release

Among the possible explanations for our findings, DA release in at least one brain region expressing D3 receptor may *decrease* in response to a novel environment. The D3 receptor's high affinity for DA predicts that at resting DA concentrations, the D3 receptor is occupied to an appreciable extent (Sokoloff *et al*, 1992; Richtand *et al*, 2001b; Strange, 2001). Thus, in an acclimated animal, where DA concentrations may be at resting levels, it is less likely that a D3 agonist would have a demonstrable behavioral effect. While DA release increases in the prefrontal cortex, nucleus accumbens, and striatum in response to novelty (Feenstra *et al*, 1995; Feenstra and Botterblom, 1996; Berridge *et al*, 1999; Zapata *et al*, 2001), increased DA release in at least one brain region may be transient relative to the time of exposure to D3 receptor agonist and our period of behavioral observation. Additionally, increased DA release may not be uniform, sparing at least some region expressing D3 receptor. A recent study of the effect of novelty on accumbens DA release determined that DA release increased transiently, occurring only during the brief period of entry into a novel environment, and increased only in the accumbal shell and shell–core transition zone, but not in the accumbal core or overlying neostriatal regions (Rebec, 1998). Alternatively, projections from regions of increased DA release (eg prefrontal cortex) might inhibit DA release in another brain region expressing D3 receptor, such as islands of Calleja. Thus, several hypothetical models could allow D3 agonists to exert an inhibitory effect under conditions of decreased DA release (novelty), while agonist treatment would be without measurable effect upon receptors closer to saturation (acclimation).

Studies examining prefrontal cortical and accumbens DA release in both habituated and nonhabituated animals may allow for elucidation of the mechanisms responsible for the appearance of selective effects of D3 receptor agonists in novel environments. Characterization of antagonists selective for the D3 receptor, and more detailed characterization of the exploratory behavior of the D3 mutant mouse, may shed further light on the potential role for the D3 receptor in novelty-stimulated locomotion.

CONCLUSIONS

We demonstrate inhibition of novelty-stimulated locomotion by 7-OH-DPAT and PD 128907 doses below 10 μg/kg in WT, but not in D3 receptor knockout mice. Based on these findings, we conclude that D3 receptor stimulation inhibits novelty-stimulated locomotion, clarifying a behavioral effect mediated by the D3 DA receptor. We suggest D3-selective doses of both 7-OH-DPAT and PD 128907, which may allow further clarification of the behavioral effects of D3 receptor stimulation. Decreased DA release under novel environmental conditions in a brain region expressing D3 DA receptors provides one potential mechanism for the observed results. Further study is needed to test this suggestion.

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