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Dopamine Receptor Mediation of the Exploratory/ Hyperactivity Effects of Modafinil

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Modafinil (2-((diphenylmethyl)sulfinyl)acetamide) is described as an atypical stimulant and is a putative cognition enhancer for schizophrenia, but the precise mechanisms of action remain unclear. Receptor knockout (KO) mice offer an opportunity to identify receptors that contribute to a drug-induced effect. Here we examined the effects of modafinil on exploration in C57BL/6J mice, in dopamine drd1, drd2, drd3, and drd4 wild-type (WT), heterozygous (HT), and KO mice, and in 129/SJ mice pretreated with the drd1 antagonist SCH23390 using a cross-species test paradigm based on the behavioral pattern monitor. Modafinil increased activity, specific exploration (rearing), and the smoothness of locomotor paths (reduced spatial d) in C57BL/6J and 129/SJ mice (increased holepoking was also observed in these mice). These behavioral profiles are similar to that produced by the dopamine transporter inhibitor GBR12909. Modafinil was ineffective at increasing activity in male drd1 KOs, rearing in female drd1 KOs, or reducing spatial d in all drd1 KOs, but produced similar effects in drd1 WT and HT mice as in C57BL/6J mice. Neither dopamine drd2 nor drd3 mutants attenuated modafinil-induced effects. Drd4 mutants exhibited a genotype dose-dependent attenuation of modafinil-induced increases in specific exploration. Furthermore, the drd1 KO effects were largely supported by the SCH23390 study. Thus, the dopamine drd1 receptor appears to exert a primary role in modafinil-induced effects on spontaneous exploration, whereas the dopamine drd4 receptor appears to be important for specific exploration. The modafinil-induced alterations in exploratory behavior may reflect increased synaptic dopamine and secondary actions mediated by dopamine drd1 and drd4 receptors.
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INTRODUCTION

The psychostimulant modafinil (2-((diphenylmethyl)sulfinyl)acetamide) is currently approved by the United States Food and Drug Administration as a schedule IV agent to treat excessive daytime sleepiness in narcolepsy, shift work sleep disorder, and obstructive sleep apnea/hypopnea syndrome (Minzenberg and Carter, 2008). Despite the increasing off-label use of modafinil, which includes studies assessing its use in the treatment of neuropsychiatric disorders such as schizophrenia (Minzenberg and Carter, 2008; Turner *et al*, 2004; Young *et al*, 2009b), its mechanism(s) of action remain unclear.

Many psychostimulants, such as amphetamine, methylphenidate, and GBR-12909, inhibit the dopamine transporter (DAT) leading to increased extracellular dopamine levels (Greenhill, 2006). Initial *in vitro* binding studies suggested that this mechanism may not be the case for modafinil as it exhibited only a weak binding potential for the DAT (Mignot *et al*, 1994). Investigations into the putative mechanism of action of modafinil have suggested contributions of norepinephrine (de Saint Hilaire *et al*, 2001), glutamate (Ferraro *et al*, 1997, 1998, 1999), γ-aminobutyric acid (Tanganelli *et al*, 1992, 1994), histamine (Scammell *et al*, 2000), and serotonin (Tanganelli *et al*, 1992) receptors.

Evidence from *in vivo* studies is increasing, however, for the role of the DAT in modafinil-induced stimulation. A positron emission tomography (PET) study in monkeys revealed that modafinil may exhibit significant binding ($\sim$50%) to the DAT in the striatum and norepinephrine transporter (NET) in the thalamus (Madras *et al*, 2006). More recently, a PET imaging study in man confirmed modafinil-induced inhibition of the DAT at therapeutic doses (Volkow *et al*, 2009). Measurements of dopamine levels in monkeys provide yet further support for this mechanism (Andersen *et al*, 2010). Moreover, DAT knockout (KO) mice are unresponsive to modafinil-induced wakefulness, in contrast with their wild-type (WT) littermates (Wisor *et al*, 2001), although dopamine D1 and D2 receptor (drd1 and drd2, respectively) downregulation

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in these mice (Fauchey *et al*, 2000; Jones *et al*, 1999) may have confounded the results (Qu *et al*, 2008). Dopamine drd1 and drd2 receptors may, however, be important for the downstream actions of DAT inhibition (Young, 2009; Zolkowska *et al*, 2009). More recently, it was observed that modafinil increases motivation for reward in mice, consistent with the selective DAT inhibitor GBR12909 (Young and Geyer, 2010) and with studies in man (Stoops *et al*, 2005). The effects of these two stimulants were, however, blunted in drd1 heterozygous (HT) mice (which have a 50% reduced expression of drd1). Given the limited binding of GBR12909 at drd1, it was suggested that the drd1 interaction was a downstream effect DAT inhibition (Young and Geyer, 2010).

Studies examining the interactive effects of modafinil with dopamine receptors have focused on the drd1 and drd2, with little attention paid to drd3 or drd4. We previously described the behavioral pattern monitor (BPM) as a useful method to differentiate the mechanisms of action of diverse stimulants, such as NMDA-mediated PCP, DAT/NET-mediated amphetamine or GBR12909, or muscarinic acetylcholine receptor-mediated scopolamine (Geyer *et al*, 1986; Perry *et al*, 2009; Young *et al*, 2010a, 2007b). The BPM provides multiple measures, which can be reduced to three principal factors—diversive exploration or the amount of activity, inspective exploration of specific stimuli, and spatiotemporal patterns of locomotion (Paulus and Geyer, 1993). Previously, we combined the use of these measures and multiple dopamine drd KO mutant mice (drd1, drd2, and drd3) to delineate the dopaminergic contributions to the behavioral effects of MDMA (Risbrough *et al*, 2006). Moreover, we have used the mouse BPM to differentiate the effects of the DAT/NET inhibitor amphetamine from those of the selective DAT inhibitor GBR12909 (Perry *et al*, 2009). Furthermore, we have described the availability of this paradigm for testing in man, using the same measures used in rodents to differentiate the exploratory patterns observed in patients with bipolar disorder and schizophrenia (Perry *et al*, 2009; Young *et al*, 2007b).

Here, we utilized the mouse BPM to understand the contribution of various dopamine receptors to the underlying neurobiology of modafinil-induced hyperactivity. We examined the effects of modafinil on the spontaneous exploration of C57BL/6J mice drd1, drd2, drd3, and drd4 WT, HT, and KO littermate mice backcrossed for at least 10 generations to a C57BL/6J background, as well as in 129/SJ mice pretreated with the drd1 antagonist SCH23390 (Simon *et al*, 1995; McNamara *et al*, 2003). We have included HT mice in these studies given the utility of gene dosage effects in delineating receptors that mediate a variety of behaviors (Young *et al*, 2007a; Young and Geyer, 2010).

METHODS

Animals

To assess the dose–response effects of modafinil, male C57BL/6J mice (20–30 g) were obtained from Jackson laboratories and tested at approximately 4 months of age. To assess the effects of the drd1 antagonist SCH23390 pretreatment on modafinil-induced alterations in exploration, male 129/SJ mice (20–30 g) were obtained from Jackson laboratories and tested at approximately 3 months of age. Drd1, drd2, drd3, and drd4 WT, HT, and KO mice (constitutive gene deletion background mice) were used in the remaining experiments. The drd2 mice (B6.129S2-Drd2tm1Low/J) and drd4 mice were originally generated at the Oregon Health and Science University, backcrossed onto the C57BL/6J background strain for 17 and 24 generations, respectively, and genotyped as described (Kelly *et al*, 1998; Rubinstein *et al*, 1997). Stocks of drd1 mice (B6.129S4-Drd1atm1Lcd/J; Drago *et al*, 1994) and D3 mice (B6.129S4-Drd3tm1Dac/J; Accili *et al*, 1996) were obtained from the mutant mouse repository at the Jackson Laboratory (Bar Harbor, ME), backcrossed onto the C57BL/6J background for 10–12 generations, and genotyped as described (Ralph-Williams *et al*, 2002). Study mice were bred using HT pairs and housed at the University of California San Diego (UCSD) vivarium, where they were kept in a climate-controlled, reversed light-cycle environment (lights on at 2000 h and off at 0800 h). Male and female mice were housed separately ($n = 1$–4 per cage), with food (Harlan Teklab, Madison, WI) and water being available *ad libitum*, except during behavioral testing. Procedures were approved by the UCSD Institutional Animal Care and Use Committee and conformed to NIH Guidelines. Testing began at approximately 3 months of age, with mice weighing between 20 and 30 g. Mice were brought to the laboratory 60 min before testing during their dark cycle between 0900 and 1700 h.

Drug Treatment

Modafinil and SCH23390 were purchased from Sigma (St Louis, MO). Modafinil was suspended with 1% methylcellulose and 5% Tween by sonicating for 1 h at 50 °C. The injection volume of modafinil (intraperitoneal injection) was 10 ml/kg immediately before testing. The injection volume of SCH23390 (subcutaneous injection) was 5 ml/kg 20 min before testing.

Mouse BPM

Spontaneous locomotor and exploratory behavior was examined in 10 mouse BPM chambers as described previously (Halberstadt *et al*, 2009; Risbrough *et al*, 2006). Each chamber is illuminated from a single light source above the arena (producing 350 lx in the center, and 92 lx in the four corners). The arena consisted of a $30.5 \times 61 \times 38\,cm^3$ area with a Plexiglas hole board floor equipped with three floor holes and eight wall holes (Young *et al*, 2010b). Nose poking behavior was detected using an infrared photobeam. The location of the mouse was recorded every 0.1 s using a grid of 12×24 infrared photobeams located 1 cm above the floor recorded. The position of the mouse was defined across nine unequal regions (Flicker and Geyer, 1982; Young *et al*, 2010c). Rearing behavior was recorded using an array of 16 infrared photobeams 2.5 cm above the floor aligned with the long axis of the chamber.

Exploratory Assessment

A between-subject dose response of modafinil (32, 64, and 128 mg/kg) compared with vehicle and saline was first conducted in C57BL/6J mice ($n = 8$ per group). C57BL/6J mice were used because the dopamine mutant mice to be tested were backcrossed onto this strain. These doses were chosen based on previous publications showing wake-inducing and motivation-increasing effects (Wisor *et al*, 2001; Young and Geyer, 2010). Vehicle and modafinil (32 mg/kg) were used for all subsequent crossover studies in dopamine mutant mice (see Table 1 for sample sizes). Finally, we assessed whether the drd1 antagonist SCH23390 (1.5, 5, or 15 μg/kg) would attenuate the effects of modafinil (32 mg/kg) in 129/SJ mice. These doses were chosen based on previous publications showing that 7.5 and 25 μg/kg SCH23390 reduced activity alone in mice (Simon *et al*, 1995; McNamara *et al*, 2003), and to avoid any drd5 activity (McNamara *et al*, 2003). All mice were BPM naïve before testing. At the start of each test session, mice were placed in the bottom left-hand corner of the chamber, facing the corner, and the test session started immediately.

Numerous measures are recorded in the BPM, and three independent factors of activity, specific exploration, and locomotor patterns have been observed (Paulus and Geyer, 1993). Thus, the primary measures of interest were transitions (activity), holepoking and rearing (specific exploration), plus the spatial scaling exponent 'd' and the spatial coefficient of variation (CV) (locomotor patterns). Spatial d quantifies the geometrical structure of the locomotor path, where a value of 2 represents highly circumscribed localized movement and 1 represents straight-line distance-covering movements (Paulus and Geyer, 1991). Spatial CV represents the variation of transitions within the nine-region transition matrix (with 40 permissible transitions). Repetition of certain transitions between the nine regions of the chamber increases the spatial CV value and reflects a more consistent or perseverative pattern of locomotion (Geyer *et al*, 1986). These two measures are useful to compare given the different patterns that they produce and that they can be differentially affected by psychostimulants. For example, amphetamine decreases spatial d and spatial CV, whereas MDMA and scopolamine decrease spatial d, but increase spatial CV (Geyer *et al*, 1986; Perry *et al*, 2009; Risbrough *et al*, 2006; Young *et al*, 2007b).

Table 1 Sample Sizes of Dopamine Mutant Mice by Sex and by Gene Dose

Dopamine receptor	Male			Female		
	WT	HT	KO	WT	HT	KO
D1	19	20	6	10	10	5
D2	9	5	5	11	10	10
D3	1	4	6	6	7	11
D4	7	15	11	10	3	13

Abbreviations: WT = wild type, 100%; HT = heterozygous; 50%, KO = knockout, 0%.

Statistics

The variables from each experiment were analyzed using an analysis of variance. Treatment was a between-subjects factor for the C57BL/6J study, performance was binned into three 20-min time periods and analyzed as a within-subjects factor. For the crossover mutant studies, treatment was a within-subjects factor, genotype and sex as between-subjects factors, with performance analyzed over the entire 60 min. Sex was not included as a factor in drd3 mutant mice given the limited number of male WT mice. For the SCH23390 study, pretreatment and treatment (modafinil) were between-subjects factor. Alpha level was set to 0.05. Significant main effects were analyzed using Tukey's *post hoc* analyses. The data were analyzed using the Biomedical Data Programs statistical software (Statistical Solutions, Saugus, MA).

RESULTS

Effects of Modafinil on Spontaneous Exploration in C57Bl/6J Mice

Activity. Modafinil increased transitions ($F(4,32) = 37.2$, $p < 0.0001$; Figure 1a). Tukey's *post hoc* analyses confirmed that all three doses induced significantly higher transitions compared with vehicle and saline, whereas 128 mg/kg was also significantly higher than 32 mg/kg ($p < 0.05$). Vehicle- and saline-treated mice did not differ ($p > 0.1$).

Specific exploration. Modafinil did not affect holepoking ($F(4,32) = 1.8$, NS; Figure 1b), but did increase rearing ($F(4,32) = 6.7$, $p < 0.0005$; Figure 1c). Tukey's *post hoc* analyses revealed that all three doses of modafinil significantly increased rearing compared with both vehicle and saline ($p < 0.05$), whereas vehicle- and saline-treated mice did not differ ($p > 0.1$).

Locomotor pattern. Modafinil decreased spatial d ($F(4,32) = 6.2$, $p < 0.001$; Figure 1d) and increased spatial CV ($F(4,32) = 3.44$, $p < 0.05$; Figure 1e). *Post hoc* analyses revealed that modafinil at 64 and 128 mg/kg significantly lowered spatial d compared with vehicle- and saline-treated mice ($p < 0.05$). Only 128 mg/kg modafinil increased spatial CV ($p < 0.05$). Vehicle- and saline-treated mice did not differ on any measure ($p > 0.1$).

Effects of D1, D2, D3, and D4 Receptor Gene Deletion on Modafinil-Induced Alterations in Activity

D1 mice. Modafinil increased activity as measured by transitions ($F(1,64) = 117$, $p < 0.0001$; Figure 2a). This modafinil-induced increase in transitions was not observed for every group; however, because no effect was observed in male KO mice ($F(2,64) = 4.6$, $p < 0.05$; Figure 2b). *Post hoc* analyses confirmed that a modafinil-induced increase in transitions was observed for every genotype and sex ($p < 0.05$), except drd1 male KO mice ($p > 0.1$).

D2 mice. Modafinil increased transitions, irrespective of genotype ($F(1,44) = 235$, $p < 0.0001$; Figure 2c). A main effect of genotype was also observed ($F(2,44) = 3.4$,

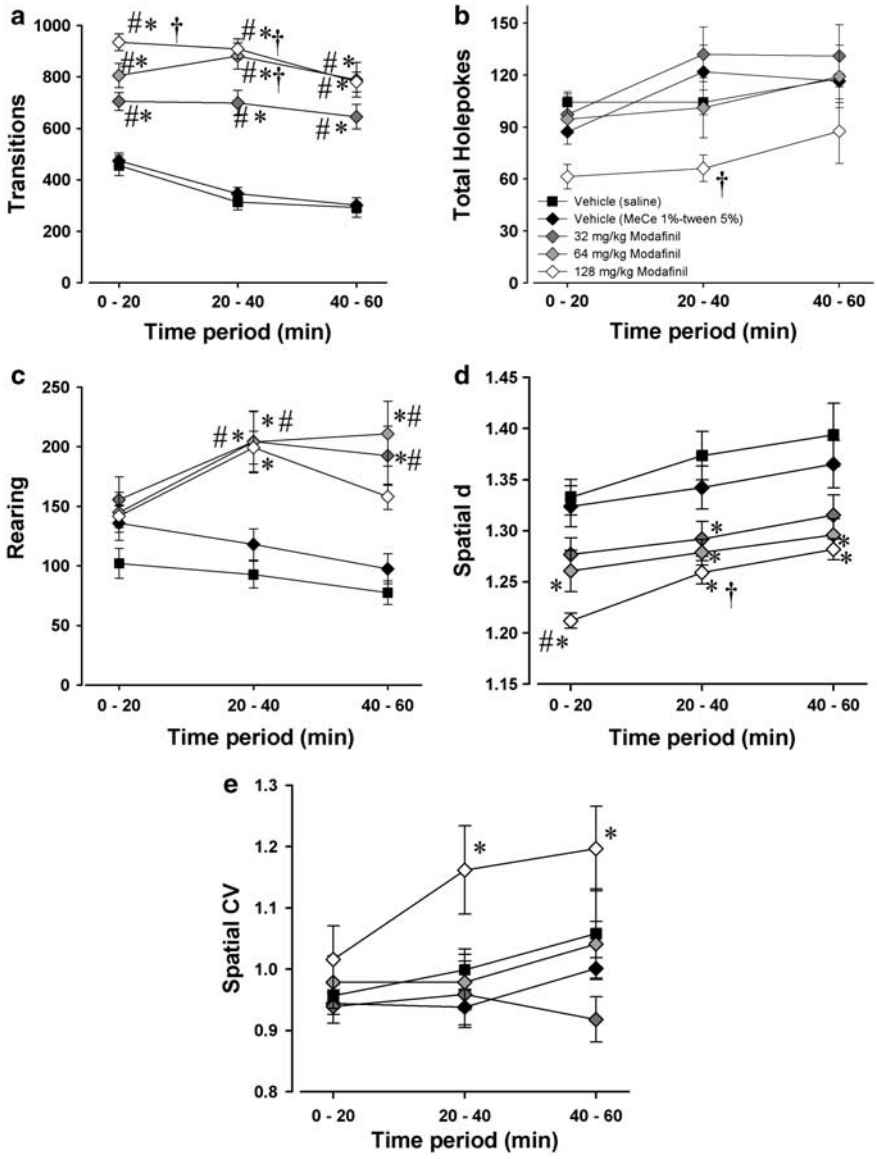

Figure 1 Dose–response of modafinil-induced alterations in spontaneous exploration. The effects of modafinil (32, 64, and 128 mg/kg) on exploration were compared with both vehicle- and saline-treated mice in the Behavioral Pattern Monitor across a 60 min period binned into three 20 min intervals. Modafinil increased activity as measured by transitions (a) at all three doses. Although modafinil did not affect holepoking, except for a nonsignificant reduction by 128 mg/kg, (b) a robust modafinil-induced increase in specific exploration as measured by rearing in the last two time bins was observed for every dose (c). Finally, mice treated with modafinil exhibited a more linear pattern of movement compared with control mice as measured by spatial d (d), whereas the increased predictability in path patterns (increased spatial CV) was only observed at the highest dose (e). Vehicle- and saline-treated mice did not differ in any measure, supporting the conclusion that modafinil affects multiple aspects of spontaneous exploration. Data presented as mean ± SEM. $^{*}p<0.05$ when compared with vehicle, $^{\#}p<0.05$ when compared with saline, and $^{\dagger}p<0.05$ when compared with 32 mg/kg modafinil.

$p<0.05$). No genotype × drug interactions or main effect of sex were observed however ($F<1.1$, NS). *Post hoc* analyses revealed that WT mice exhibited higher transitions than KO mice ($p<0.05$), whereas WT and KO mice did not differ from HT mice ($p>0.1$).

D3 mice. Modafinil administration significantly increased transitions ($F(1,32)=150$, $p<0.0001$; Figure 2d), irrespective of genotype ($F<1$, NS), nor was there a drug × genotype interaction for any measure ($F<1$, NS).

D4 mice. Administration of modafinil significantly increased transitions ($F(1,28)=70.7$, $p<0.0001$; Figure 2e). No significant drug × genotype interaction was observed for transitions ($F(2,28)=2.1$, $p=0.14$). No main effects of sex were observed for any measure ($F<1$, NS).

Effects of D1, D2, D3, and D4 Receptor Gene Deletion on Modafinil-Induced Alterations in Specific Exploration

D1 mice. Modafinil administration increased total holepokes ($F(1,64)=5.3$, $p<0.05$; Figure 3a and b) and rearing ($F(1,64)=24.4$, $p<0.0001$; Figure 4a and b), irrespective of sex or genotype as no interactions were observed ($F<1$, NS). Main effects of genotype were observed for total holepoking ($F(2,64)=27.7$, $p<0.0001$), and rearing ($F(2,64)=5.0$, $p<0.001$). *Post hoc* analyses revealed that drd1 KO mice exhibited fewer holepokes and rearings than

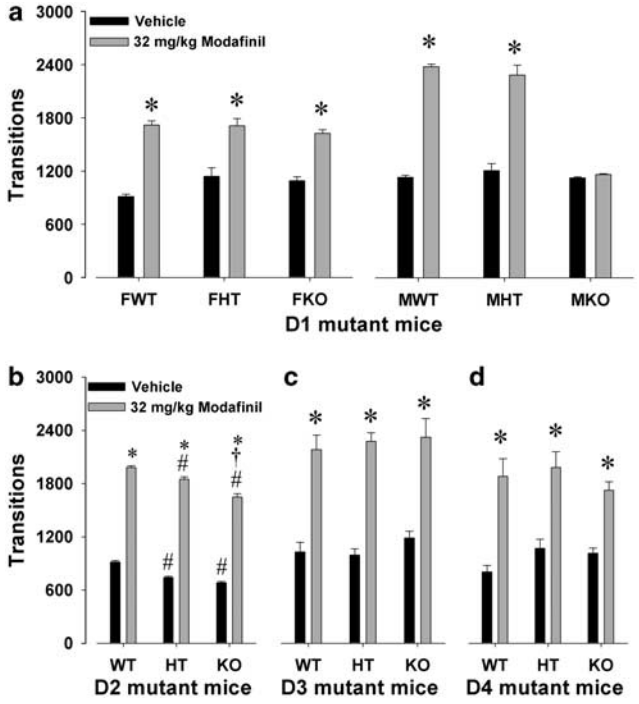

Figure 2 Modafinil-induced effects on activity in dopamine receptor mutant mice. The effects of modafinil (32 mg/kg) on activity in D1, D2, D3, and D4 wild-type (WT), heterozygous (HT), and knockout (KO) mice were compared with vehicle treatment. Modafinil increased activity in all drd1 mutant mice, except male drd1 KO mice, with no effect of drd1 mutation on activity (a). Modafinil increased activity in drd2 mutant mice, irrespective of genotype, with drd2 HT and KO mice exhibiting less activity compared with WT mice under both vehicle and modafinil administration (b). Modafinil increased activity in drd3 (c) and drd4 (d) mutant mice, irrespective of genotype. Data were presented as mean ± SEM. $^{*}p<0.05$ when compared with corresponding vehicle-treated mice, $^{\#}p<0.05$ when compared with WT mice, and $^{\dagger}p<0.05$ when compared with HT mice.

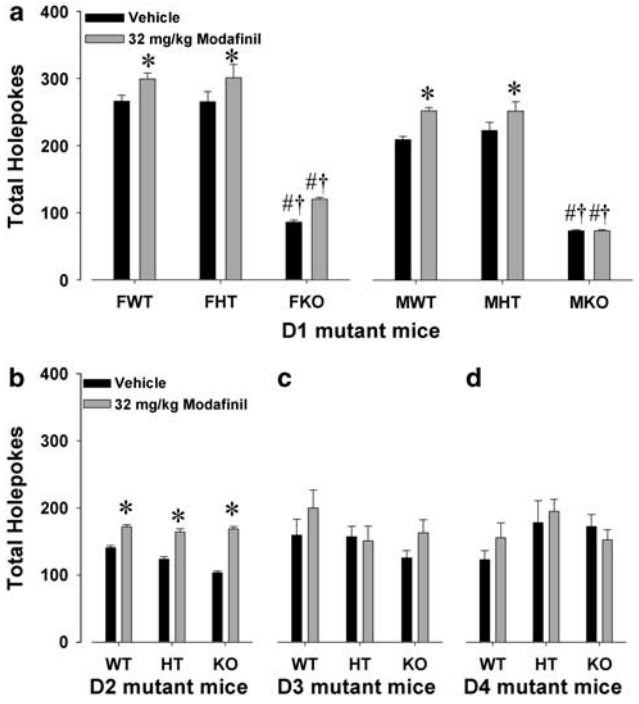

Figure 3 Modafinil-induced effects on specific exploration (holepoking) in dopamine receptor mutant mice. The effects of modafinil (32 mg/kg) on holepoking in D1, D2, D3, and D4 wild-type (WT), heterozygous (HT), and knockout (KO) mice were compared with vehicle treatment. Modafinil increased total holepokes in WT and HT, but not KO drd1 mutant mice, irrespective of sex. (a) Drd1 KO mice exhibited fewer holepokes when compared with WT and HT mice. (b) Modafinil increased holepoking in drd2 mutant mice, (c) but not significantly in drd3 and (d) not at all in drd4 mutant mice. Data presented as mean ± SEM. $^{*}p<0.05$ when compared with corresponding vehicle-treated mice, $^{\#}p<0.05$ when compared with WT mice, and $^{\dagger}p<0.05$ when compared with HT mice.

WT or HT mice ($p<0.05$), whereas WT and HT mice did not differ ($p>0.05$), irrespective of drug administration. Female mice exhibited more total holepoking ($F(1,64)=5.1$, $p<0.05$; Figure 3a) when compared with male mice, whereas male mice exhibited a trend toward more rearing ($F(1,64)=3.3$, $p=0.076$; Figure 3b).

D2 mice. Modafinil increased total holepoking ($F(1,44)=29.8$, $p<0.0001$; Figure 3c), irrespective of genotype ($F<1.3$, NS). No effect of genotype was observed for holepoking ($F<1.1$, NS). For rearing, significant main effects of genotype ($F(2,44)=5.5$, $p<0.01$), and drug ($F(1,44)=90$, $p<0.0001$), were observed as was a genotype × drug interaction ($F(2,44)=3.7$, $p<0.05$; Figure 4c). Tukey's *post hoc* analyses revealed that vehicle-administered KO mice exhibited less rearing than both WT and HT mice ($p<0.05$). Modafinil increased rearing with WT and KO mice no longer differing ($p>0.05$), whereas HT exhibited more rearing compared with KO mice ($p<0.05$), and a trend to greater rearing compared with WT mice ($p<0.1$).

D3 mice. There was a trend toward modafinil-induced increase in total holepoking ($F(1,32)=3.4$, $p=0.07$; Figure 3d). No effect of genotype ($F<1$, NS) or genotype × drug interaction ($F<1.2$, NS) was observed. Modafinil significantly increased rearing ($F(1,32)=17.7$, $p<0.0005$; Figure 4d). Although there was a trend toward a genotype effect on rearing ($F(2,32)=2.8$, $p=0.078$), with KO and HT mice exhibiting higher values compared with WT mice, *post hoc* analyses revealed these differences were not significant ($p>0.05$). Moreover, there was no genotype × drug interaction on rearing ($F<1$, NS).

D4 mice. Modafinil administration did not affect total holepoking ($F<1$, NS; Figure 3e). Modafinil did increase rearing behavior ($F(1,28)=8.9$, $p<0.01$; Figure 4e). A drug × genotype interaction was observed for total holepoking ($F(2,28)=4.0$, $p<0.05$) and rearing ($F(2,28)=5.5$, $p<0.01$). Tukey's *post hoc* analyses for total holepoking and rearing revealed no significant effect of genotype within vehicle or drug treatment ($p>0.1$). Analyses of drug effect within genotype revealed that modafinil exhibited a genotype dose-dependent increase in rearing, as it increased rearing in WT mice ($p<0.005$), exhibited a trend toward increases in HT mice ($p<0.1$), and did not increase rearing in KO mice ($p>0.1$).

Effects of D1, D2, D3, and D4 Receptor Gene Deletion on Modafinil-Induced Alterations in Locomotor Patterns

D1 mice. Modafinil administration lowered spatial d ($F(1,64)=29.6$, $p<0.0001$) without affecting spatial CV

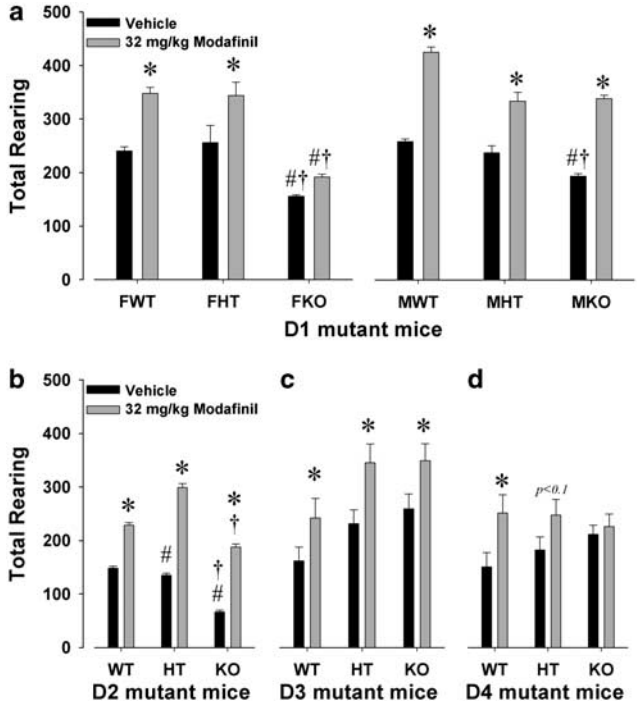

Figure 4 Modafinil-induced effects on specific exploration (rearing) in dopamine receptor mutant mice. The effects of modafinil (32 mg/kg) on rearing in D1, D2, D3, and D4 wild-type (WT), heterozygous (HT), and knockout (KO) mice were compared with vehicle treatment. Modafinil increased rearing in all drd1 mutant mice, except female drd1 KO mice. Drd1 KO mice exhibited reduced rearing compared with WT and HT mice (a). Modafinil increased rearing in drd2 mutant mice, irrespective of genotype. Vehicle-treated HT and KO mice exhibited reduced rearing compared with WT mice, whereas KO mice exhibited fewer rearings than HT mice under both vehicle and modafinil treatment (b). Modafinil increased rearing in drd3 mutant mice, irrespective of genotype (c). Modafinil increased rearing in a genotype-dependent manner in drd4 mutant mice, where increases were observed in WT mice, a trend observed for HT mice, but no modafinil-induced increase in rearing was observed in KO mice (d). Data presented as mean ± SEM. $^{*}p<0.05$ when compared with corresponding vehicle-treated mice, $^{\#}p<0.05$ when compared with WT mice, and $^{\dagger}p<0.05$ when compared with HT mice.

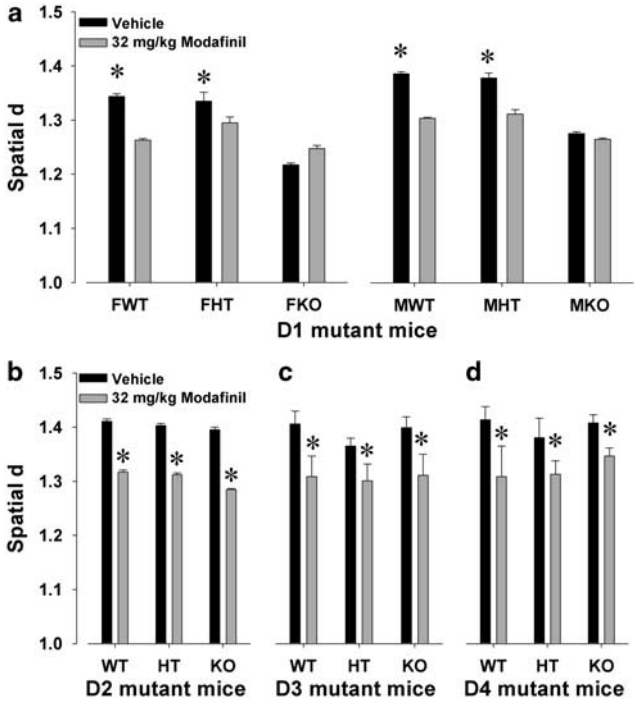

Figure 5 Modafinil-induced effects on linear movement (spatial d) in dopamine receptor mutant mice. The effects of modafinil (32 mg/kg) on spatial d in D1, D2, D3, and D4 wild-type (WT), heterozygous (HT), and knockout (KO) mice were compared with vehicle treatment. Modafinil reduced spatial d in drd1 WT and HT mice only, with no effect on KO mice. Drd1 KO mice exhibited reduced rearing compared with WT and HT mice (a). Modafinil reduced spatial d, irrespective of genotype in drd2 (b), drd3 (c), and drd4 (d) mutant mice. Data presented as mean ± SEM. $^{*}p<0.05$ when compared with corresponding vehicle-treated mice, $^{\#}p<0.05$ when compared with WT mice, and $^{\dagger}p<0.05$ when compared with HT mice.

($F<1$, NS). A drug × genotype interaction was observed for spatial d ($F(2,64)=10.2$, $p<0.0005$; Figure 5a and b), with *post hoc* analyses revealing that modafinil reduced spatial d in WT and HT ($p<0.05$), but not in KO mice ($p>0.05$). A drug × sex interaction was observed for spatial CV ($F(1,64)=11.2$, $p<0.005$; Figure 6a and b) with *post hoc* analyses revealing that modafinil reduced spatial CV in male, but not female mice. Female mice exhibited a lower spatial CV than male mice ($F(1,64)=8$, $p<0.01$).

D2 mice. No significant main effect of genotype was observed for spatial d or spatial CV ($F<1.3$, NS). Modafinil administration significantly lowered spatial d ($F(1,44)=51.3$, $p<0.0001$; Figure 5c) and spatial CV ($F(1,44)=17.1$, $p<0.0005$; Figure 6c). No significant genotype × drug interaction was observed for spatial d ($F<1.3$, NS) or spatial CV ($F(2,44)=2.5$, $p=0.095$). Female mice exhibited lower spatial d when compared with male mice ($F(1,44)=4.3$, $p<0.05$).

D3 mice. Modafinil administration significantly lowered spatial d ($F(1,32)=34.0$, $p<0.0001$; Figure 5d) and spatial CV ($F(1,32)=14.3$, $p<0.0001$; Figure 6d), with no effect of genotype or genotype × drug interaction observed for any measure ($F<1.1$, NS).

D4 mice. Modafinil administration significantly lowered spatial d ($F(1,28)=8.2$, $p<0.005$; Figure 5e), but did not affect spatial CV ($F<1.4$, NS; Figure 6e). No genotype × drug interaction was observed for spatial d or spatial CV ($F<1$, NS), nor was a sex effect observed for either measure ($F<1$, NS).

Effects of the Drd1 Antagonist SCH23390 Pretreatment on Modafinil-Induced Alterations in Exploration in 129/SJ Mice

Activity. Modafinil increased activity ($F(3,72)=47.4$, $p<0.0001$), whereas SCH23390 exhibited a dose-dependent reduction in activity ($F(3,72)=13.8$, $p<0.0001$). An interaction between these treatments was also observed ($F(3,72)=6.2$, $p<0.001$; Figure 7a). Tukey's *post hoc* analyses revealed that SCH23390 only decreased activity in vehicle-treated mice at the highest dose ($p<0.005$), whereas it attenuated modafinil-induced increases in activity at the two lowest doses compared with modafinil alone ($p<0.05$).

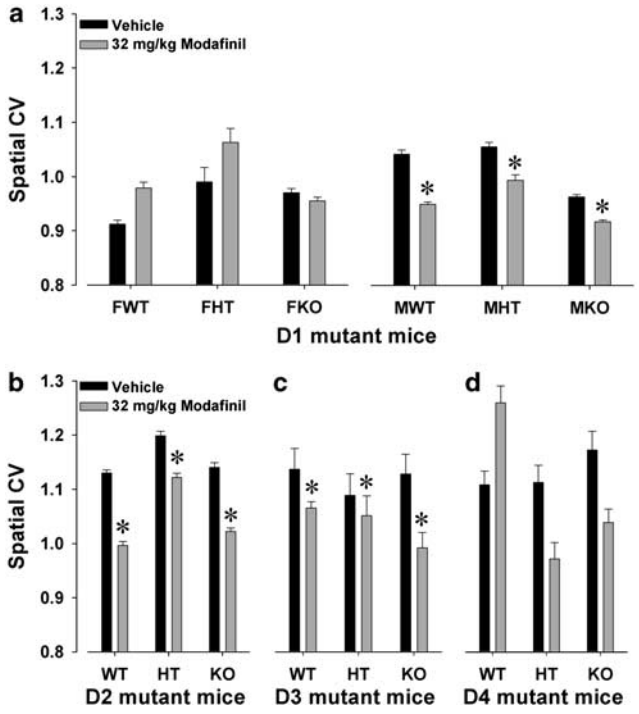

Figure 6 Modafinil-induced effects on predictability of locomotor pattern (spatial CV) in dopamine receptor mutant mice. The effects of modafinil (32 mg/kg) on spatial CV in D1, D2, D3, and D4 wild-type (WT), heterozygous (HT), and knockout (KO) mice were compared with vehicle treatment. Modafinil reduced spatial CV in male drd1 mutant mice, but not in female mice, irrespective of genotype (a). Modafinil reduced spatial CV in drd2 (b) and drd3 (c) mutant mice, irrespective of genotype, whereas no effect of modafinil was observed on drd4 mutant mice (d). Data presented as mean ± SEM. *$p<0.05$ when compared with corresponding vehicle-treated mice.

Specific exploration. Modafinil increased holepoking ($F(1,72)=23.4$, $p<0.0001$), whereas SCH23390 dose dependently decreased holepoking ($F(3,72)=9.0$, $p<0.0001$). No interaction between these treatments was observed ($F(3,72)=1.8$, $p=0.2$; Figure 7b). Tukey's *post hoc* analyses revealed that SCH23390 decreased holepoking compared with saline-treated mice at the highest dose only ($p<0.05$). Both the medium and high doses attenuated modafinil-induced increases in holepoking compared with modafinil-treated mice only ($p<0.05$). Modafinil also increased rearing in these mice ($F(1,72)=15.4$, $p<0.0005$), whereas SCH23390 alone decreased rearing ($F(3,72)=4.4$, $p<0.05$), and an interaction between the two treatments was observed ($F(3,72)=2.8$, $p<0.05$; Figure 7c). Tukey's *post hoc* analyses revealed that while the highest dose of SCH23390 reduced rearing, the medium and highest doses attenuated modafinil-induced increases in rearing compared with controls ($p<0.05$).

Locomotor patterns. For spatial d, main effects of modafinil ($F(1,72)=5.8$, $p<0.05$) and SCH23390 ($F(3,72)=9.5$, $p<0.0001$) were observed with no interaction ($F<1$, NS; Figure 7d). Although modafinil decreased spatial d, irrespective of SCH23390 dose, SCH23390 increased spatial d ($p<0.05$). In terms of spatial CV, a main effect of SCH23390 was observed ($F(3,72)=6.7$, $p<0.001$), but no effect of modafinil or interaction ($F<1$, NS; Figure 7e) was observed.

DISCUSSION

These studies revealed that modafinil affected several aspects of spontaneous exploration that were altered in dopamine receptor mutant mice (see Table 2 for summary). Modafinil not only increased activity in C57BL/6J and 129/SJ mice, but also increased specific exploration (rearing in the former, holepoking and rearing in the latter), and reduced spatial d. This profile of effects was largely unchanged in dopamine drd3 receptor-null mutant mice, but null mutation of the dopamine drd1, drd2, or drd4 receptors differentially altered this pattern. Modafinil-induced increases in activity were absent in male drd1 KO mice. Modafinil-induced increases in specific exploration were observed as increased holepoking and rearing in drd1 and drd2 mutant mice, with a trend effect in drd3 mutant mice, although modafinil-induced increases in holepoking were absent in drd1 KO mice and increases in rearing were absent in female drd1 KO mice. In support of these findings, the drd1 antagonist SCH23390 attenuated modafinil-induced increases in activity and holepoking, at doses that did not affect these behaviors alone. A gene–dose-related reduction of modafinil-induced increases in specific exploration was observed in dopamine drd4 mutant mice where increases were observed in WT mice, attenuated in HT mice, and absent in KO mice. Finally, modafinil-induced reductions in spatial d were absent in dopamine drd1 KO mice, but present in every other mutant line. Thus, while the full behavioral profile of modafinil-induced effects appears to require at least some drd1 expression, drd4 receptor expression levels more selectively modulate modafinil-induced increases in specific exploration in a gene–dosage-related manner.

Mutation of the dopamine drd2 and drd3 receptors altered the effects of modafinil on specific exploration only. The primary difference between the effects of modafinil on these mice and C57BL/6J mice was that increases in specific exploration were observed in terms of holepoking and rearing in the former (consistent with 129/SJ mice), but only rearing in the latter. The modafinil-induced increase in specific exploration was also expressed as increased holepoking and rearing in drd1 mutants, but only as rearing in drd4 mutants. It is interesting to note that previous work found that GBR12909 increased specific exploration in C57BL/6J mice as measured by rearing, but did not affect holepoking (Perry *et al*, 2009), which is the background strain for these mutant lines. When administered to 129 mice however, from whose stem cells these mutant mice were derived, GBR12909 increased specific exploration as measured by holepoking and rearing (Young *et al*, 2010b), consistent with drd1, drd2, drd3, and 129 mice in this study. The effects in drd4 mutant mice were most consistent with those in C57BL/6J mice likely because the drd4 mutant line had been backcrossed the most times onto that strain compared with drd1, drd2, and drd3 mutant lines. Therefore, the effects of increased holepoking observed in drd1, drd2, and drd3 mice may well reflect the remaining influence of their 129 background. Further evidence of background strain influence on the expression of modafinil effects arises from modafinil-induced reduction in spatial CV (ie reduced predictability of path patterns) in drd1 male, drd2, and drd3 mice, but not in C57BL/6J or drd4 mutant

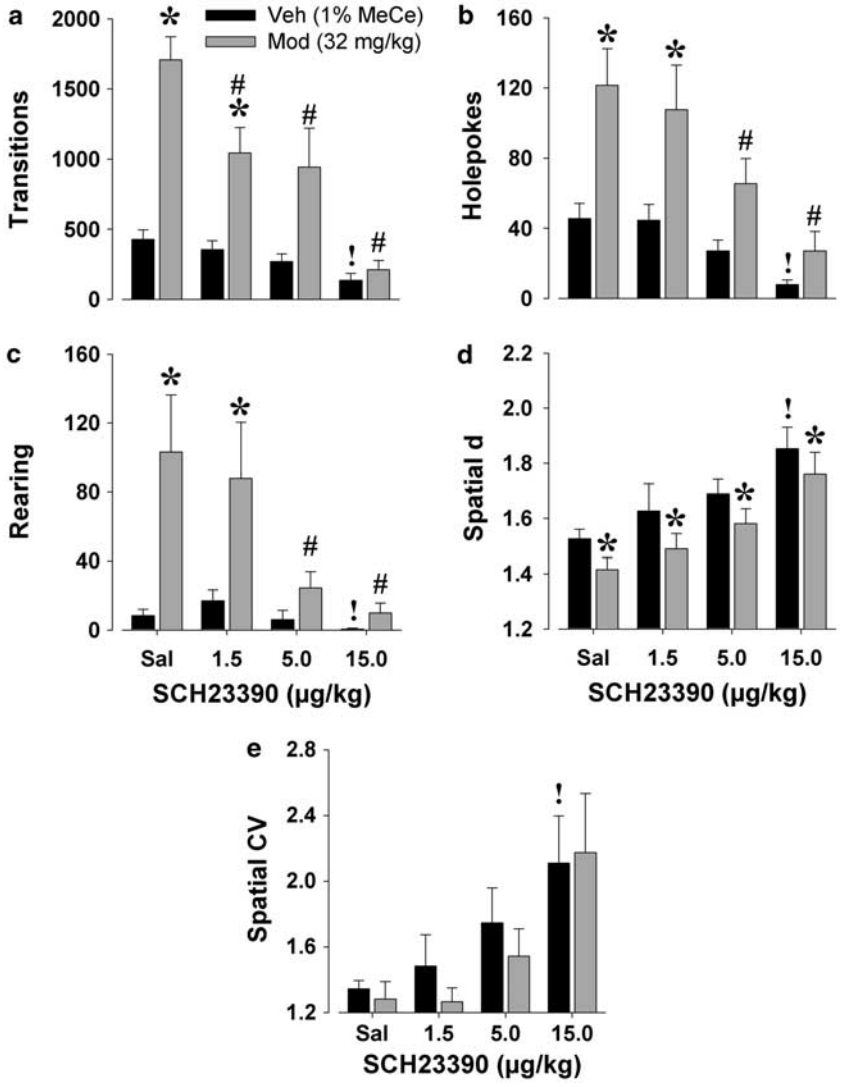

Figure 7 Effects of pretreatment of the D1 receptor antagonist SCH23390 on the modafinil-induced alteration in exploration in 129/SJ mice. The effects of pretreatment with saline or the D1 receptor antagonist SCH23390 (1.5, 5, and 15 µg/kg) on modafinil-induced (32 mg/kg) alterations in exploratory behavior in 129/SJ mice were assessed. Modafinil increased activity as measured by transitions that was attenuated by SCH23390 at 1.5 and 5 µg/kg. The highest dose of SCH23390 (15 µg/kg) not only reversed modafinil-induced hyperactivity, but also affected activity alone (a). This pattern was consistent for specific exploration (holepoking, b; and rearing, c), where the two middle doses attenuated modafinil-induced increases without affecting behavior alone, whereas the highest dose not only reversed the effects of modafinil, but also reduced specific exploration alone. Modafinil consistently induced linear movement (reduced spatial d), irrespective of SCH23390 dose, whereas at 15 µg/kg, SCH23390 increased meandering localized movement (increased spatial d, d). Modafinil was without affect on the predictability of movement from one region to another (no change in spatial CV), but SCH23390 made movements less predictable (increased spatial CV) at 15 µg/kg (e). Data presented as mean ± SEM. *Modafinil different from vehicle-treated mice within the same dose of SCH23390 ($p<0.05$), #Modafinil ± SCH23390 pretreatment differed from modafinil ± saline pretreatment ($p<0.05$), and !SCH23390 dose differed from vehicle + saline-pretreated mice ($p<0.05$).

mice at 32 mg/kg. Thus, it appears that the expression of modafinil-induced increased specific exploration appears to be strain dependent in a manner consistent with GBR12909 (Young *et al*, 2010a, b; Zolkowska *et al*, 2009). A higher dose of modafinil (128 mg/kg) increased spatial CV in C57BL/6J mice, thus the 32 mg/kg modafinil-induced reduction in spatial CV in drd1, drd2, and drd3 mutant mice may reflect a shift in the dose response curve affecting spatial CV in these mice. This effect is unlikely however, because 32 mg/kg did not reduce holepoking in these mice as was observed in C57BL/6J mice at 128 mg/kg. Future studies examining a dose–response curve in these mice could confirm this hypothesis. The consistency of strain-specific effects between modafinil and GBR 12909 support the hypothesis that these drugs have a similar mechanism of action, specifically DAT inhibition (Zolkowska *et al*, 2009).

Although dopamine drd2 receptor KO and HT mice exhibited altered rearing behavior when compared with WT littermates, modafinil affected every genotype consistently. Thus, despite altered DAT levels in dopamine drd2 receptor KO mice, DAT inhibition effects have proven normal in these mice in these studies as in previous reports (Dickinson *et al*, 1999). Dopamine drd3 receptor mutant and WT mice were also affected similarly by modafinil. Although previous work showed that the dopamine drd3 receptor agonist pramiprexole reduced specific exploration

Table 2 Summary of Effects of Modafinil (32 mg/kg) on Spontaneous Exploration in C57BL/6J, 129/SJ, and Dopamine Receptor Mutant Mice

	Gene	Activity	Exploration		Locomotor patterns	
			Pokes	Rears	Spatial d	Spatial CV
C57BL/6J	N/A	↑	NS	↑	↓	NS
129/SJ	N/A	↑[a]	↑[a]	↑[a]	↓	NS
D1	WT	↑	↑	↑	↓[b]	↓♂
	HT	↑	↑	↑	↓[b]	↓♂
	KO	↑♀	↑[b]	↑[b]♂	NS[b]	↓♂
D2	WT	↑[c]	↑	↑[b]	↓	↓
	HT	↑[c]	↑	↑[b]	↓	↓
	KO	↑[c]	↑	↑[b]	↓	↓
D3	WT	↑	↑	↑	↓	↓
	HT	↑	↑	↑	↓	↓
	KO	↑	↑	↑	↓	↓
D4	WT	↑	NS	↑	↓	NS
	HT	↑	NS	~↑	↓	NS
	KO	↑	NS	NS	↓	NS

Shading added to highlight consistency of C57BL/6J mice and the mutant line with the largest (24) number of backcrosses onto the C57BL/6J line.
NS denotes no significant effect of modafinil.
[a]Effect was attenuated by low doses of SCH23390 (1.5–5 μg/kg).
[b]KO mice exhibited lower scores cf. HT and WT mice during vehicle administration.
[c]WT mice exhibited higher scores cf. KO mice during vehicle administration.
♂Effect occurred in male mice only.
♀Effect occurred in female mice only.

in mice (Chang *et al*, 2010), the present data support a lack of influence of the drd3 receptor on the effects of modafinil. Thus, the mutations that primarily modulated the effects of modafinil on exploratory behavior were from the dopamine drd1 and drd4 receptors.

The degree to which the dopamine drd1 receptor is necessary for stimulant-induced hyperactivity likely depends on the primary mechanism of action of the stimulant. Cocaine and amphetamine, which exhibit primary effects via inhibition of the DAT and NET, do not increase activity in drd1 KO mice (Crawford *et al*, 1997; Miner *et al*, 1995), although conflicting reports exist (McDougall *et al*, 2005). In these studies, modafinil did not increase the activity in male dopamine drd1 receptor KO mice, nor rearing in female dopamine drd1 receptor KO mice. The influence of sex on the effects of stimulants on mutant mice is not always reported, clouding the reasons for sex differences in these studies. Sex can alter several gene-related behaviors including DAT-mediated changes in sexual behavior or dopamine drd1 influences on learning (Bay-Richter *et al*, 2009; Guo *et al*, 2007). One possible mechanism for sex-related differences in the effect of modafinil is that females exhibit more DAT compared to males (Harrod *et al*, 2004). This sex difference was observed in rats however, and has yet to be assessed in mice. Thus, the precise mechanism(s) underlying the sex differences in this study remain unclear and require further specific assessment. The serotonin transporter inhibitor MDMA increased activity in both male and female dopamine drd1KO mice (Risbrough *et al*, 2006), suggesting that the drd1 receptor does not mediate serotonergic stimulant-induced effects on activity at least. The drd1 antagonist SCH23390 pretreatment study provided support for drd1 receptor mediation of some modafinil-induced alterations in exploration. For example, SCH23390 attenuated modafinil-induced hyperactivity, consistent with previous reports (Simon *et al*, 1995), and increased holepoking at doses that did not affect these behaviors alone. This SCH23390-induced attenuation of modafinil-stimulated behavior—at doses that did not affect the behavior alone—was also observed for rearing. The numbers of rears recorded were so low, however, that this attenuation may have been confounded by floor effects. Although this study supported the drd1 KO data, SCH23390 affected all aspects of exploration at the highest dose tested (15 μg/kg), suggesting that there may also have been some additive effects of this pretreatment.

Reports with regard to modafinil-induced effects on activity in man are limited. Interestingly, modafinil increased activity in patients with schizophrenia as measured by a wrist-worn actigraph, although the interpretation of these findings is unfortunately limited because healthy comparison subjects were not included (Farrow *et al*, 2006). The utility of the BPM used in these studies goes beyond measures of the amount of activity by assessing a profile of exploratory behavior. This multivariate approach has proven useful in differentiating between stimulants in rats (Geyer *et al*, 1986) and mice (Young *et al*, 2010a), and also in characterizing human psychiatric and healthy populations (Perry *et al*, 2009; Young *et al*, 2007b). In these studies, modafinil not only increased activity, but also increased specific exploration (increased rearing/holepoking) and increased the linearity of the movement patterns of the animals (reduced spatial d). This modafinil-induced reduction in spatial d is consistent with amphetamine (Perry *et al*, 2009), GBR12909 (Young *et al*, 2010a), and MDMA, (Risbrough *et al*, 2006), as well as in isolation-reared mice (Gresack *et al*, 2010), an animal model related to schizophrenia. The modafinil-induced reduction in spatial d was absent in dopamine drd1 KO mice, although this effect may have been confounded by floor effects because these mice exhibited low spatial d even after vehicle treatment, as reported previously (Risbrough *et al*, 2006), and these data were not supported by the drd1 antagonist study which increased spatial d. Although floor effects in the behavior of dopamine drd1 KO have limited the interpretation of modafinil-induced effects previously (Young and Geyer, 2010), MDMA significantly reduced spatial d in drd1 KO mice (Risbrough *et al*, 2006), suggesting that serotonin-mediated reduction in spatial d may still be possible for these mice, but that modafinil-induced reduction is not. These drd1 KO and antagonist data therefore support a role for the dopamine drd1 receptor in mediating the linearity of movement (spatial d) during exploration.

Although mutation of the drd4 receptor did not affect modafinil-induced alterations in activity or spatial d, a significant interaction was observed with specific exploration. A gene–dosage effect on specific exploration (rearing) was observed in drd4 mutant mice, where modafinil increased rearing in WT mice, which was attenuated in

HT mice, but reduced in KO mice. The gene–dosage attenuation of modafinil-induced increases in specific exploration is one of the few studies to include drd4 HT mice, which exhibit reduced drd4 expression levels compared with WT mice. These findings complement research that links the dopamine D4 receptor to novelty seeking and impulsivity in human beings (Ebstein *et al*, 1996; Munafo *et al*, 2008; Tsuchimine *et al*, 2009). Moreover, previous studies have shown a phenotype of attenuated novelty-seeking behavior in drd4 KO mice (Dulawa *et al*, 1999). The importance of specific exploration in psychiatric populations was highlighted recently in differentiating between patients with BD mania and schizophrenia (Perry *et al*, 2010). Moreover, despite GBR12909 and amphetamine sharing a similar mechanism of DAT and NET inhibition (although the former is more selective for DAT and the latter for NET), these drugs could be differentiated in the BPM in terms of increased and decreased specific exploration, respectively (Perry *et al*, 2009; Xu *et al*, 2000). These differences in effect profiles provide further evidence for mechanistic differences between modafinil and amphetamine, where modafinil- and amphetamine-induced hyperactivity can be differentially affected by dopamine drd1 and drd2 receptor antagonists (Simon *et al*, 1995).

Although drug-induced alterations in activity, specific exploration, sleep–wake cycle, and motivation can be altered by dopaminergic manipulations (see above), other behaviors may be retained or differentially affected. For example, dopamine drd1 KO mice exhibit normal conditioned place preference for cocaine (Miner *et al*, 1995), and modafinil-induced speeding of stop reaction times was unaffected by general dopamine antagonists in rats (Eagle *et al*, 2007). Moreover, modafinil- and GBR12909-induced increases in motivation were blunted in dopamine drd1 receptor HT mice (Young and Geyer, 2010), whereas no attenuation of modafinil-induced alteration in spontaneous exploration was observed in drd1 HT mice in these studies. The only dopamine receptor not covered by these studies was the drd5 receptor, although drd5 mutant mice exist (Holmes *et al*, 2001). Drd5 mutant mice do not exhibit altered cocaine-induced alterations in activity or discriminative ability, although these cocaine effects were antagonized by a D1 family antagonist (Elliot *et al*, 2003). Thus, it is unlikely that modafinil-induced alterations in activity would be affected by reduced expression of the drd5 receptor. Other aspects of modafinil-induced effects on exploration such as rearing or spatial d may differ in drd5 mutant mice however. The specific contributions of dopamine receptor subtypes to drug-induced alterations in behavior appear to vary by the behavior assessed.

This mutant study data indicate that the dopamine drd1 receptor is required for the majority of modafinil-induced effects on exploration, including activity and specific exploration, albeit with some sex differences observed. These data are supported by the drd1 antagonist pretreatment study in which modafinil-induced increases in activity and holepoking were attenuated by SCH23390 at doses that did not affect these measures alone. Although the modafinil-induced reduction in spatial d was also absent in drd1 KO mice, this effect was likely to be due to floor effects because MDMA reduced spatial d in these mice and SCH23390 did not affect the modafinil-induced reduction in spatial d. The drd4 receptor may also be important, however, for modafinil-induced increases in specific exploration. The drd2 and drd3 receptors do not appear to be required for any modafinil-induced alterations in exploration. Given the sex $\times$ drug $\times$ genotype interactions observed in drd1 mutants however, increasing the sample sizes for each sex could reveal an effect of drd2 and/or drd3 receptor subtypes. Although these data highlight the importance of the drd1 and drd4 receptors in mediating modafinil-induced effects, these drd1 and drd4 effects could be downstream of the direct effects of DAT inhibition of modafinil (Young, 2009; Zolkowska *et al*, 2009). The similarity of pattern of effects on exploration between modafinil and the selective DAT inhibitor GBR12909 in C57 and 129 mice, as can also seen for motivation, support a DAT inhibition mechanism of action for modafinil (Madras *et al*, 2006; Volkow *et al*, 2009; Young and Geyer, 2010). Given the interest in this drug as a putative treatment for cognitive disruption in psychiatric disorders such as schizophrenia (Turner *et al*, 2004), and apparent differences in drd1 mediation of effects in other behaviors (see above), future studies should examine the effects of gene deletion on other behaviors relevant to cognition and schizophrenia (Young *et al*, 2009b). For example, future studies could investigate modafinil-induced effects on vigilance (Turner *et al*, 2003; Young *et al*, 2009a), attentional set-shifting (Young *et al*, 2010d), and prepulse inhibition (Doherty *et al*, 2008; Powell *et al*, 2008) in these mice.

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DISCLOSURE

The authors declare no conflict of interest.

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