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ORIGINAL INVESTIGATION

Dopaminergic mediation of the discriminative stimulus functions of modafinil in rats

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Abstract
Rationale Modafinil is a wake-promoting drug with FDA approval for the treatment of excessive daytime sleepiness that has been prescribed for ADHD and recently assessed as a potential treatment for psychostimulant dependence. Previous research indicates that modafinil modestly increases locomotor activity and produces similar discriminative stimulus effects to psychostimulants in rodents, although the subjective effects of modafinil are reportedly distinct from those of cocaine or amphetamine in humans with a history of psychostimulant abuse.
Objectives The current study employed drug discrimination procedures in rats to examine the pharmacological actions contributing to modafinil's discriminative stimulus functions.
Methods Eight male Sprague–Dawley rats were trained to discriminate intragastric administration of 256 mg/kg modafinil from vehicle (5 % arabic gum) under a FR 20 schedule of food reinforcement. Substitution tests were conducted with various dopaminergic agents (d-amphetamine, cocaine, PNU-91356A, GBR 12909, methylphenidate) and nondopaminergic agents (nicotine, ethanol). Antagonist tests were conducted with the selective D1 antagonist, SCH 39166, and the nonselective D2 antagonist, haloperidol.
Results Rats trained to discriminate modafinil displayed complete stimulus generalization to cocaine, methylphenidate, and GBR 12909 and the discrimination was completely blocked by both SCH 39166 and haloperidol. Evidence for significant partial substitution was obtained with d-amphetamine, PNU-91356A, and nicotine.
Conclusions Results strongly support the role of dopaminergic mechanisms in the discriminative stimulus functions of modafinil, although further evaluation regarding the contribution of other neurotransmitter systems to these effects should be continued. The findings are discussed in light of clinical research efforts with modafinil as a treatment for psychostimulant dependence.

Keywords Modafinil · Drug discrimination · Cocaine · GBR 12909 · Amphetamine · Methylphenidate · Rats

Modafinil is an alertness-promoting drug manufactured by Cephalon® with FDA approval for the treatment of narcolepsy. It is also reportedly effective in the treatment of chronic fatigue syndrome (Turkington et al. 2004), obstructive sleep apnea/hypopnea syndrome, and shift work sleep disorder (Keating and Raffin 2005) and has been investigated to treat fatigue in patients with neurological disorders (Sheng et al. 2013) such as Parkinson's disease (Hogl et al. 2002), amyotrophic lateral sclerosis (Carter et al. 2005), and dementia (Howcroft and Jones 2005). Furthermore, modafinil's wake-promoting (Hermant et al. 1991; Silvestri et al. 2002; Webb et al. 2006) and cognitive-enhancing effects (Turner et al. 2003) are similar to those of traditional psychomotor stimulants (e.g., amphetamine, cocaine) apparently without the side effects typically associated with these substances (Deroche-Gamonet et al. 2002; Hermant et al. 1991; Lin et al. 2000), indicating it is a promising candidate for agonist replacement therapy for psychostimulant dependence.

In a review of preclinical, human laboratory, and clinical research, Herin et al. (2010) summarized the rationale for agonist-like pharmacotherapy for stimulant dependence;

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medications with effects similar to the abused drug, but exhibiting a lower abuse liability may normalize neurochemistry and stabilize behavior and subsequently reduce drug use. As such, modafinil's reported low abuse liability and similar pharmacological actions to psychostimulants support its use in the treatment of psychostimulant dependence. In the conditioned place preference paradigm, modafinil does not produce a preference for the drug-paired context (Deroche-Gamonet et al. 2002; Quisenberry et al. 2013b) nor does it potentiate d-amphetamine-induced place preference in rats (Quisenberry et al. 2013b). Further, modafinil is not self-administered in rats (Deroche-Gamonet et al. 2002) or humans unless under specific behavioral demands (Stoops et al. 2005). Reports regarding modafinil's actions on dopamine transporter (DAT) binding sites and evidence for its longer duration of action and lower efficacy compared to cocaine support the use of modafinil as an agonist replacement therapy for cocaine dependence (Loland et al. 2012; Federici et al. 2013). However, clinical studies of modafinil for the treatment of cocaine (Dackis et al. 2005; Schmitz et al. 2012; Anderson et al. 2009), amphetamine (Mann and Bitsios 2008), or methamphetamine (Shearer et al. 2009) dependence and for cognitive deficits associated with a history of methamphetamine abuse (Dean et al. 2011; Ghahremani et al. 2011) have yielded equivocal results.

Further development of modafinil as a treatment option for psychostimulant dependence requires a better understanding of the pharmacological actions responsible for its behavioral and subjective effects. This information is critically important for predicting potential interactions with other central nervous system (CNS) stimulants. As noted above, the DAT is implicated in modafinil's actions (Minzenberg and Carter 2008; Wisor et al. 2001). Investigations utilizing in vitro techniques to assess modafinil-induced DAT binding (Loland et al. 2012; Nguyen et al. 2011) along with results from in vivo assays suggest dopamine's involvement in modafinil's CNS effects (Minzenberg and Carter 2008; Wisor et al. 2001; Volkow et al. 2009; Dopheide et al. 2007; Zolkowska et al. 2009; Rowley et al. 2013). Evidence for modafinil-induced increases in locomotor activity in Marmosets (Van Vilet et al. 2006), Rhesus monkeys (Andersen et al. 2010), and rats (Zolkowska et al. 2009; Ward et al. 2004; Young et al. 2011) is also indicative of its stimulant-like mechanism of action.

Drug discrimination is a widely accepted behavioral assay with considerable pharmacological specificity for determining CNS mechanisms of drug action. To date, three studies found that modafinil engendered full substitution for cocaine (Paterson et al. 2010; Loland et al. 2012; Newman et al. 2010), while three other studies found modafinil to produce only partial substitution for cocaine (Gold and Balster 1996; Rush et al. 2002a; Dopheide et al. 2007). Additionally, modafinil produced only partial substitution for d-amphetamine, but potentiated the effects of low doses of d-amphetamine (Dopheide et al. 2007; Quisenberry et al. 2013a) and cocaine (Dopheide et al. 2007). Furthermore, human laboratory studies of modafinil's reinforcing and subjective effects indicate that oral modafinil at clinically effective doses does not appear to have strong reinforcing effects and produces subject-rated effects distinct from psychostimulants (Malcolm et al. 2006; Rush et al. 2002b; Warot et al. 1993). Considered together, these findings suggest that modafinil can produce subjective effects similar to those of cocaine or amphetamine in some individuals, but these effects are typically differentiated by humans with a history of psychostimulant abuse.

Further understanding of the mechanisms of action responsible for modafinil's subjective effects may be garnered by training animals to discriminate modafinil and assessing other substances for stimulus generalization and antagonism. The primary aim of this study was to examine the contribution of the DAT to modafinil's discriminative stimulus functions by training rats to discriminate modafinil and subsequently assessing DAT inhibitors and other dopaminergic and nondopaminergic agents for stimulus generalization and DA antagonists for blockade of the training stimulus.

Methods

Subjects Eight adult male Sprague–Dawley rats (Charles River, Portage, MI) were singly housed in polycarbonate cages lined with corncob bedding (Harlan Teklad, Conrad, Iowa) in a vivarium maintained on a 12:12-light/dark cycle with lights on from 7:00 a.m. to 7:00 p.m. Water was provided ad libitum in home cages and once daily feedings of Purina® 5001 rodent laboratory chow (Richmond, Indiana) were given to maintain animals at 85 to 90 % of free-feeding weights. All procedures were reviewed and approved by the Western Michigan University Institutional Animal Care and Use Committee and complied with the Guide for the Care and Use of Laboratory Animals (2011).

Apparatus Training and testing sessions were conducted in eight standard operant conditioning chambers (ENV-001; MED Associates Inc., Georgia, VT), housed within sound- and light-attenuating shells. Each chamber was equipped with three removable levers and a food pellet dispenser located on the front panel, a 28-V house light, and a fan. Reinforcement for lever pressing consisted of the delivery of 45 mg Dustless Precision Pellets® (Product# F0021, BioServ, Flemington, NJ). Experimental events and data collection were programmed using Version IV Med-PC software (MED Associates Inc., St. Albans, VT).

Drugs (±) Modafinil was synthesized in the laboratory of Dr. Thomas Prisinzano using previously described methods (Prisinzano et al. 2004). Suspensions were prepared fresh each

day in a 5 % arabic gum solution (Sigma-Aldrich, St. Louis, MO) and administered by gavage (i.g.) in a volume of 10 ml/kg 30 min prior to training sessions. The DA releaser, d-amphetamine-hemisulfate (Sigma-Aldrich, St. Louis, MO), and the D_2 agonist, PNU-91356A (Pharmacia & Upjohn, Inc., Kalamazoo MI), were dissolved in 0.9 % NaCl and administered via intraperitoneal (i.p.) injection 10 min prior to test sessions. The DAT inhibitor, GBR 12909 bismethanesulfonate monohydrate, was prepared in the Chemical Biology Research Branch, National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism. GBR 12909 was dissolved in sterile water and administered i.p. 30 min prior to test sessions. Another DAT inhibitor, cocaine-hydrochloride (National Institute on Drug Abuse, Bethesda, MD), and the nicotinic cholinergic receptor agonist, (−)-nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO), were dissolved in 0.9 % NaCl and administered i.p. 15 min before test sessions. Another DAT inhibitor, methylphenidate-hydrochloride, was obtained by prescription from Sindecuse Health Center pharmacy (Kalamazoo, MI) in the form of 5 mg tablets that were crushed and dissolved in sterile water and administered i.g. in a 10-ml/kg volume 30 min before test sessions. Ethanol, a GABA agonist, was diluted in saline and administered via i.g. 30 min before test sessions. SCH 39166 (Shering-Plough Corporation, Bloomfield, NJ), a D_1 dopamine antagonist, was dissolved in 0.9 % NaCl and administered i.p. 10 min prior to modafinil (256 mg/kg) administration and 40 min before test sessions. Haloperidol (Sigma-Aldrich, St. Louis, MO), a non-selective D_2 dopamine antagonist, was dissolved in 0.9 % NaCl and administered i.p. 30 min prior to modafinil (256 mg/kg) administration and 60 min before test sessions.

Training procedures Preliminary training and drug discrimination training procedures were similar to those described in our previous published work (e.g., Quisenberry et al. 2013a). Animals underwent preliminary training to establish lever pressing on a fixed ratio 20 (FR 20) schedule of reinforcement, initially with only the center lever present and subsequently only with the left lever or right lever present under either drug or vehicle stimulus conditions. Once responding was reliably maintained on the FR 20 schedule under both stimulus conditions, discrimination training commenced with both left and right levers present. Drug injections consisted of 256 mg/kg modafinil (i.g. 30 min) and vehicle injections consisted of a 5 % arabic gum solution. Half the animals were reinforced for responses on the left lever following modafinil and for responses on the right lever following vehicle administration. Conditions were reversed for the remaining animals.

The modafinil training dose and pretreatment interval were selected based on previous findings that this dose produced nearly complete substitution for d-amphetamine in rats (Quisenberry et al. 2013a). The selection of a 30-min pretreatment interval is supported by a previous report by Dopheide et al. (2007), who assessed the time course of modafinil using drug discrimination procedures. The highest dose they tested (128 mg/kg) produced only partial substitution for low doses of d-amphetamine and cocaine, with maximal drug lever responses occurring between 30 and 120 min.

Modafinil and vehicle training sessions were administered in a non-systematic order, with the limitation that no animal received more than two consecutive drug or two consecutive vehicle sessions throughout the study. The chambers and levers were wiped clean with isopropyl alcohol after each session to reduce the influence of olfactory stimuli on lever selection (Extance and Goudie 1981). Criteria for stimulus discrimination were 80 % correct lever responses prior to the delivery of the first reinforcer and for the remainder of each training session for a minimum of 8 of 10 consecutive discrimination training sessions.

Substitution and antagonists tests Stimulus generalization tests commenced when each subject met the criteria described above. In between test sessions, animals were administered no less than one drug training session and one vehicle training session and were required to exhibit 80 % response accuracy on the first FR and for the remainder of the training session under both conditions to continue testing. If an animal did not meet these criteria, training sessions continued until discrimination criteria were met for each stimulus condition on two consecutive days. Substitution tests were conducted with the following test compounds in the order listed: modafinil (0, 16, 32, 64, 128, 256, and 384 mg/kg); d-amphetamine (0, 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg); PNU-91356A (0.01, 0.03, 0.1, and 0.3 mg/kg); GBR 12909 (0, 5, 10, 20, and 30 mg/kg); nicotine (0, 0.1, 0.2, 0.4, and 0.8 mg/kg); cocaine (0, 1.25, 2.5, 5, and 10 mg/kg); and methylphenidate (0, 1.25, 2.5, 5, 10 mg/kg). Antagonist tests were then conducted with SCH 39166 (0, 0.03, 0.1, 0.3 mg/kg, i.p.) and haloperidol (0, 0.125, 0.25, 0.5 mg/kg, i.p.) in combination with 256 mg/kg modafinil. Lastly, ethanol (0.75, 1.5, 2.0 g/kg, i.g.) was assessed as a negative control for substitution.

Individual test doses of each compound were administered in a counterbalanced order among animals. At each dose level, half of the animals were tested on a day following a drug training session and the other half were tested on a day following a vehicle training session. Test sessions were similar to discrimination training sessions with the exception that no reinforcers were delivered and rats were immediately removed from the chambers following the completion of 20 consecutive responses on either lever or after 20 min had elapsed, whichever occurred first.

Data analysis Stimulus generalization was quantified as the percentage of total responses emitted on the drug-appropriate lever. Complete stimulus generalization was defined as a

group mean of 80 % or higher on any given dose. A group average between 20 and 80 % drug-lever selection was considered partial substitution. Complete stimulus antagonism was defined as a group mean of 20 % or lower on any given dose. Response rate was expressed as the number of responses per second, calculated by dividing the total number of responses on both levers by the number of seconds to complete a test session. Means (±S.E.M.) for the percentage of drug-lever responses and response rate were calculated for each test dose and dose–response curves were plotted from these data. For test compounds producing dose-dependent increases in drug lever selection, separate one-way repeated measures analysis of variance (ANOVA) tests were conducted to assess the main effect of drug dose on the percentage of drug-lever responses and on response rate. Test sessions in which animals failed to emit at least 20 responses were excluded from statistical analyses of the percentage drug-lever selection, but were included in statistical analyses of response rate. Statistical analyses were performed using Prism GraphPad version 6.0 (GraphPad, San Diego, CA).

Results

Modafinil established stimulus control in all eight animals within an average of 36 (±2.4) discrimination training sessions (range, 23–43). However, two animals failed to consistently maintain the discrimination following the completion of substitution tests with modafinil and d-amphetamine. The modafinil training dose was subsequently increased to 384 mg/kg for these two animals and stimulus control was re-established. Due to the difference in training dose, statistical analysis and graphic representation of subsequent test results excluded these two animals, although testing did continue with these animals. Data from these two animals were included for the modafinil and d-amphetamine substitution tests because they were completed prior to the increase in training dose.

The modafinil dose–response curve and dose–response curves generated from substitution tests with drugs that produced complete substitution following at least one dose (cocaine, methylphenidate, GBR 12909) are displayed in the left panel and those that produced partial substitution (PNU-91356A, nicotine, d-amphetamine) are depicted in the right panel in Fig. 1. Modafinil produced a dose-dependent increase in drug-lever responses with full substitution at the training dose. A repeated measures one-way ANOVA revealed a significant effect of modafinil dose on percentage of drug-lever responses [F (6, 42)=5.29, $p<0.001$]. Tukey post-hoc tests were significant between the 256 mg/kg dose and vehicle ($p<0.001$) and between the 256 and 16 mg/kg dose ($p<0.05$). There were also significant differences between 384 mg/kg and vehicle ($p<0.01$). A one-way repeated measures ANOVA on response rate following modafinil administration was not statistically significant.

The cocaine dose–response curve depicts full substitution for modafinil with 2.5 mg/kg (94 %) and 10 mg/kg (80 %). A one-way repeated measures ANOVA on percent modafinil-lever selection revealed a significant main effect of cocaine dose [F (4, 29)=4.18, $p<0.05$]. Tukey post-hoc tests were significant between the vehicle and 2.5 mg/kg ($p<0.05$) conditions. Response rate following cocaine administration decreased in a dose-dependent fashion, although a one-way repeated measures ANOVA on response rate following cocaine was not significant.

Methylphenidate (MPH) produced dose-dependent increases in percent drug-lever responding with full substitution at both 5 mg/kg (88 %) and 10 mg/kg (100 %). A one-way repeated measures ANOVA revealed a significant main effect of MPH dose [F (4, 12)=13.05, $p<0.001$]. Post-hoc comparisons showed significant differences between vehicle and 5 mg/kg ($p<0.01$) and between vehicle and 10 mg/kg ($p<0.01$) in addition to differences between 1.25 and 5 mg/kg ($p<0.001$) and between 1.25 and 10 mg/kg ($p<0.05$). A one-way repeated measures ANOVA on response rate following MPH administration was not significant.

GBR 12909 produced dose-dependent increases in drug-appropriate responding, with full substitution (86 %) following the administration of 30 mg/kg. A one-way repeated measures ANOVA on percent modafinil-lever responses revealed a significant effect of GBR 12909 dose [F (4, 20)=3.47, $p<0.05$]. Tukey post-hoc tests were significant between percent drug-lever responses following 30 mg/kg and vehicle ($p<0.05$). GBR 12909 also produced dose-dependent decreases in response rate. A one-way repeated measures ANOVA on response rate was statistically significant [F (4, 20)=6.15, $p<0.01$] with significant Tukey post-hoc tests between vehicle and 30 mg/kg ($p<0.001$), between 5 and 30 mg/kg ($p<0.05$), and between 20 and 30 mg/kg GBR 12909 ($p<0.05$).

d-Amphetamine produced dose-dependent increases in the percentage of drug-lever responses, with significant partial substitution (74 %) at 3.0 mg/kg. However, four of the eight animals failed to meet the response requirement at this dose. Therefore, this dose was eliminated from the statistical analysis of percent drug-lever responses but was included in the statistical analysis of response rate. A one-way repeated measures ANOVA excluding the 3 mg/kg dose revealed a significant effect of d-amphetamine dose on percentage of drug-lever responses [F (4, 28)=3.76, $p<0.05$]. Post-hoc tests were significant between 1.0 mg/kg and vehicle ($p<0.05$) and between 0.03 and 1.0 mg/kg d-amphetamine ($p<0.05$). A one-way repeated measures ANOVA on response rate including all dose levels was statistically significant [F (5, 35)=14.98, $p<0.001$] with significant Tukey post-hoc tests between vehicle and 1.0 mg/kg ($p<0.05$) and 3.0 mg/kg ($p<0.001$).

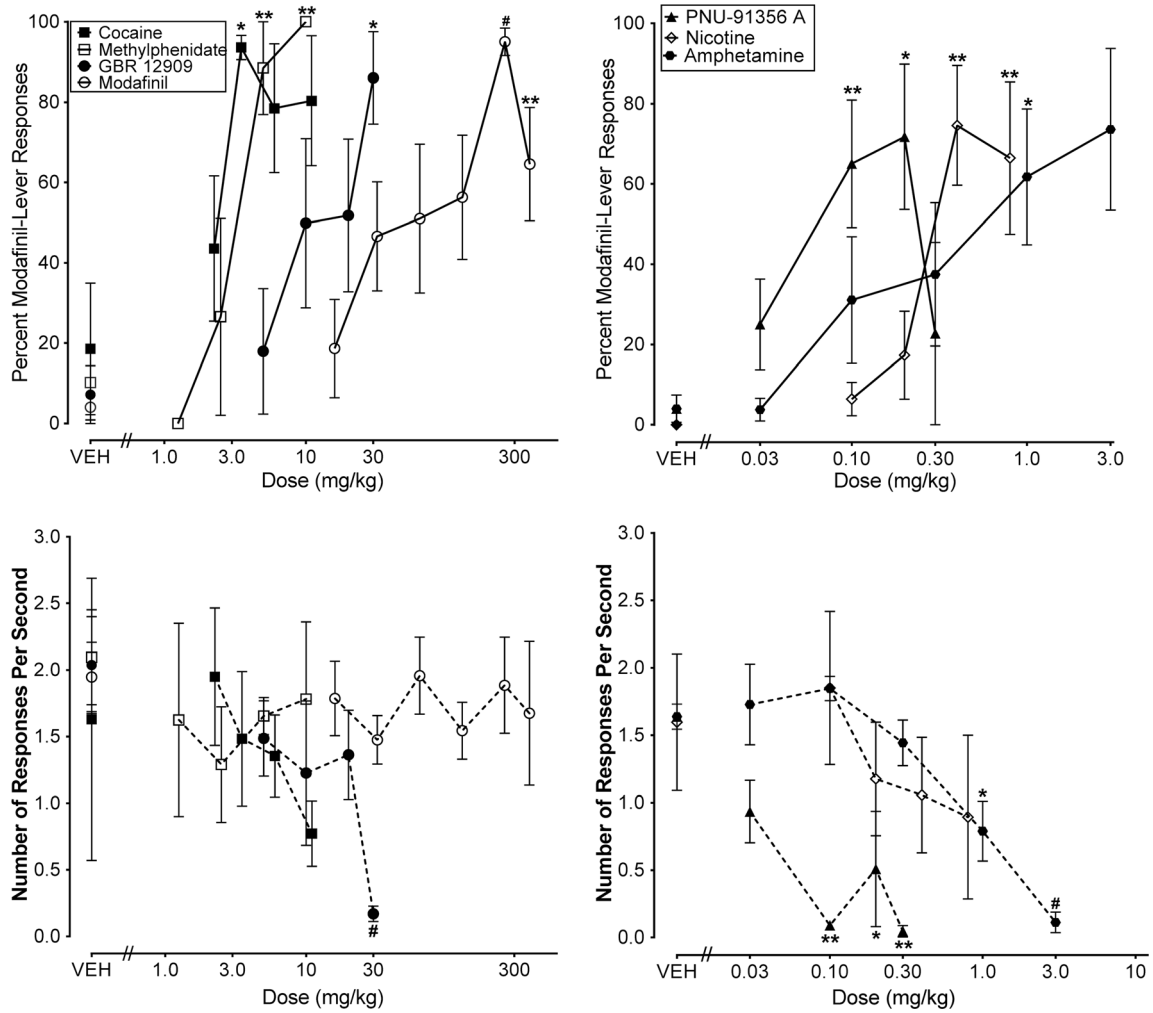

Fig. 1 Dose–response curves generated from the results of substitution tests with cocaine (n=6), methylphenidate (n=4), GBR 12909 (n=6), and modafinil (n=8) in the *left panel* and with PNU-91356A (n=5), nicotine (n=6), and d-amphetamine (n=8) in the *right panel*. Mean (±S.E.M.) percent drug-lever selection is shown in the *upper two graphs*; mean (±S.E.M.) response rate is depicted in the *lower two graphs*. Symbols indicate statistically significant post-hoc tests compared to vehicle (*p<0.05, **p<0.01, #p<0.001)

Significant differences were also found between 0.10 and 1.0 m/kg (p<0.01) and between 0.10 and 3.0 mg/kg (p<0.001). The 0.3 mg/kg dose was only significantly different from the 3.0 mg/kg dose (p<0.001).

Responding by the majority of animals was severely disrupted by the highest dose test of the D_2 agonist PNU-91356A (0.3 mg/kg), and this dose was excluded from statistical analysis of percent drug-lever responses. Substantial partial substitution for modafinil was observed with 0.1 mg/kg (63 %) and 0.2 mg/kg (67 %) PNU-91356A. A one-way repeated measures ANOVA on percent modafinil-lever selection revealed a significant main effect of PNU-91356A dose [F (3, 12)=7.22, p<0.01] and Tukey post-hoc tests were significant between vehicle and 0.1 mg/kg PNU-91356A (p<0.01) and between vehicle and 0.2 mg/kg PNU-91256A (p<0.05). This test compound reduced response rate in a dose-dependent fashion. A one-way repeated measures ANOVA on response rate was significant [F (4, 16)=7.69, p<0.01] and Tukey post-hoc tests revealed a statistically significant difference between vehicle and 0.1 mg/kg (p<0.01), between vehicle and 0.2 mg/kg dose (p<0.05), and between vehicle and 0.3 mg/kg dose (p<0.01).

Of particular interest, partial substitution for modafinil was produced by nicotine administration, with a group mean of 74 % drug-lever selection following 0.4 mg/kg. A one-way repeated measures ANOVA revealed a significant effect of nicotine dose on percentage of drug-lever responses [F (4, 20)=8.11, p<0.001]. Tukey post-hoc tests were significant between vehicle and 0.4 mg/kg (p<0.01), between vehicle and 0.8 mg/kg (p<0.01), and between 0.2 mg/kg and 0.4 mg/kg (p<0.05). Visual analysis of response rate data after nicotine administration suggests a dose-dependent decrease in responding; however, a one-way repeated measures ANOVA found no statistically significant effects of nicotine on response rate. As a negative control, oral ethanol administration was assessed for substitution to modafinil and none

of the doses tested produced significant drug-appropriate responding, as shown in Table 1.

Figure 2 displays the results of antagonist tests with the D_1 dopamine receptor antagonist, SCH 39166, or the non-selective D_2 dopamine receptor antagonist, haloperidol administered in combination with the modafinil training dose. Percent modafinil-lever selection is presented in the upper panel and response rate is displayed in the lower panel. Dose-dependent decreases in percent modafinil-lever selection were evident with both antagonists. A repeated measures one-way ANOVA revealed a significant effect of SCH 39166 dose on percentage of drug-lever responses [F (3, 15)=18.29, p<0.001]. Tukey post-hoc tests were significant between the vehicle and 0.1 mg/kg (p<0.001) and between vehicle and 0.3 mg/kg (p<0.001). A repeated measures one-way ANOVA revealed no significant effect of SCH 39166 dose on response rate.

A repeated measures one-way ANOVA revealed a significant effect of haloperidol dose on percentage of drug-lever responses [F (3, 9)=4.99, p<0.05]. Tukey post-hoc tests were significant between vehicle and the 0.5 mg/kg dose (p<0.01). Statistical analysis of response rate with a one-way repeated measures ANOVA also revealed a significant effect of dose [F (3, 12)=9.63, p<0.01]. Tukey post-hoc tests were significant between 0.5 mg/kg and all other dose levels (p<0.01).

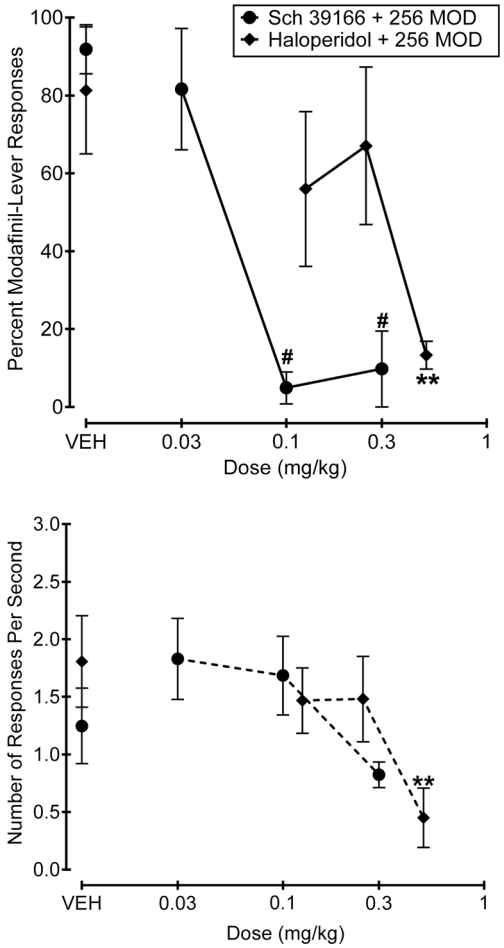

Fig. 2 Dose–response curves generated from the results of antagonist tests with SCH 39166 + 256 mg/kg modafinil (n=6) and haloperidol + 256 mg/kg modafinil (n=6). Mean (±S.E.M.) percent drug-lever selection is represented in the *top panel* and mean (±S.E.M.) response rate is shown in *bottom panel*. Symbols indicate statistically significant post-hoc tests compared to vehicle + 256 mg/kg modafinil (*p<0.05, **p<0.01, #p<0.001)

Discussion

This study investigated the involvement of dopaminergic actions in the discriminative stimulus effects of the wake-promoting agent, modafinil. It was determined that 256 mg/kg modafinil (i.g. 30 min) establishes discriminative stimulus control in rats. Although maintenance of this discrimination was weak in two of the eight animals assessed, increasing the training dose to 384 mg/kg in these animals successfully re-established discrimination. More importantly, the DAT inhibitors, GBR 12909, methylphenidate, and cocaine all engendered complete substitution, implicating the involvement of DAT inhibition in mediating modafinil's discriminative stimulus effects. In contrast, the psychostimulant d-amphetamine, which exerts its actions primarily through increasing DA release, failed to produce complete substitution at a dose that markedly suppressed responding. These results are consistent with two previous reports (Dopheide et al. 2007; Quisenberry et al. 2013a) that modafinil produces only partial substitution in rats trained to discriminate d-amphetamine.

Table 1 Results of negative control tests with ethanol

Ethanol dose (g/kg)	Percent modafinil lever			Responses per second		
	Mean	S.E.M.	*N*	Mean	S.E.M.	*N*
0.75	0.87	0.87	5	1.20	0.46	5
1.5	13.01	11.52	5	0.54	0.44	5
2	0.95	0.95	3	0.12	0.04	3

The complete blockade of modafinil discrimination by the D_1 antagonist, SCH 39166, and the non-selective D_2 antagonist, haloperidol, also strongly support the role of dopaminergic mechanisms in the discriminative stimulus functions of modafinil. Furthermore, the current results confirm those of previous studies using in vitro techniques that the DAT and both D_1 and D_2 DA receptors are involved in modafinil's neuropharmacological actions (Loland et al. 2012; Nguyen et al. 2011; Korotkova et al. 2007) and behavioral effects (Wisor et al. 2001; Zolkowska et al. 2009; Rowley et al. 2013).

While it is tempting to conclude from the current data that modafinil's discriminative stimulus effects are mediated primarily by its actions as a DAT inhibitor, significant partial

substitution by d-amphetamine and by the nicotinic cholinergic agonist, nicotine, should not be ignored. Although nicotine is not a direct dopamine agonist, it increases DA efflux in the nucleus accumbens and DA antagonists have been shown to block its discriminative stimulus effects (Di Chiara 2000). Furthermore, a previous report that the nicotinic receptor antagonist, mecamylamine, did not change modafinil-induced DA efflux suggests that the cholinergic system is not involved in modafinil's mechanism of action (Dopheide et al. 2007). The current findings suggest modafinil and nicotine may have similar discriminative stimulus effects and, thus, the extent to which dopaminergic actions contribute to these similarities warrant further investigation. For example, it may be of interest to determine if modafinil discrimination is enhanced by nicotine or vice versa. Such investigations may have particular relevance regarding modafinil's behavioral and subjective effects in smokers versus nonsmokers.

Although the present results strongly support the role of DAT inhibition in modafinil's discriminative stimulus functions, future investigations should evaluate the contribution of other neurotransmitter systems to these effects. While cocaine and d-amphetamine are well known to act upon the DA system, they also have actions on noradrenergic and serotonergic systems (Glennon and Young 2011). For example, DAT inhibitors readily substitute for cocaine and both DAT and 5-HT transporter inhibitors enhance the discriminative stimulus effects of cocaine in rats trained to discriminate low doses of cocaine (Kleven and Koek 1998). Additionally, evaluation of the discriminative stimulus effects of d-amphetamine revealed substitution with NE transport inhibitors (Kamien and Woolverton 1989), suggesting this is a primary mechanism involved in maintaining the discriminative stimulus effects of d-amphetamine. Given that the NE system appears to be important to the locomotor-activating effects of modafinil (Hermant et al. 1991), further investigations of the shared discriminative stimulus functions between modafinil and other noradrenergic agonists are warranted. Additionally, in consideration of the presumed involvement of orexin in modafinil's wake-promoting effects (Willie et al. 2005), exploration of the orexin system's role in modafinil's discriminative stimulus functions may also be of interest.

Limitations of the current study warrant some discussion. Different injection methods and vehicles were used in previous drug discrimination investigations of modafinil, which could result in different substitution patterns among various studies. Moreover, the use of intragastric modafinil delivery in the current study could have led to variable and inconsistent drug absorption and may be partly responsible for variability in responding among animals. For example, stimulus generalization following 384 mg/kg modafinil was lower than that obtained with the training dose, due to two animals that responded mainly on the vehicle lever in substitution tests with this dose.

Drug discrimination in nonhumans is a useful tool to inform the potential therapeutic utility of medications for the treatment of substance dependence (Li et al. 2006). As such, the current results that modafinil shares similar discriminative stimulus functions with cocaine may be relevant to recent clinical evaluations of modafinil for treating psychostimulant dependence. Of particular importance, modafinil produces different subject-rated effects in participants with and without a history of psychostimulant use (Malcolm et al. 2006; Rush et al. 2002a, b; Warot et al. 1993). Humans without a history of psychostimulant use appear to be more likely to characterize the effects of modafinil as amphetamine-like (Makris et al. 2007; Stoops et al. 2005), while findings are generally consistent that participants with a history of psychostimulant abuse can readily distinguish the effects of modafinil from either cocaine or amphetamine (Malcolm et al. 2006; Rush et al. 2002a, b).

Consistent with the idea that previous exposure to psychostimulants can influence the behavioral effects of modafinil, drug self-administration studies with nonhumans found that modafinil fails to function as a reinforcer in rats without a history of cocaine self-administration (Deroche-Gamonet et al. 2002), but appears to be reinforcing in rhesus monkeys previously trained to self-administer cocaine (Gold and Balster 1996). The impact of drug-use history on modafinil discrimination should be further assessed in both preclinical and clinical research settings. For example, nonhuman self-administration and drug discrimination procedures could be employed sequentially to assess the impact of amphetamine or cocaine self-administration training on their subsequent discrimination and stimulus generalization to modafinil. Studies of this sort may be a worthwhile pursuit as a preclinical assessment of drug use history on the subjective effects of modafinil.

Evidence that psychostimulant users can discriminate the effects of modafinil from those of other psychostimulants does not preclude the possibility that modafinil could be established as a therapeutic agent to assist in recovery from psychostimulant dependence. Based on the rationale for agonist-like pharmacotherapy for stimulant dependence summarized by Herin et al. (2010), the current findings coupled with previous reports of modafinil's low abuse liability (Jasinski 2000; Rush et al. 2002b; Myrick et al. 2004; Stoops et al. 2005) and findings that modafinil's actions on DAT binding have a longer duration of action with lower efficacy compared to cocaine (Loland et al. 2012) support continued research with modafinil as a substitution therapy for psychostimulant dependence. However, substantial evidence for modafinil's clinical efficacy in this regard is currently lacking. Despite initial promising results in a small number of cocaine-dependent patients over a brief time period (Dackis et al. 2005), a more recent double-blind, placebo-controlled randomized clinical trial failed to indicate clinical efficacy of modafinil as a

treatment for cocaine dependence (Schmitz et al. 2012). Another investigation of modafinil's effectiveness on reducing cocaine use, the percentage of non-use days significantly increased when participants who were also alcohol-dependent were removed from analysis (Anderson et al. 2012) and results from a sample of participants seeking treatment for methamphetamine dependence indicated modest but promising effects of modafinil (Shearer et al. 2009).

In sum, this is the first report that a high dose of oral modafinil (256 mg/kg) can be established as a discriminative stimulus and that stimulus generalization is robust with DAT inhibitors, while DA antagonists block modafinil's discriminative stimulus effects. These data suggest that discriminative stimulus functions of modafinil are mediated by the dopaminergic system and provide a foundation on which to build new experiments. History of psychostimulant use, in both humans and animals, appears to be an important factor in the discrimination and substitution of modafinil for other psychostimulants. Thus, continued research on modafinil as an agonist therapy for psychostimulant dependence must carefully consider how individual differences in substance use history might influence treatment outcome. Exploration of the impact of previous drug use history on modafinil's reinforcing and subjective effects may benefit from the use of preclinical models, including drug self-administration methods and drug discrimination methods in tandem. Further research on the behavioral and neuropharmacological effects of modafinil in populations with different substance use history is essential to evaluating its therapeutic efficacy and potential risks as a relapse-prevention treatment for psychostimulant dependence.

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Conflict of interest The authors report no conflicts of interest.

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