Modafinil evokes striatal [3H]dopamine release and alters the subjective properties of stimulants

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Abstract

Modafinil is a mild psychostimulant used for the treatment of sleep and arousal-related disorders, and has been considered a pharmacotherapy for cocaine and amphetamine dependence; however, modafinil’s mechanism of action is largely unclear. The present study investigated modafinil using drug discrimination and slice superfusion techniques. Rats were trained to discriminate cocaine (1.6 or 5 mg/kg) or amphetamine (0.3 mg/kg) from saline injection for food reinforcement. Modafinil (64–128 mg/kg) substituted partially for both cocaine doses and amphetamine. Pretreatment with a lower modafinil dose (32 mg/kg) augmented the discriminative stimulus properties of cocaine (1.6 mg/kg dose group) and amphetamine. In neurochemical experiments, modafinil (100–300 μM) evoked [3H]overflow from rat striatal slices preloaded with [3H]dopamine in a concentration-dependent manner; however, modafinil was less potent and efficacious than amphetamine and nicotine. The dopamine transporter inhibitor nomifensine (10 μM) blocked modafinil-evoked [3H]overflow, and concentrations of modafinil (≤100 μM) that did not have intrinsic activity attenuated amphetamine (1 and 3 μM)-evoked [3H]overflow. Modafinil-evoked [3H]overflow was not altered by the nicotinic acetylcholine receptor antagonist mecamylamine, and modafinil did not alter nicotine-evoked [3H]overflow, indicating that nicotinic acetylcholine receptors likely are not important for modafinil’s mechanism of action. The present results indicate that modafinil evokes dopamine release from striatal neurons and is a psychostimulant that is pharmacologically similar to, but much less potent and efficacious than, amphetamine.

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1. Introduction

Modafinil (diphenyl-methyl sulphinil-2-acetamide; Provigil, Alertec and Vigiler) is a wake-promoting psychostimulant that is approved by the U.S. Food and Drug Administration for the treatment of narcolepsy, shift work sleep disorder and as an adjunctive treatment for obstructive sleep apnea/hypopnea syndrome (Ballon and Feifel, 2006). Modafinil also is effective for attenuating the symptoms of Attention-Deficit Hyperactivity Disorder, symptoms of dysphoric mood disorders, negative symptoms of schizophrenia, and cognitive deficits found in organic brain syndromes and Alzheimer’s Disease (Nasr, 2004; Ballon and Feifel, 2006; Krebs et al., 2006).

A concern with the use of traditional psychostimulants, such as amphetamines, for psychiatric disorders is their relatively-high abuse and dependence liability. However, research in humans suggests that modafinil has less of an abuse liability than amphetamines or cocaine (Warot et al., 1993; Jasinski and Kovacevic-Ristanovic, 2000; Myrick et al., 2004). In a drug discrimination assay, modafinil induced a state that was distinct from amphetamine and cocaine (Warot et al., 1993; Rush et al., 2002). Moreover, modafinil has recently been considered as a treatment to minimize the withdrawal symptoms from long-term amphetamine or cocaine use (Dackis et al., 2003, 2005).
Modafinil has a similar behavioral profile in rodents and humans to increase arousal and improve cognitive processes. For example, modafinil (30–300 mg/kg) increased wakefulness and enhanced performance in serial-reversal and working memory tasks in rats (Edgar and Seidel, 1997; Beracochea et al., 2001). Regarding drug abuse and dependence, modafinil was not self-administered, nor did it induce conditioned place preference (Deroche-Garnon et al., 2002), supporting the human research suggesting that modafinil has low addiction liability. In rats in which cocaine (10 mg/kg) was trained as a discriminative stimulus, modafinil (250 mg/kg) partially substituted (~ 67% of responses on the cocaine-paired lever) for the cocaine stimulus (Gold and Balster, 1996).

Despite the behavioral efficacy of modafinil, its mechanism of action has not been defined clearly. Modafinil research has focused on orexin receptors, adrenoceptors and serotonin receptors (Wisor and Eriksson, 2005; Ballon and Feifel, 2006). However, the role of each receptor is unclear, and it appears that none of these candidates are solely responsible for modafinil’s effects. Another possible mechanism is elevation of extracellular dopamine levels, the mechanism of action traditionally attributed to cocaine and amphetamine’s efficacy (Pierce and Kumaresan, 2006). Cocaine and amphetamine function as an inhibitor and substrate of the dopamine transporter, respectively (Chen and Reith, 2002; Sulzer et al., 2005). Mignot and colleagues determined that modafinil, at concentrations of 10 μM and greater, bound to the dopamine transporter and inhibited [3H]dopamine uptake in rat brain neurons (Mignot et al., 1994). Madras and colleagues recently reported that modafinil inhibited (IC50 value=6 μM) [3H]dopamine uptake in human dopamine transporter expressed in human embryonic kidney cells, and occupied dopamine transporter in monkey striatum after injection (5 mg/kg, i.v.) (Madras et al., 2006). Microdialysis studies determined that modafinil (100 mg/kg) increased extracellular dopamine levels in brain (Ferraro et al., 1996), although modafinil was less potent (~ 50-fold) than amphetamine (Nishino et al., 1998). It is important to note that not all modafinil research indicates a role for dopamine (Akaoka et al., 1991; De Sereville et al., 1994; Simon et al., 1995). For example, in a superfusion assay, modafinil (10 μM) was ineffective to evoke [3H]overflow from mouse striatal synaptosomes preloaded with [3H]dopamine (Simon et al., 1995). However, based on the research investigating modafinil and the dopamine transporter (Mignot et al., 1994; Madras et al., 2006), higher modafinil concentrations (≥ ~ 10 μM) may have been necessary to observe [3H]overflow.

A mechanism that has not received attention is acetylcholine activity, specifically that of nicotinic acetylcholine receptors. The nicotinic acetylcholine receptor agonist nicotine shares some behavioral properties with modafinil in human and animals (Edgar and Seidel, 1997; Beracochea et al., 2001; Le Foll and Goldberg, 2006) and evokes dopamine release from brain (Mansvelder et al., 2002). As such, it is possible that nicotinic acetylcholine receptors mediate the effect of modafinil to change behavior and evoke neurotransmitter release.

The objective of the present study was to investigate the mechanism of action for modafinil, using behavioral and in vitro techniques. In drug discrimination experiments using rats, a high or low cocaine dose or amphetamine was trained as the discriminative stimulus. Subsequently, modafinil substitution for the stimulus and the effect of modafinil pretreatment on the stimulus were determined. The drug discrimination experiments were performed to understand better modafinil’s interaction with amphetamine and cocaine in a behavioral assay. To understand modafinil’s effects in a neurochemical assay, the concentration-response profile for modafinil to evoke [3H]overflow from rat striatal slices preloaded with [3H]dopamine was determined. The profiles for amphetamine and nicotine also were determined as comparison drugs. To investigate the interaction of modafinil with the dopamine transporter, the effect of the dopamine transporter inhibitor nomifensine on modafinil-evoked [3H]overflow, and the effect of modafinil on amphetamine-evoked [3H]overflow were determined. To investigate the interaction of modafinil with nicotinic receptors, the effects of the antagonist mecamylamine on modafinil-evoked [3H]overflow and modafinil on nicotine-evoked [3H] overflow were determined.

2. Materials and methods

2.1. Animals

The University of Missouri Institutional Animal Care and Use Committee approved the present procedures. Male Sprague–Dawley rats (Harlan, Indianapolis IN) were housed two subjects per cage and received ad libitum access to tap water. For the [3H]overflow experiment, rats received ad libitum access to standard rodent chow. For the drug discrimination experiment, rats received restricted access to chow (~ 80% of daily consumption by rats that receive ad libitum access), and as a consequence, rat body weights were maintained at ~ 400 g. The colony was maintained under a 12-hour/12-hour light/dark cycle and all experiments were conducted during the light phase.

2.2. Drugs

Throughout the manuscript, drug concentrations and doses represent the free base weight.

d-Amphetamine SO4, cocaine HCl, and nomifensine C21H26O6 were purchased from Sigma Chemical Co. (St. Louis MO). For the drug discrimination assay, modafinil (Cephalon, Frazer PA) was purchased from Hy-Vee Pharmacy (Macomb IL). Modafinil was purchased from Sigma for the [3H]overflow assay. Ascorbic acid, glucose and pargyline HCl were purchased from Acros Organics (Fairlawn NJ). Radiolabeled dopamine (dihydroxyphenylethylamine 3.4-([ring-2,5,6-3H]) was purchased from PerkinElmer Life Sciences (Boston MA). All other chemicals were purchased from Fisher Scientific (Pittsburgh PA).

For the drug discrimination assay, amphetamine and cocaine were dissolved in saline (0.9% w/v) vehicle. The drug injection volume was 1 ml solution/kg body weight. Cocaine was administered i.p. and amphetamine was administered s.c. Modafinil was dissolved in a water vehicle containing C3H6OS (DMSO,
0.1% v/v) and administered p.o. via gavage at a volume of 1 ml solution/kg body weight.

2.3. Drug discrimination assay

Standard operant chambers (ENV-001; Med Associates, Georgia VT) were used. The sidewalls of the chamber were aluminum, the front and back walls were clear Plexiglas, and the floor consisted of stainless steel rods. A recessed receptacle (5 x 4.2 cm) was located at the bottom-center of one of the sidewalls of the chamber and response levers were located on either side of the receptacle. Responses made on the active lever were reinforced, and responses made on the other, inactive lever were recorded but had no scheduled consequence (i.e., were not reinforced). Completion of the response requirement resulted in delivery of a food pellet (20 mg; Bio-Serv, Frenchtown NJ) into the receptacle. All stimulus and response events were recorded and controlled by a computer running Med Associates’ Med PC-IV Software.

Rats were initially trained to respond on the levers in the operant chamber. Responding on either lever was maintained by a fixed-ratio (FR) 1 schedule of pellet delivery. Once responding was established on FR 1, the ratio requirement for delivery was gradually increased to FR 10. Subsequently, rats were assigned randomly to 1 of 3 groups in which the discriminative stimulus was 0.3 mg/kg amphetamine (n=6 rats), 1.6 mg/kg cocaine (n=6 rats) or 5 mg/kg cocaine (n=7 rats), and training commenced. The amphetamine dose was selected because it is within the range of doses typically used in rodent drug discrimination studies (Stolerman et al., 1995). The cocaine doses are less than the typical cocaine dose (10 mg/kg) used in other experiments (Stolerman et al., 1995; Schechter, 1997). Our previous research demonstrated that these doses can serve as a discriminative stimulus. Furthermore, specific generalization to the stimulus was observed with drugs from various pharmacological classes (Cunningham et al., 2006).

In these training sessions, rats were administered drug or saline, returned to the home cage for 10 min, and then placed in the chamber where only one of the levers was active (i.e., delivered pellets). Following administration of drug, one lever was active, and following administration of saline, the other lever was active. The lever designated as drug-paired was counterbalanced within each stimulus group. Each session was terminated after 15 min or 300 total responses, whichever occurred first. Only one training session was conducted daily, and the presentation of drug or saline across sessions adhered to the following pattern of saline (S) and drug (D): S, D, S, S, D, S, S, S, D, D.

Within a session, performance was assessed on the first completed ratio. A correct lever selection was recorded when the rat made 10 responses on the injection-appropriate lever with no more than 5 responses on the incorrect lever. The criterion for acquisition (stimulus control) for each rat was correct lever selection on 8 out of 10 successive daily sessions. These criteria for stimulus control were selected from previous studies conducted by the experimenter (Nation et al., 2000). After all rats reached stimulus control, substitution and drug interaction tests began. In the substitution and interaction test sessions, both levers were active and 10 responses on either lever resulted in pellet delivery. At least five daily training sessions followed each test session. The order of saline and drug administration on these subsequent training sessions followed the pattern described for initial training toward stimulus control.

Substitution tests examined responding after administration of different modafinil doses. Rats were administered 32, 64 or 128 mg/kg modafinil or vehicle and returned to the home cage. Following a 10–240 min delay, rats were placed in the operant chamber. After delivery of a pellet or 15 min, rats were removed from the box. This procedure was followed at a 10, 30, 60, 120 and 240 min delay after the modafinil or vehicle injection. As such, each modafinil dose was evaluated at each of the five time points in a single daily session. The modafinil doses were selected based on previous experiments using rodents (Gold and Balster, 1996; Edgar and Seidel, 1997; Beracochea et al., 2001); however, the 128 mg/kg dose was the maximal dose because it was the limit of solubility in vehicle.

In the interaction tests, the effect of pretreatment with modafinil or vehicle on cocaine and amphetamine was determined. Rats were administered 32 mg/kg modafinil or vehicle, and returned to the home cage for 50 min. Rats in the 1.6 mg/kg and 5 mg/kg cocaine groups were administered cocaine (0.016–1.6 mg/kg and 0.16–5 mg/kg, respectively), and those in the amphetamine group were administered amphetamine (0.01–0.3 mg/kg). After the cocaine or amphetamine injection, rats were returned to home cage for 10 min, and then placed in the operant chamber. The effect of modafinil or vehicle on a different cocaine or amphetamine dose was determined on a subsequent session. The modafinil dose and the duration between modafinil injection and placement in the operant chamber were selected based on the results of the substitution tests (Figs. 1 and 2), as a dose and duration that did not result in substitution for the discriminative stimulus.

For the substitution and interaction tests, response rates were the first dependent measure and were calculated by dividing the total number of responses on both levers by the total session time. If no responses were performed on either lever, a rate of zero was assigned. Separate statistical analyses were performed for each of the three discriminative stimulus groups. For the substitution test, data were analyzed via 2-way repeated-measures analysis of variance (RM-ANOVA; SPSS, version 10.0, Chicago IL) with Modafinil Dose and Time as within-subject factors. For the interaction test, response rate data were analyzed via 1-way repeated-measures ANOVA with Cocaine or Amphetamine Dose as a within-subject factor. Tukey post hoc tests were performed when appropriate (P<0.05). The second dependent measure was the percentage of responses on the cocaine- or amphetamine-paired lever (the number of responses on the drug-paired lever divided by the total number of responses on the drug- and saline-paired levers). Full substitution was defined as greater than 80% of responses performed on the amphetamine- or cocaine-paired lever and partial substitution was defined as 40–80% of responses performed on the drug-paired lever (Appel et al., 1982). ED50 values were determined via linear regression (Graph Pad Prism, version 3.03, San Diego CA). For the interaction tests, the
slopes and intercepts (elevations) of the lines were calculated, and significant differences in these values in the presence and absence of modafinil were revealed via t-tests.

2.4. [3H]Overflow assay

Rats were euthanized via rapid decapitation and striata were dissected and sliced (750 μm thick slices). Slices were incubated in oxygenated buffer (in mM, 108 NaCl, 25 NaHCO₃, 11.1 glucose, 4.7 KCl, 1.3 CaCl₂, 1.2 MgSO₄, 1.0 Na₂HPO₄, 0.11 ascorbic acid, 0.004 EDTA) in a metabolic shaker at 37 °C for 30 min. Slices were transferred to fresh buffer, [3H]dopamine (0.1 μM) was added, and slices were incubated for an additional 30 min. Each slice was then transferred to 1 of 12 reaction chambers (0.2 ml) bounded by glass microfiber filters (GF/B, Whatman, Maidstone England) in an automated superfusion system (Suprafusion 2500, Brandel, Gaithersburg MD). Pargyline (10 μM) was included in buffer to inhibit monoamine oxidase (Westerink and Kikkert, 1986). Slices were superfused at a rate of 0.75 ml/min. After 65 min, sample collection commenced at a rate of 1 sample per 2 min.

[3H]Overflow Experiment 1 determined the effect of modafinil, amphetamine and nicotine. Five baseline samples were collected. Modafinil (0.1–300 μM), amphetamine (1–10 μM) and nicotine (10–100 μM) were added and superfusion continued for 6 min. The modafinil concentrations were selected from the range where modafinil was active in vitro (Mignot et al., 1994; Madras et al., 2006), and the amphetamine and nicotine concentrations were selected based on our previous [3H]overflow experiments and the literature (Teng et al., 1997; Miller et al., 2005). One slice was superfused in the absence of drug and represented a control condition. Slices were then superfused with only buffer for 20 min. Upon completion of superfusion, slices and filters were removed from the reaction chamber. Radioactivity in superfusate samples and slices/filters was measured by liquid scintillation spectroscopy (LS 6500 Scintillation Counter, Beckman-Coulter, Fullerton CA; counting efficiency ≈ 60%).

Fractional release for each superfusate sample was calculated by dividing the [3H] collected in each 2 min sample by the total [3H] present in the tissue at the time of sample collection. Basal...
\[ ^{[3} \text{H}] \text{outflow} \text{ was calculated from the average of fractional release in the five samples just before the addition of the compound. The fractional samples greater than baseline were summed and basal outflow was subtracted to determine total \[ ^{[3} \text{H}] \text{overflow}. \]

For \[ ^{[3} \text{H}] \text{Overflow Experiment 1}, separate series of analyses were performed for modafinil, amphetamine and nicotine. Each set of total \[ ^{[3} \text{H}] \text{overflow} \text{ data was analyzed via 1-way RM-ANOVA with Modafinil Concentration, Amphetamine Concentration or Nicotine Concentration as within-subject factors. Fractional \[ ^{[2} \text{H}] \text{release} \text{ data (i.e., the time course) were analyzed via 2-way RM-ANOVA with Time and Modafinil Concentration, Amphetamine Concentration or Nicotine Concentration as within-subject factors. Tukey post hoc comparisons were performed when appropriate (P<0.05).}

\[ ^{[3} \text{H}] \text{Overflow Experiment 2} \text{ determined the concentration-response function for modafinil in the presence of an excess concentration (10 \mu M) of the dopamine transporter inhibitor nomifensine. The 10 \mu M concentration is \sim 67-fold greater than the IC}_{50} \text{ value (0.15 \mu M) for nomifensine to inhibit \[ ^{[3} \text{H}] \text{dopamine uptake in rat brain (Hunt et al., 1974). The procedure was similar to that for \[ ^{[3} \text{H}] \text{Overflow Experiment 1}; however, buffer contained nomifensine for the entire period of superfusion. Total \[ ^{[3} \text{H}] \text{overflow} \text{ data were analyzed via 1-way RM-ANOVA with Modafinil Concentration as a within-subject factor. Fractional \[ ^{[2} \text{H}] \text{release} \text{ data were analyzed via 2-way RM-ANOVA with Modafinil Concentration and Time as within-subject factors. A supplemental analysis was performed to compare the concentration-response function for modafinil in the presence and absence of nomifensine (comparison of \[ ^{[3} \text{H}] \text{Overflow Experiments 1 and 2). Comparison of the control conditions (superfusion in the absence of modafinil) revealed that there was greater (\sim 110\%) basal \[ ^{[3} \text{H}] \text{overflow} \text{ in the presence than in the absence of nomifensine (Table 1). As such, total \[ ^{[3} \text{H}] \text{overflow} \text{ data were calculated as a percent of basal \[ ^{[3} \text{H}] \text{overflow}. These data were analyzed via 2-way RM-ANOVA with Modafinil Concentration as a within-subject factor and Nomifensine Concentration as a between-group factor.}

\[ ^{[3} \text{H}] \text{Overflow Experiment 3} \text{ determined the effect of modafinil on amphetamine-evoked \[ ^{[3} \text{H}] \text{overflow}. Buffer did not contain nomifensine for this and each subsequent experiment. After the collection of five baseline samples, modafinil (0.01–30 \mu M) was added and superfusion continued for 10 min. Amphetamine (1 or 3 \mu M) was added for 6 min. Slices were then superfused with buffer that did not contain modafinil or amphetamine for 14 min. The modafinil concentrations were selected as those that did not have intrinsic activity (Fig. 4) and the amphetamine concentrations were determined from the results of our previous research (Miller et al., 2005). One control slice was superfused with only amphetamine, and a second control slice was superfused with only buffer. Total \[ ^{[3} \text{H}] \text{overflow} \text{ data were analyzed via 2-way RM-ANOVA with Modafinil Concentration as a within-subject factor and Amphetamine Concentration as a between-group factor. Fractional \[ ^{[2} \text{H}] \text{release} \text{ data were analyzed via 3-way RM-ANOVA with Modafinil Concentration and Time as within-subject factors and Amphetamine Concentration as a between-group factor.}

\[ ^{[3} \text{H}] \text{Overflow Experiment 4} \text{ determined if modafinil-evoked \[ ^{[3} \text{H}] \text{overflow} \text{ is altered by the nicotinic acetylcholine receptor antagonist mecamylamine (Papke et al., 2001). After the collection of 5 baseline samples, mecamylamine (0.1–10 \mu M), within the concentration used to inhibit the effect of nicotine in the slice superfusion assay (Teng et al., 1997), was added to buffer. Slices were superfused with mecamylamine for 6 min, modafinil (100 \mu M) was added to buffer, and samples were collected for an additional 6 min. Slices were then superfused for 14 min with buffer that did not contain mecamylamine or modafinil. One control slice was superfused with only modafinil, and a second control slice was superfused without mecamylamine or modafinil.}

\[ ^{[3} \text{H}] \text{Overflow Experiment 5} \text{ determined the effect of modafinil on nicotine-evoked \[ ^{[3} \text{H}] \text{overflow}. After the collection of five baseline samples, modafinil (0.01–30 \mu M) was added and superfusion continued for 10 min. Nicotine (10 \mu M) was added for 6 min. Slices were then superfused with buffer that did not contain modafinil or nicotine for 14 min. The modafinil concentrations were selected as those that did not have intrinsic activity (Fig. 4) and the nicotine concentration was selected from \[ ^{[3} \text{H}] \text{Overflow Experiment 1 (Fig. 5). One control slice was superfused with only nicotine, and a second control slice was superfused without modafinil or nicotine.}

For \[ ^{[3} \text{H}] \text{Overflow Experiments 4 and 5}, total \[ ^{[3} \text{H}] \text{overflow} \text{ data were analyzed via 1-way RM-ANOVA with Mecamylamine Concentration and Modafinil Concentration as within-subject factors, respectively.}

\section{3. Results}

\subsection{3.1. Drug discrimination training}

The effect of drugs selective for nicotinic acetylcholine receptors on the discriminative stimulus properties of cocaine
and amphetamine were initially examined in the rats used in the present drug discrimination experiments. These data and a detailed description of the acquisition of stimulus control are presented and described in our earlier manuscript (Cunningham et al., 2006). Briefly, there was a significant difference among the stimulus groups in the session in which stimulus control was achieved. Rats in the amphetamine stimulus group achieved stimulus control faster (median = 26 sessions) than rats in the 1.6 and 5 mg/kg cocaine stimulus groups (median values = 41 and 35 sessions, respectively). There were no significant differences between the two cocaine stimulus groups.

3.2. Modafinil substitutes partially for the cocaine and amphetamine discriminative stimulus

Rats were administered 32, 64 or 128 mg/kg modafinil or vehicle and then placed in the operant chamber after a 10, 30, 60, 120 and 240 min delay to determine if modafinil substitutes for the discriminative stimulus. The dose-response curves for the cocaine groups are presented in Fig. 1 and the curves for the amphetamine group are presented in Fig. 2.

Analysis of response rate data revealed significant main effects of Modafinil Dose \( (F(2,28) = 21.32, P<0.001) \) and Time \( (F(4,56) = 8.42, P<0.001) \). The Modafinil Dose × Time interaction was not significant, and there were no significant differences in response rates among the three discriminative stimulus groups. Post hoc tests determined that, overall, response rates were higher after administration of 64 and 128 mg/kg modafinil, than after administration of 32 mg/kg modafinil or vehicle. Also, response rates were lower at the 10 min point than at the 60, 120 or 240 min time points. Response rates at the 30 min time point were not significantly different from rates at any of the other time points, and there were no differences among the 60, 120 or 240 min time points.

Regarding the percentage of responses on the cocaine- or amphetamine-paired levers, vehicle did not substitute for any of the three stimuli at any time point. The 32 mg/kg modafinil dose did not substitute for either cocaine discriminative stimuli or for the amphetamine discriminative stimulus at any time point. The 64 mg/kg modafinil dose substituted partially for the 1.6 mg/kg cocaine stimulus at the 30, 60 and 120 min time points, and for the amphetamine SD at the 120 min time point. The 64 mg/kg modafinil dose did not substitute for the 5 mg/kg cocaine stimulus at any time point. The 128 mg/kg modafinil dose substituted partially for the 1.6 mg/kg cocaine stimulus at the 60 and 120 min time points. The 128 mg/kg modafinil dose

![Graphs showing dose-response curves for cocaine and amphetamine groups.](image-url)
substituted partially for the 5 mg/kg cocaine stimulus at the 60 min time point and for the amphetamine stimulus at the 30 min time point.

3.3. Modafinil augments the discriminative stimulus properties of cocaine and amphetamine

In the interaction tests, rats were administered modafinil (32 mg/kg) or vehicle followed by cocaine or amphetamine and these data are presented in Fig. 3. Regarding response rate data, the main effect of Modafinil Dose was not significant, and there were no significant differences in response rates among the three discriminative stimulus groups.

Regarding the percentage of responses on the drug-paired lever, when rats in the 1.6 mg/kg cocaine stimulus group were administered vehicle before cocaine, the ED$_{50}$ value for cocaine was 0.85 mg/kg. However, the ED$_{50}$ value for cocaine was 0.13 mg/kg when rats were administered modafinil before cocaine. There was no significant difference in the slope of the lines between the absence (mean=44%/mg, S.E.M.=±10%/mg) and presence (mean=38%/mg, S.E.M.=±9%/mg) of modafinil pretreatment. There was a significant difference in the intercepts between the absence (mean=4%, S.E.M.=±3%) and presence (mean=26%, S.E.M.=±23%) of modafinil pretreatment (t(38)=13.32, P<0.01). This suggests that modafinil pretreatment shifted the cocaine dose-response curve to the left.

For the 5 mg/kg cocaine stimulus group, identical ED$_{50}$ values (1.4 mg/kg) were calculated in the absence and presence of modafinil pretreatment. There was no difference between the absence (mean=20%/mg, S.E.M.=10%/mg) and presence (mean=17%/mg, S.E.M.=13%/mg) of modafinil in the slope of the lines, and there was no difference between the absence (mean=11%, S.E.M.=±18%) and presence (mean=17%, S.E.M.=±14%) of modafinil pretreatment in the intercepts. This indicates that modafinil did not alter the cocaine dose-response curves for this cocaine dose.

For the amphetamine stimulus group, when rats were administered vehicle before amphetamine, the ED$_{50}$ value for amphetamine was 0.13 mg/kg. The ED$_{50}$ value for amphetamine was 0.04 mg/kg when rats were administered modafinil before amphetamine. There was no significant difference in the slope of the lines between the absence (mean=334%/mg, S.E.M.=±58%/mg) and presence (mean=276%/mg, S.E.M.=±84%/mg) of modafinil pretreatment. There was, however, a significant difference in the intercepts between the absence (mean=0%, S.E.M.=±2%) and presence (mean=23%, S.E.M.=±13%) of modafinil pretreatment (t(27)=3.32, P<0.05). This difference suggests that modafinil pretreatment shifted the amphetamine dose-response curve to the left.
3.4. Modafinil, amphetamine and nicotine evoke $[3^\text{H}]$overflow

In $[3^\text{H}]$Overflow Experiment 1, the concentration-response profile for modafinil to evoke $[3^\text{H}]$overflow from rat striatal slices preloaded with $[3^\text{H}]$dopamine was determined. Total $[3^\text{H}]$ overflow and fractional $[3^\text{H}]$ release (i.e., the time course) are presented in Fig. 4. Regarding the measure of total $[3^\text{H}]$ overflow, a significant main effect of Modafinil Concentration was found ($F(6,48)=5.47, P<0.001$). Post hoc tests revealed that $[3^\text{H}]$overflow was greater in the presence of 100 and 300 μM modafinil than in the control condition (superfusion in the absence of modafinil). Regarding fractional $[3^\text{H}]$ release, a significant Modafinil Concentration × Time interaction was found ($F(114,912)=5.15, P<0.001$). Post hoc tests determined that there was greater fractional $[3^\text{H}]$ release for 300 μM modafinil than for control at the 16, 18 and 20 min time points. There was greater fractional $[3^\text{H}]$ release for 100 μM than for control at the 18 and 20 min time points. None of the other modafinil concentrations significantly increased $[3^\text{H}]$ release, relative to control, at any time point.

Amphetamine-evoked $[3^\text{H}]$overflow was also determined, and fractional $[3^\text{H}]$ release data are presented in Fig. 5. For the total $[3^\text{H}]$overflow measure, a significant main effect of Amphetamine Concentration was found ($F(2,16)=48.63, P<0.001$). The 10 μM amphetamine concentration (mean=25.5%, S.E.M.=± 3.1%) evoked more $[3^\text{H}]$overflow than the control condition (superfusion in the absence of amphetamine; mean =0.4%, S.E.M.=± 0.4%). Similarly, there was greater overflow in the presence of 1 μM amphetamine (mean=3.4%, S.E.M.=± 1.6%) than in the control. Regarding fractional $[3^\text{H}]$ release, a significant Amphetamine Concentration × Time interaction was found ($F(38,304)=31.07, P<0.001$). Post hoc tests determined that there was greater

Fig. 6. Nomifensine diminishes modafinil-evoked $[3^\text{H}]$overflow from rat striatal slices preloaded with $[3^\text{H}]$dopamine. Experiments were conducted in the absence and presence of nomifensine (0 or 10 μM, respectively). Rat striatal slices were superfused with buffer 75 min. Subsequently, modafinil was added for 6 min, and then slices were superfused with buffer that did not contain modafinil for 20 min. Due to differences between the control condition (superfusion in the absence of modafinil) in the presence and absence of modafinil, total $[3^\text{H}]$overflow data were calculated as a percent of the respective control (total $[3^\text{H}]$overflow data are presented in Table 1). Data in the figure represent the mean (±S.E.M.) percent of control measure. Asterisks denote a significant difference from the 0 μM nomifensine condition at the respective modafinil concentration ($n=9–10$ rats/group).

Fig. 7. Modafinil attenuates amphetamine (1 and 3 μM)-evoked $[3^\text{H}]$overflow from rat striatal slices preloaded with $[3^\text{H}]$dopamine. Slices were superfused with buffer for 75 min, modafinil (0.01–30 μM) was added for 10 min, amphetamine (1 or 3 μM) was added for 6 min, and then slices were superfused with buffer that did not contain modafinil or amphetamine for 14 min. The top panel depicts mean (±S.E.M.) total $[3^\text{H}]$overflow after the addition of amphetamine. Asterisks denote a significant difference from the respective control condition (superfusion in the presence of 1 or 3 μM amphetamine and absence of modafinil). The center panel depicts mean (±S.E.M.) fractional $[3^\text{H}]$ release for the experiment with 1 μM amphetamine and the bottom panel depicts $[3^\text{H}]$release for the experiment with 3 μM amphetamine. On the abscissa for the center and bottom panels, the time point designated as zero represents the start of sample collection. The downward arrow on the left marks the addition of modafinil, and the downward arrow on the right represents the addition of amphetamine. The upward arrow represents the removal of modafinil and amphetamine ($n=5–6$ rats/experiment).
fractional [3H] release for the 10 μM amphetamine concentration than for the control at the 14–40 min time points. There was significantly greater fractional [3H] release for the 1 μM amphetamine concentration than for the control at the 20 min time point.

The effect of nicotine was determined, and fractional [3H] release data are presented in Fig. 5. Analysis of total [3H] overflow data revealed a significant main effect of Nicotine Concentration (F(2,16)=36.81, P<0.001). Post hoc tests determined that the 100 μM nicotine concentration (mean=8.4%, S.E.M.=± 1.2%) evoked more [3H]overflow than the control condition (superfusion in the absence of nicotine; mean=0.4%, S.E.M.=± 0.4%). Also, there was greater overflow in the presence of 10 μM nicotine (mean=1.1%, S.E.M.=± 0.2%) than in the control. A significant Nicotine Concentration × Time interaction was found for fractional [3H] release (F(38,304)=36.71, P<0.001). Post hoc tests determined that there was greater [3H] release for the 100 μM nicotine concentration than for control at the 14–24 min time points. There was greater [3H] release for the 10 μM nicotine concentration than for the control only at the 20 min time point.

3.5. Modafinil-evoked [3H]overflow is diminished in the presence of nomifensine

[3H]Overflow Experiment 2 determined the concentration-response profile for modafinil in the presence of nomifensine. Total [3H]overflow data are presented in Table 1. A significant main effect of Modafinil Concentration was found (F(6,48)=3.46, P<0.001) and post hoc tests determined that 300 μM modafinil significantly increased [3H]overflow, relative to the control condition (superfusion in the absence of modafinil). Regarding fractional [3H] release, the Modafinil Concentration × Time interaction was not significant.

A second series of analyses were performed to compare the concentration-response curves for modafinil from [3H]Overflow Experiments 1 and 2 (i.e., the concentration-response profile in the absence and presence of nomifensine). As described, total [3H]overflow data (Table 1) were calculated as a percent of control ([3H]overflow in the absence of modafinil). These transformed data are presented in Fig. 6. Significant main effects of Modafinil Concentration (F(6,96)=6.96, P<0.001) and Nomifensine Concentration (F(1,16)=14.64, P<0.01), and a significant interaction of Modafinil Concentration × Nomifensine Concentration (F(6,96)=2.84, P<0.05) were found. Post hoc comparisons determined that there was greater [3H]overflow in the absence, than in the presence, of nomifensine at the 30, 100 and 300 μM modafinil concentrations.

3.6. Modafinil diminishes amphetamine-evoked [3H]overflow

The effect of modafinil on amphetamine-evoked [3H] overflow was determined in [3H]Overflow Experiment 3. Total [3H]overflow and fractional [3H] release are presented in Fig. 7. Regarding the measure of total [3H]overflow, significant main effects of Amphetamine Concentration (F(1,7)=19.50, P<0.001) and Modafinil Concentration (F(5,35)=2.62, P<0.05) and a significant Amphetamine Concentration × Modafinil Concentration interaction (F(5,35)=3.07, P<0.05) were found. Consistent with our previous experiment, amphetamine increased [3H]overflow. Overall, 1 and 3 μM amphetamine evoked greater [3H]overflow than superfusion with only buffer (control). Regarding the interaction of modafinil and amphetamine, the 0.01, 0.1, 10 and 30 μM modafinil concentrations significantly diminished [3H]overflow, relative to the control slice superfused only with 1 μM amphetamine. Only the 30 μM modafinil concentration significantly diminished [3H]overflow evoked by the 3 μM amphetamine concentration.

With respect to the time course, significant main effects of Modafinil Concentration (F(5,35)=2.67, P<0.05), Time (F(19,133)=25.53, P<0.001), and an Amphetamine Concentration × Modafinil Concentration × Time interaction (F(95,665)=1.57, P<0.01) were found. For the 1 μM amphetamine concentration, post hoc tests did not reveal significant differences among the modafinil concentrations at any time point. For the 3 μM amphetamine concentration, post hoc tests determined that there was significantly less fractional [3H]overflow in the presence of 30 μM modafinil and amphetamine than in the control at the 26, 28 and 30 min time points. There was less [3H]overflow in the presence of 1 and 10 μM modafinil and amphetamine than in the control at the 28 min time point.

3.7. Mecamylamine does not alter modafinil-evoked [3H] overflow

The effect of mecamylamine on modafinil-evoked [3H] overflow was determined. Addition of 100 μM modafinil to buffer increased total [3H]overflow (mean=2.75%, S.E.M.=±0.50%), relative to the slice superfused in the absence of mecamylamine and modafinil (mean=0.39%, S.E.M.=±0.10%). However, 0.1 μM (mean=2.03%, S.E.M.=±0.37%), 1 μM (mean=2.99%, S.E.M.=±0.42%) and 10 μM (mean=2.51%, S.E.M.=±0.90%) mecamylamine did not significantly alter this effect of modafinil.

3.8. Modafinil does not alter nicotine-evoked [3H] overflow

The effect of modafinil on nicotine-evoked [3H] overflow was determined. Addition of nicotine (10 μM) increased total [3H]overflow (mean=2.09%, S.E.M.=±0.72%), relative to the slice superfused without nicotine (mean=0.02%, S.E.M.=±0.02%). However, 0.1 μM (mean=1.48%, S.E.M.=±0.50%), 1 μM (mean=1.54%, S.E.M.=±0.45%), 10 μM (mean=1.41%, S.E.M.=±0.27%), and 30 μM (mean=1.65%, S.E.M.=±0.99%) modafinil did not significantly alter this effect of nicotine.

4. Discussion

The present study investigated modafinil, a clinically used psychostimulant that is reported to have relatively-low abuse potential, in behavioral and neurochemical assays (Warot et al.,...
1993; Gold and Balster, 1996; Jasinski and Kovacevic-Ristanovic, 2000; Myrick et al., 2004). Despite modafinil’s clinical successes and its efficacy to alter behavior in the preclinical laboratory, the mechanism of action for modafinil in brain has not been clearly defined.

The drug discrimination procedure was used to measure the subjective properties of modafinil in rats that were trained to discriminate cocaine or amphetamine from vehicle. The drug discrimination assay can be used as a behavioral test to investigate drug mechanism (Colpaert, 1999), and the use of amphetamine and cocaine allowed for the interaction of modafinil with a typical dopamine transporter substrate and inhibitor respectively, to be determined. The rats in the 1.6 mg/kg cocaine stimulus group were trained with a cocaine dose that is markedly lower than the dose range (≤10 mg/kg) typically used in rat drug discrimination experiments (Schechter, 1997). Our previous research (Cunningham et al., 2006) and the present substitution and interaction tests revealed differences between the two cocaine stimulus groups. In the present substitution tests when rats were administered vehicle followed by different cocaine doses, the cocaine dose-response curve for the 1.6 mg/kg cocaine stimulus group was to the left of the curve for the 5 mg/kg cocaine stimulus group (ED50 value = 0.85 mg/kg) was to the left of the curve for the 5 mg/kg cocaine stimulus group (ED50 value = 1.4 mg/kg). Also, the threshold cocaine dose for partial and full substitution was higher for the 5 mg/kg cocaine stimulus group than for the 1.6 mg/kg cocaine stimulus group. Overall, these data indicate that a unique subjective state was induced by the 1.6 and 5 mg/kg cocaine doses.

The 64 and 128 mg/kg modafinil doses increased response rates, relative to responding after administration of vehicle or 32 mg/kg modafinil. The increase is consistent with other animal research (Edgar and Seidel, 1997) and the classification of modafinil as a psychostimulant. Other studies determined that modafinil doses greater than 128 mg/kg produced a decrease in behavior (Gold and Balster, 1996). This modafinil dose-dependent increase and then decrease in response rates results in an “inverted-U” curve that has been observed for other psychostimulants (Branch, 1984; Harland et al., 1989).

Modafinil only partially substituted for the cocaine or amphetamine discriminative stimulus. The substitution was time-dependent and most pronounced 60–120 min after modafinil administration. The majority of the responses were performed on the saline-paired lever early (10 min) and late (240 min) after modafinil administration. The partial substitution also was modafinil dose dependent. There was greater substitution of modafinil to the 1.6 mg/kg cocaine dose than to the 5 mg/kg cocaine dose. Gold and Balster (1996) reported only partial substitution of modafinil (150–250 mg/kg) for rats trained with a stimulus of 10 mg/kg cocaine. For the present research, 128 mg/kg modafinil was the upper bound of doses examined, and full substitution might have been observed if higher (>128 mg/kg) doses were tested. However, it is important to note that at doses above 128 mg/kg, modafinil decreased response rates (Gold and Balster, 1996), and it would be difficult to attribute substitution to a specific modafinil effect. Considered overall, these data suggest that modafinil produces a subjective state that is only slightly similar to cocaine and amphetamine in rodents. In human studies, modafinil produced an interoceptive state distinct from these stimulants (Warot et al., 1993; Rush et al., 2002). Overall, this pattern is consistent with the classification of modafinil as a mild psychostimulant.

The 32 mg/kg modafinil dose, which did not substitute for the amphetamine or cocaine discriminative stimulus, augmented the interoceptive properties of amphetamine and the 1.6 mg/kg cocaine dose in the interaction test. Pretreatment with modafinil shifted the dose-response curve ~6-fold to the left for cocaine and ~3-fold for amphetamine. Thus, a modafinil dose, which did not substitute for the state induced by amphetamines or cocaine, enhanced the psychological state produced by the more-potent psychostimulants. This effect of modafinil to augment the stimulus properties of cocaine and amphetamine had not been reported. The 32 mg/kg modafinil dose that produced the shift in the dose-response curve is lower than the threshold dose range to observe changes in dopamine activity brain (Ferraro et al., 1996; Nishino et al., 1998). As such, it is possible that the low-dose interactions are not dopamine-mediated, a finding germane to the present [3H]overflow research.

Modafinil (≥100 μM) evoked [3H]overflow from rat striatal slices in a concentration-dependent manner. Research on the effect of modafinil to increase extracellular levels is controversial. In some in vivo assays, modafinil (64–256 mg/kg) did not increase extracellular dopamine levels (Akaoka et al., 1991; De Sereville et al., 1994) and inhibited the activity of midbrain dopamine neurons (Korotkova et al., 2007). Also, a relatively-low modafinil concentration (10 μM) did not evoke [3H]dopamine release from mouse striatal synaptosomes (Simon et al., 1995). However, the present results are consistent with in vivo microdialysis experiments in which administration of a behaviorally-active modafinil dose (100 mg/kg, p.o. or 5 mg/kg, i.v.) increased extracellular dopamine concentrations (Ferraro et al., 1996; Nishino et al., 1998). Interestingly, the results from these latter studies, in which systemic modafinil administration increased dopamine levels and/or altered transporter function, suggest that the present [3H]overflow analysis investigated modafinil concentrations that would be active in behavioral assays (such as the present drug experiment experiments) at higher doses (64–128 mg/kg).

Modafinil was compared to amphetamine and nicotine in the [3H]overflow assay. The threshold concentrations for amphetamine and nicotine were 1 and 10 μM, respectively, while the threshold concentration for modafinil was 100 μM. Although the effect of higher (>10 μM) amphetamine concentrations was not determined, overall comparison of the data with the three drugs suggests that modafinil evoked much less [3H]dopamine than nicotine or amphetamine. Thus, the present research indicates that modafinil evokes dopamine, but it is less potent and efficacious than other psychostimulants. This low in vitro potency and efficacy may explain why relatively-high (≥100 mg/kg, p.o. or 5 mg/kg, i.v.) modafinil doses are necessary to observe a change in behavior (Gold and Balster, 1996; Edgar and Seidel, 1997; Beracochea et al., 2001).

Modafinil, at concentrations (≤30 μM) that did not have intrinsic activity, blocked the activity of the dopamine transporter substrate amphetamine to evoke [3H]overflow.
Furthermore, the dopamine transporter inhibitor nomifensine completely blocked the \[^{3}H\]overflow evoked by 100 μM modafinil and attenuated (∼55%) the \[^{3}H\]overflow evoked by 300 μM modafinil. These results suggest that modafinil has affinity for the transporter and that the effect of modafinil to evoke dopamine release is mediated by the dopamine transporter. This finding is in contrast to a recent report in which nomifensine (1 μM) did not interfere with the effect of modafinil (50 μM) to inhibit firing of midbrain dopamine neurons (Korotkova et al., 2007). However, the present results are consistent with modafinil’s affinity for the dopamine transporter in several binding assays (Mignot et al., 1994; Nishino et al., 1998; Madras et al., 2006). Modafinil has been considered to be a dopamine transporter inhibitor (Mignot et al., 1994), like cocaine or bupropion. Dopamine transporter inhibitors have affinity for the transporter, but are ineffective to evoke dopamine release from neurons (Chen and Reith, 2002). For example, in our preliminary experiments, cocaine (≤100 μM) and bupropion (≤10 μM) did not evoke \[^{3}H\] overflow from striatal slices. As a substrate, amphetamine has an affinity for the transporter, but alters transporter function and evokes dopamine release (Sulzer et al., 2005). In our experiment, amphetamine was efficacious to evoke \[^{3}H\] overflow, as was modafinil. Taken together, our data suggest that modafinil is a substrate for the dopamine transporter. However, the efficacy and potency of modafinil is much weaker than that of amphetamine. This finding of weak substrate activity agrees with recent electrophysiological work in midbrain dopamine neurons (Korotkova et al., 2007), although Korotkova and colleagues suggest that an interaction with D2 dopamine receptors is the primary target for modafinil.

Regarding the comparison of the present behavioral and neurochemical results, higher modafinil doses (64–128 mg/kg) substituted for the amphetamine or cocaine discriminative stimulus, which is consistent with the efficacy of higher modafinil concentrations (≥100 μM) to evoke \[^{3}H\]overflow. For lower modafinil doses and concentrations, there appears to be an inconsistency between the behavioral and neurochemical findings. A low modafinil dose (32 mg/kg) augmented the discriminative stimulus properties of amphetamine, but lower modafinil concentrations (≤30 μM) blocked amphetamine-evoked \[^{3}H\]overflow. The reason for this dichotomy is not known. However, it is unclear if the effects of the lower modafinil dose on behavior are strictly related to a change in brain dopamine levels, as this low dose was ineffective in in vivo microdialysis experiments studying extracellular dopamine levels (Akaoka et al., 1991; De Sereville et al., 1994), and lower concentrations were ineffective to evoke \[^{3}H\] overflow. Also, although the present research indicates that modafinil alters the function of the dopamine transporter, research on modafinil also suggests a role for orexin receptors, adrenergic receptors and serotonin receptors (Ballon and Feifel, 2006). Modafinil may also regulate the activity of dopamine neurons indirectly, through regulation of norepinephrine neurons or D2 dopamine receptors (Wisor and Eriksson, 2005; Korotkova et al., 2007).

Modafinil and nicotine similarly reduce fatigue, increase alertness and have cognitive-enhancing effects. Nicotine increases extracellular dopamine concentrations, and this effect of nicotine is maximally inhibited by mecamylamine (Teng et al., 1997; Mansvelder et al., 2002). As such, it was of interest to determine if modafinil interacts with nicotine acetylcholine receptors, similar to nicotine. Mecamylamine, within the concentration range where it completely inhibits nicotine-evoked \[^{3}H\]overflow (Teng et al., 1997), did not alter modafinil-evoked \[^{3}H\]overflow. This indicates that modafinil-evoked \[^{3}H\]overflow is not mediated by nicotinic acetylcholine receptors. The interaction of modafinil with nicotine-evoked \[^{3}H\]overflow also was determined. Modafinil did not augment or inhibit the effect of nicotine, indicating further that modafinil does not interact with nicotinic acetylcholine receptors. Modafinil’s interaction with nicotinic acetylcholine receptor-selective ligands was not examined in the present drug discrimination study. While the present \[^{3}H\]overflow data are “negative”, they are significant considering modafinil’s multifaceted interaction with multiple molecular targets in brain (e.g., adrenoceptors, orexin receptors and serotonin receptors).

Modafinil is approved for the treatment of sleep disorders, and has demonstrated efficacy for other psychopathologies, including treating the withdrawal syndrome from psychostimulant dependence (Dackis et al., 2003; Nasr, 2004; Dackis et al., 2005; Ballon and Feifel, 2006; Krebs et al., 2006). The present findings suggest that the clinical efficacy of modafinil may be related to its interaction with the dopamine transporter. Our data indicate that modafinil evokes dopamine release from striatal neurons. Although this research did not address directly the reinforcing or addictive properties of modafinil, these results do support previous findings in humans and animals that modafinil has a low abuse liability (Warot et al., 1993; Gold and Balster, 1996; Jasinski and Kovacevic-Ristanovic, 2000; Myrick et al., 2004).

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References


