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ARTICLE

Modafinil potentiates cocaine self-administration by a dopamine-independent mechanism: possible involvement of gap junctions

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Modafinil and methylphenidate are medications that inhibit the neuronal reuptake of dopamine, a mechanism shared with cocaine. Their use as "smart drugs" by healthy subjects poses health concerns and requires investigation. We show that methylphenidate, but not modafinil, maintained intravenous self-administration in Sprague-Dawley rats similar to cocaine. Both modafinil and methylphenidate pretreatments potentiated cocaine self-administration. Cocaine, at self-administered doses, stimulated mesolimbic dopamine levels. This effect was potentiated by methylphenidate, but not by modafinil pretreatments, indicating dopamine-dependent actions for methylphenidate, but not modafinil. Modafinil is known to facilitate electrotonic neuronal coupling by actions on gap junctions. Carbenoxolone, a gap junction inhibitor, antagonized modafinil, but not methylphenidate potentiation of cocaine self-administration. Our results indicate that modafinil shares mechanisms with cocaine and methylphenidate but has a unique pharmacological profile that includes facilitation of electrotonic coupling and lower abuse liability, which may be exploited in future therapeutic drug design for cocaine use disorder.

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INTRODUCTION

Racemic modafinil (modafinil; Provigil®) and methylphenidate (Ritalin®) are clinically available for treatment of narcolepsy and attention deficit disorders, respectively. These medications share with cocaine their primary pharmacological target, the blockade of neuronal dopamine (DA) reuptake through the DA transporter (DAT). Indeed, clinically relevant doses of modafinil increase DA levels in the human brain by blocking DAT, similar to therapeutic doses of methylphenidate [1]. These findings support a primary role for DA and DAT in the therapeutic actions of both modafinil [1] and methylphenidate [2].

Actions at DAT have also been implicated in the abuse liability of psychostimulants [3], initially leading to conclusions that drugs that block DAT may have potential for abuse [3]. However, there is evidence that atypical DAT blockers bind with high affinity to DAT, but do not possess cocaine-like reinforcing effects [4]. In this regard, while methylphenidate shares neuro-behavioral activities with cocaine [5], the effects of modafinil differ substantially. In preclinical tests, modafinil and its enantiomers differ from cocaine in their binding at DAT [6, 7], which is minimally influenced by DAT conformation. On the other hand, cocaine and methylphenidate have higher DAT affinity when it is in a conformation open as opposed to closed to the extracellular space [6, 7]. In addition, modafinil and methylphenidate have been evaluated in clinical studies as medications for psychostimulant use disorders, and in the case of modafinil, with some success [8, 9]. Further, modafinil did not show abuse liability in individuals with cocaine use disorder [10], and to our knowledge, there have been no systematic reports of modafinil abuse, nor identifiable symptoms of withdrawal after its chronic use [11].

Other than stimulating brain DA levels, modafinil has been shown to affect the levels of various neurotransmitters in several brain regions [12] that may play a role in its pharmacological actions. Recent studies have shown that by acting through gap junctions, modafinil plays a facilitative role in electrotonic coupling effects in neuronal and astroglia cells [13, 14]. For instance, modafinil has been proposed to modulate electrical coupling by actions through connexin 30-mediated gap junctions [14, 15]. Interestingly, in preclinical studies gap junctions have been found to play a role in the effects of modafinil on sleep and cognitive functions [13, 15, 16], which are impaired in individuals with substance use disorder [17–20]. Furthermore, modafinil

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treatment has been suggested to attenuate impaired sleep and cognitive functions in psychostimulant addicted subjects [21–23].

Recently, use of modafinil, methylphenidate and other psychostimulants as "smart drugs" to increase intellectual performance among healthy individuals has been debated [24, 25]. Of concern is their use without prescription and their potential for abuse or to facilitate the abuse of other psychostimulants, such as cocaine.

In order to investigate the potential influence of smart drugs like modafinil and methylphenidate on concurrent or subsequent use of illicit substances, we evaluated the following in rats: (1) the reinforcing effects of modafinil compared with methylphenidate using intravenous drug self-administration procedures; (2) the potential of modafinil and methylphenidate to enhance the self-administration of cocaine; (3) how these findings relate to stimulation of extracellular DA levels measured by in vivo microdialysis with probes implanted in the nucleus accumbens shell (NAS) (Supplementary Fig. S1), a mesolimbic region implicated in the reinforcing effects of drugs [26, 27]; and (4) the potential contribution of facilitation of neuronal electrical coupling through gap junctions [28] to the self-administration of cocaine after pretreatment with modafinil or methylphenidate, by administering carbenoxolone, a gap junction inhibitor, that has been shown to block modafinil's facilitation of electrical coupling, in vitro and in vivo [13, 14, 16]. Notably, carbenoxolone has also shown some behavioral effects dependent on stimulation of DA receptors in striatal areas [29].

MATERIALS AND METHODS

Subjects

Experimentally naïve, adult, male Sprague-Dawley rats (Taconic Farms, Germantown, NY, or Charles River, Wilmington, MA), 275–350 g, were habituated for at least one week before the start of experiments that were conducted during the light phase of a 12:12-h light-dark cycle (lights on 06:00 a.m.) or for experiments shown in Fig. 5, the dark phase in a reverse light-dark cycle (lights off at 7:00 a.m.). All animals used in the present study were maintained in an AAALAC International accredited facility in accordance with NIH Policy Manual 3040-2, Animal Care and Use in the Intramural Program (released 1 November 1999). The animal research conducted to perform this study was approved by the NIDA-IRP Animal Care and Use Committee, in accordance with the guidelines of the National Institutes of Health.

Compounds

(±)-Modafinil was synthesized [30] in the Medicinal Chemistry Section, NIDA-IRP, and dissolved in a vehicle containing DMSO 10%, Tween 80 15%, and saline 75% (V/V/V). (−)-Cocaine hydrochloride and methylphenidate hydrochloride (Sigma-Aldrich, St. Louis, MO or NIDA Drug Supply Program) were prepared fresh daily in sterile saline. In some experiments, detailed below, methylphenidate was prepared using the modafinil vehicle. Pretreatment times and doses of drugs used in the present study are described below and were chosen based on preliminary data obtained in this laboratory.

Self-administration studies

Twelve singly housed subjects were maintained at approximately 320 g by adjusting daily food rations (Scored Bacon Lover Treats, BIOSERV, Frenchtown, NJ). Water was available at all times in the home cages. During daily experimental sessions, subjects were placed in operant-conditioning chambers (modified ENV-008CT, Med Associates, St. Albans, VT) as described previously [31].

Subjects were initially trained during sessions with food reinforcement (20-mg food pellets, BIOSERV, Frenchtown, NJ) to press the right lever, and were subsequently trained under a fixed-ratio 5-response schedule of reinforcement (each fifth response produced a food pellet). Food deliveries were followed by 20-s timeout (TO) periods during which all lights were off, and responses had no scheduled consequences other than a feedback click. These training sessions lasted for 20 min or until 30 food pellets were delivered.

After subjects were responding at a rate at which 30 food pellets were obtained within each of three consecutive sessions, i.v. catheters were surgically implanted in the right or left external jugular vein as described [31].

Cocaine self-administration sessions lasted 2 h (3 h for carbenoxolone studies) and were conducted until response rates and patterns of responding showed no substantial session-to-session changes (within 25% deviations for the last three consecutive sessions). During these sessions, the LEDs above the active lever were illuminated when cocaine injections were available. Completion of five responses turned off the LEDs and activated the infusion pump, delivering a unit dose of 1.0 mg/kg. A 20-s TO period, during which LEDs were off, started with the injection. After the TO period, the LEDs were illuminated and responding had scheduled consequences again. Once response rates maintained by cocaine were stable (as above) across sessions, the session was divided into 20-min components, each preceded by a 2-min TO. This arrangement allowed the assessment of different cocaine doses/injection within each component. By adjusting injection volumes and durations, the cocaine dose per injection was incremented in the five sequential components in an ascending order as follows. In the first component, no injection was delivered [which is also referred to as extinction (EXT) because responses had no scheduled consequences other than the feedback click and each fifth response turning off the LEDs for 20 s]. The second through fifth components had doses/injection of: 0.03, 0.10, 0.32, and 1.0 mg/kg cocaine. Injection volumes in µl (and durations) for the five components were respectively 0 (0 s), 5.6 (0.32 s), 18.0 (1.0 s), 56.0 (3.2 s), and 180 (10 s), based on a body weight of 0.32 kg. A response-independent "sample" injection of cocaine at the corresponding dose was administered immediately before each component.

Training continued until stability was obtained, which consisted of: (1) at least 5.0 mg/kg of cocaine was self-administered within a session with <20% variation in the total number of cocaine injections compared with the previous session; (2) the dose of cocaine that maintained maximal response rates varied by no more than one-half log unit over two consecutive test sessions; and (3) maximum response rates were at least twofold higher than response rates maintained during EXT. This criterion was subsequently used for the remainder of the study.

When performances were stable across successive sessions, the effects of presession intraperitoneal (i.p.) injections of methylphenidate or (±)-modafinil on response rates maintained by cocaine injections were assessed, with and without carbenoxolone pretreatment. Presession treatments were separated by a minimum of 72 h and were conducted only if performances met the training criteria. All tests were conducted with a mixed order of drugs and doses.

Response rates were determined by dividing responses by elapsed time in each component, excluding the TOs that followed drug injections. A one-way, or two-way, repeated measures ANOVA was used to assess the effects of self-administered dose of cocaine, saline, (±)-modafinil, or methylphenidate (successive components) as appropriate, with a post-hoc Bonferroni *t*-test used for pairwise comparisons. For the experiments with methylphenidate, the modafinil vehicle was used.

In vivo brain microdialysis

Probes had an active dialyzing surface of 1.8–2.0 mm, and were implanted during surgical procedures [uncorrected coordinates: [32] anterior = +2.0 mm, and lateral = ±1.0 mm from bregma; vertical = −7.9 mm from dura (see Supplementary Fig. S1 for

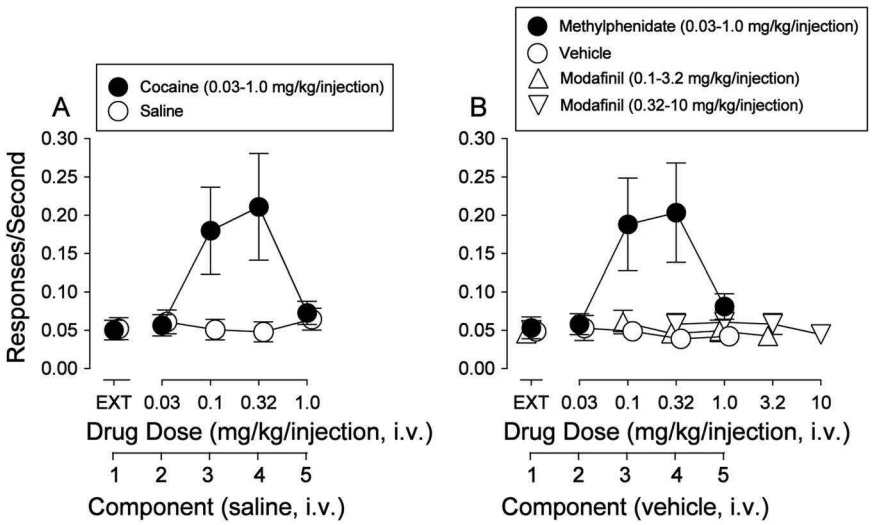

Fig. 1 Methylphenidate, but not modafinil, sustains self-administration behavior. Effects of substitution of saline, vehicle for modafinil, modafinil (lower and higher ranges of the doses), or the standard DA uptake inhibitor methylphenidate (dissolved in the modafinil vehicle), in rats trained to self-administer cocaine. Ordinates, responses per second; abscissae, dose of each drug in milligrams per kilogram per injection or saline or modafinil vehicle sequential component of the session. Each point represents the mean, with vertical bars representing S.E.M. ($n = 6$). **a** cocaine and saline. **b** vehicle, methylphenidate, modafinil (0.1–3.2 mg/kg/inj) and modafinil (0.32–10 mg/kg/inj). EXT extinction.

probe placements)] under a mixture of ketamine and xylazine anesthesia, 60.0 and 12.0 mg/kg i.p., respectively, as described [5]. During the same surgical procedure, a silastic catheter was implanted into the right external jugular vein as previously described [33]. Experiments were performed on freely moving subjects, ~22–24 h after probe implantation. Dialysates were sampled every 10 min and immediately analyzed. After reaching stable DA values (2–4 consecutive samples, <15% variability), subjects were treated with drugs. Subjects were injected during a single microdialysis session with i.v. injections of vehicle, modafinil (10, 17, 32, and 56 mg/kg), methylphenidate (0.1, 0.3, 1.0, and 3.0 mg/kg), or cocaine (0.03, 0.1, 0.3, and 1.0 mg/kg), spaced 30 min apart [34]. Modafinil (10, 17, or 32 mg/kg, i.p.), or methylphenidate (1 or 3.2 mg/kg, i.p.) pretreatments were administered to different groups of subjects 10 min before receiving cocaine, 0.03, 0.1, 0.3, and 1.0 mg/kg, i.v., or saline injections, spaced 30 min apart.

DA was detected by HPLC coupled with a coulometric detector (5200a Coulochem II, or Coulochem III, ESA, Chelmsford, MA, USA) as described [5]. Assay sensitivity for DA was 2 fmoles per sample. Data were used only from subjects for which probe tracks were within the correct NAS boundaries, confirmed by histology, as described [5] (Supplementary Fig. S1).

Microdialysis results were expressed as a percentage of basal DA values. Statistical analysis was carried out using one- or two-way ANOVA (factors: time, drug dose, or drug pretreatment) for repeated measures over time with significant results subjected to post-hoc Tukey's test. The average basal DA values in dialysates in the present experiments were 39.5 ± 6.6 fmoles (±S.E.M.) in a 10 µl sample, $n = 90$. No significant differences ($p > 0.05$) were found in basal DA concentrations across all the experimental groups (ANOVA, $F_{14,75} = 1.11$, $p = 0.364$).

RESULTS

Methylphenidate, but not modafinil, sustains intravenous self-administration

Average response rates maintained by cocaine were a bell-shaped function of injection dose, with a 0.211 ± 0.070 responses/s maximum at 0.32 mg/kg/injection. The maximum response rate was more than four-fold higher and significantly greater than the 0.050 ± 0.013 responses/s occurring in EXT (Fig. 1a, filled circles above EXT). A two-way repeated measures ANOVA indicated a significant difference in response rates vs. vehicle indicative of reinforcing effects of cocaine ($F_{1,20} = 8.81$, $p = 0.031$; post-hoc, 0.1 and 0.32 mg/kg/injection vs. EXT, t values ≥ 4.57, p values ≤ 0.001). Neither saline nor the vehicle used for modafinil maintained response rates substantially greater than those obtained in EXT (Fig. 1a, b, open circles).

As with its vehicle, modafinil (0.1–3.2 mg/kg/injection) did not maintain rates of responding appreciably greater than those obtained in EXT (Fig. 1b, open triangles up; F values ≤ 2.14; p values ≥ 0.114). When a higher range of modafinil doses was studied (0.32–10 mg/kg/inj), a largely similar outcome was obtained (Fig. 1b, open triangles down). However, modafinil at the dose of 3.2 mg/kg/injection on this occasion produced a slight increase of 0.019 responses/s (approximately 12-fold lower than the increase produced by 0.32 mg/kg/injection cocaine). A two-way repeated measures ANOVA was significant ($F_{1,20} = 8.61$, $p = 0.032$), but did not result in any significant difference in the number of modafinil infusions compared with vehicle infusions (main effect treatment $F_{2,10} = 1{,}624$, $p = 0.245$).

Like cocaine, methylphenidate maintained rates of responding substantially greater than those obtained in EXT (Fig. 1b, filled circles). A two-way repeated measures ANOVA for response rates yielded significant differences from vehicle self-administration, indicative of reinforcing effects (response rates, $F_{1,20} = 8.84$; $p = 0.031$; component/dose, $F_{4,20} = 7.01$; $p = 0.001$; and their interaction, $F_{4,20} = 6.52$; $p = 0.002$; post-hoc, 0.1 and 0.32 mg/kg/injection vs. vehicle, t values ≥ 3.90, p values ≤ 0.001).

Modafinil and methylphenidate potentiate cocaine self-administration

Presession treatments with modafinil dose-dependently shifted the cocaine self-administration dose-effect curve to the left (Fig. 2a), indicating a potentiation of cocaine self-administration. A two-way repeated measures ANOVA yielded significant effects of cocaine dose ($F_{4,60} = 7.90$, $p < 0.001$), presession treatment dose of modafinil ($F_{3,60} = 8.83$, $p < 0.001$), and a significant interaction of the two ($F_{12,60} = 6.56$, $p < 0.001$). The lowest dose of modafinil, 10 mg/kg, was inactive, while doses of 17 and 32 mg/kg left-shifted the dose-effect curve ~3- and 10-fold, respectively (Fig. 2a). Post-hoc comparisons indicated that pretreatment with modafinil (17 or 32 mg/kg) significantly increased response rates compared with vehicle pretreatment

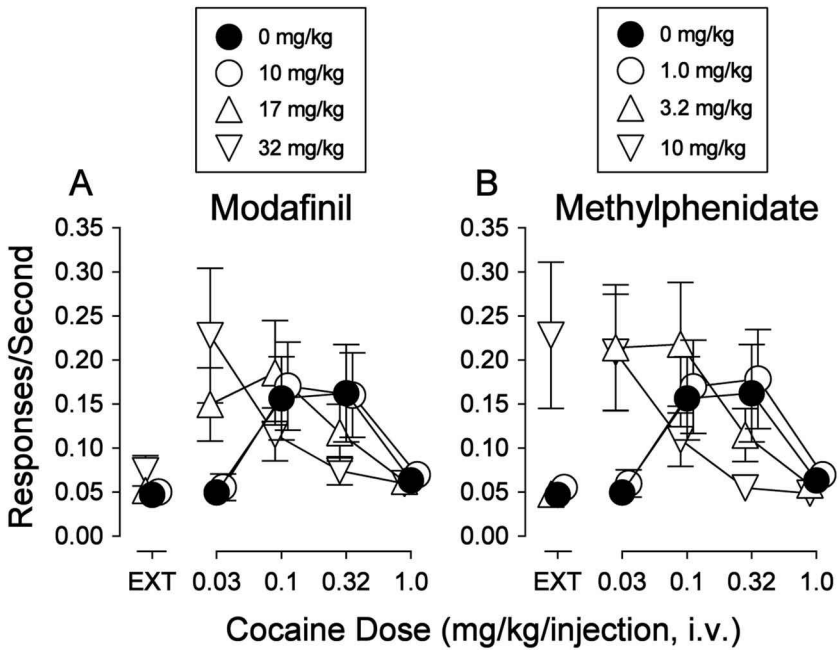

Fig. 2 Methylphenidate and modafinil potentiate cocaine self-administration. Effects of presession treatments with modafinil and methylphenidate on responding maintained by cocaine injections. Ordinates: Responses per sec. Abscissae: Cocaine injection dose in milligrams per kilogram per injection. Each point represents the mean, with vertical bars representing S.E.M. ($n = 6$). Modafinil and methylphenidate both were administered i.p. at 5 min before sessions. **a** Effects of modafinil (10, 17 and 32 mg/kg, i.v.) on cocaine self-administration. **b** Effects of methylphenidate (1.0, 3.2, and 10 mg/kg, i.v.) on cocaine self-administration. The vehicle consists of distilled water including 10% DMSO and 15% Tween 80. EXT extinction.

at the 0.032 mg/kg/injection dose of cocaine ($t \geq 4.06$, $p < 0.001$). In addition, the highest dose (32 mg/kg) of modafinil significantly decreased response rates compared with vehicle pretreatment at 0.32 mg/kg/injection of cocaine ($t = 3.59$, $p = 0.004$).

Presession treatments with methylphenidate also left-shifted the cocaine self-administration dose-effect curve (Fig. 2b). A two-way repeated measures ANOVA indicated significant effects of cocaine dose ($F_{4,60} = 6.56$, $p < 0.01$), pre-session treatment dose of methylphenidate ($F_{3,60} = 6.91$, $p < 0.01$), and an interaction of the two ($F_{12,60} = 6.57$, $p < 0.01$). These effects were dose related. The lowest dose, 1.0 mg/kg, was inactive, while doses of 3.2 and 10 mg/kg left-shifted the cocaine dose-effect curve approximately ~3- and 10-fold, respectively (Fig. 2b). Post-hoc comparisons indicated significantly increased response rates with methylphenidate (3.2 or 10 mg/kg) vs. saline pretreatment at the 0.032 mg/kg/injection dose of cocaine ($t \geq 4.43$, $p < 0.001$). In addition, the highest dose of methylphenidate, 10 mg/kg, significantly decreased response rates compared with saline pretreatment at 0.32 mg/kg/injection of cocaine ($t = 2.99$, $p < 0.05$). Further, that dose of methylphenidate also increased response rates during EXT (Fig. 2b, downward triangles above EXT; $t = 5.04$, $p < 0.001$).

Effects of modafinil and methylphenidate on extracellular NAS DA levels

Modafinil, 10–56 mg/kg, i.v. or methylphenidate, 0.1–3.0 mg/kg, i.v., administered at 30 min intervals, significantly stimulated extracellular NAS DA levels (Fig. 3a, b). Two-way repeated measures ANOVA indicated significant main effects of modafinil dose ($F_{4,44} = 6.44$, $p < 0.001$) and time ($F_{2,88} = 5.55$, $p < 0.01$), and a non-significant interaction of the two ($F_{8,88} = 0.79$, $p > 0.05$), and indicated also significant effects of methylphenidate dose ($F_{4,20} = 18.0$, $p < 0.0001$), time ($F_{2,40} = 21.2$, $p < 0.0001$), and their interaction ($F_{8,40} = 4.42$, $p < 0.05$). No significant changes were obtained with i.v. injections of modafinil vehicle or saline ($p > 0.05$).

A comparison of the maximum stimulation of DA levels produced by i.v. modafinil, 10–56 mg/kg, methylphenidate, 0.1–3.0 mg/kg, or cocaine, 0.03–3.0 mg/kg, is shown in Fig. 3c. Cocaine and methylphenidate were equipotent and equi-effective in stimulating extracellular DA in the NAS, whereas modafinil had approximately 100-fold lower potency, and was also substantially less effective in stimulating DA levels than cocaine or methylphenidate, at nontoxic doses. Nonetheless, doses of cocaine and methylphenidate that maintained response rates significantly greater than vehicle, i.e., 0.1 mg/kg (Fig. 1), produced a stimulation of DA to ~157% of basal levels (indicated by the dashed line on Fig. 3c). Each dose of modafinil studied produced increases in DA concentration greater than those produced by self-administered doses of cocaine or methylphenidate. The lowest tested dose of modafinil (10 mg/kg, i.v.), which was not self-administered, produced an increase in DA that was ~164% of basal levels (above the dashed line on Fig. 3c).

The maximal increases in DA concentrations obtained with modafinil were about 250% of basal levels compared with ~700% or ~800% increases in DA levels obtained with cocaine or methylphenidate, respectively (Fig. 3c). The highest dose of modafinil (56 mg/kg, i.v.) also produced acute toxicity with convulsions in two of six subjects, whereas increases produced by the highest doses of cocaine or methylphenidate were obtained without any grossly observable convulsant or pro-convulsant effects.

Methylphenidate, but not modafinil, potentiates cocaine-enhanced stimulation of NAS DA

Pretreatments with modafinil, 10–32 mg/kg i.p., had no significant effect on cocaine-induced stimulation of NAS DA (Fig. 4a and Supplementary Fig. S2). In contrast, pretreatments with methylphenidate (1.0 or 3.2 mg/kg, i.p., 10 min prior to cocaine) enhanced the stimulation of DA levels produced by i.v. cocaine (Fig. 4b and Supplementary Fig. S2). A two-way repeated measures ANOVA indicated significant main effects of dose ($F_{2,9} = 7.79$, $p < 0.02$), time ($F_{14,126} = 14.03$, $p < 0.0001$), and dose by time interaction ($F_{28,126} = 1.72$, $p < 0.05$).

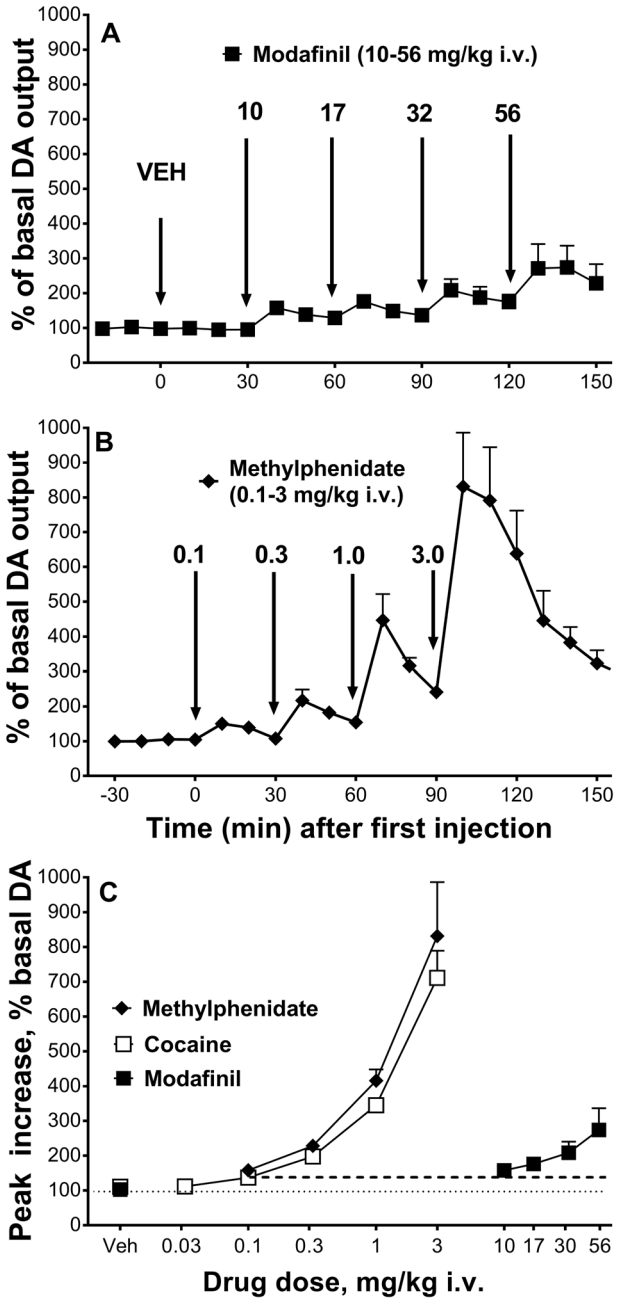

Fig. 3 Different stimulation of accumbens DA levels by methylphenidate and modafinil. Effects of i.v. administration of selected modafinil, methylphenidate, or cocaine doses spaced 30 min apart on stimulation of extracellular DA levels in dialysates from rats in which a microdialysis probe was implanted in the NAS. Results are means, with vertical bars representing S.E.M. ($n = 6$), of the amount of DA in 10-min dialysate samples, expressed as percentages of basal values. **a** Time course of modafinil administration (10–56 mg/kg, i.v.). **b** Time course of methylphenidate administration (0.1–3.0 mg/kg, i.v.). **c** Comparison of effects of various doses of modafinil, methylphenidate, and cocaine on maximal increases in extracellular DA levels in dialysates from the NAS. The dotted line indicates DA baseline (100%), whereas the dashed line indicates the maximal increase over basal DA values obtained with the lowest self-administered doses of cocaine or methylphenidate. Note that, the modafinil dose of 10 mg/kg i.v. elicited an increase in DA levels comparable to the lowest self-administered doses of cocaine and methylphenidate; however, modafinil did not maintain self-administration behavior above vehicle levels.

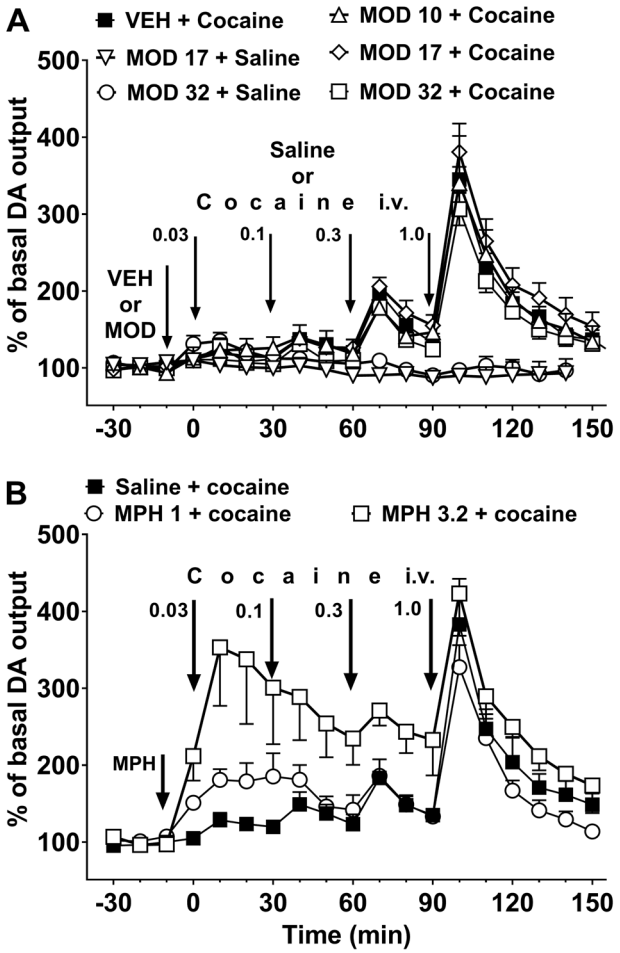

Fig. 4 Methylphenidate, but not modafinil, potentiates cocaine-induced stimulation of DA levels. Effects of **a** modafinil (10–32 mg/kg, i.p.) or **b** methylphenidate (1.0 and 3.2 mg/kg, i.p.) pretreatments on cocaine-induced stimulation of DA levels from NAS dialysates at cocaine doses (0.03–1.0 mg/kg, i.v.) that maintain self-administration behavior. Results are means, with vertical bars representing S.E.M. ($n = 4$–7), of the amount of DA in 10-min dialysate samples, expressed as percentage of basal values. VEH vehicle, MOD modafinil, MPH methylphenidate.

The enhancement produced by 1.0 or 3.2 mg/kg of methylphenidate dissipated by, respectively, ~30 or ~90 min after injection, corresponding to the time course of effects of methylphenidate administered alone (see Supplementary Fig. S2). There was no significant alteration in the dopaminergic effects of the highest dose of cocaine (Fig. 4b), which was administered about 90 min after methylphenidate. In a separate experiment (Supplementary Fig. S2), methylphenidate (3.2 mg/kg, i.p.), but not modafinil (17 mg/kg, i.p.), administered 10 min before the highest cocaine dose (1.0 mg/kg, i.v.) enhanced the stimulation of DA levels induced by cocaine (1.0 mg/kg, i.v., Supplementary Fig. S2).

Blockade of gap junctions by carbenoxolone attenuates modafinil- but not methylphenidate-induced potentiation in cocaine self-administration behavior

The differences in outcomes between methylphenidate and modafinil in combination with cocaine on NAS DA levels suggests a difference in the mechanisms of action of these two drugs beyond DAT inhibition. Since modafinil, but not methylphenidate, has been implicated in mediating neuronal electrical coupling via

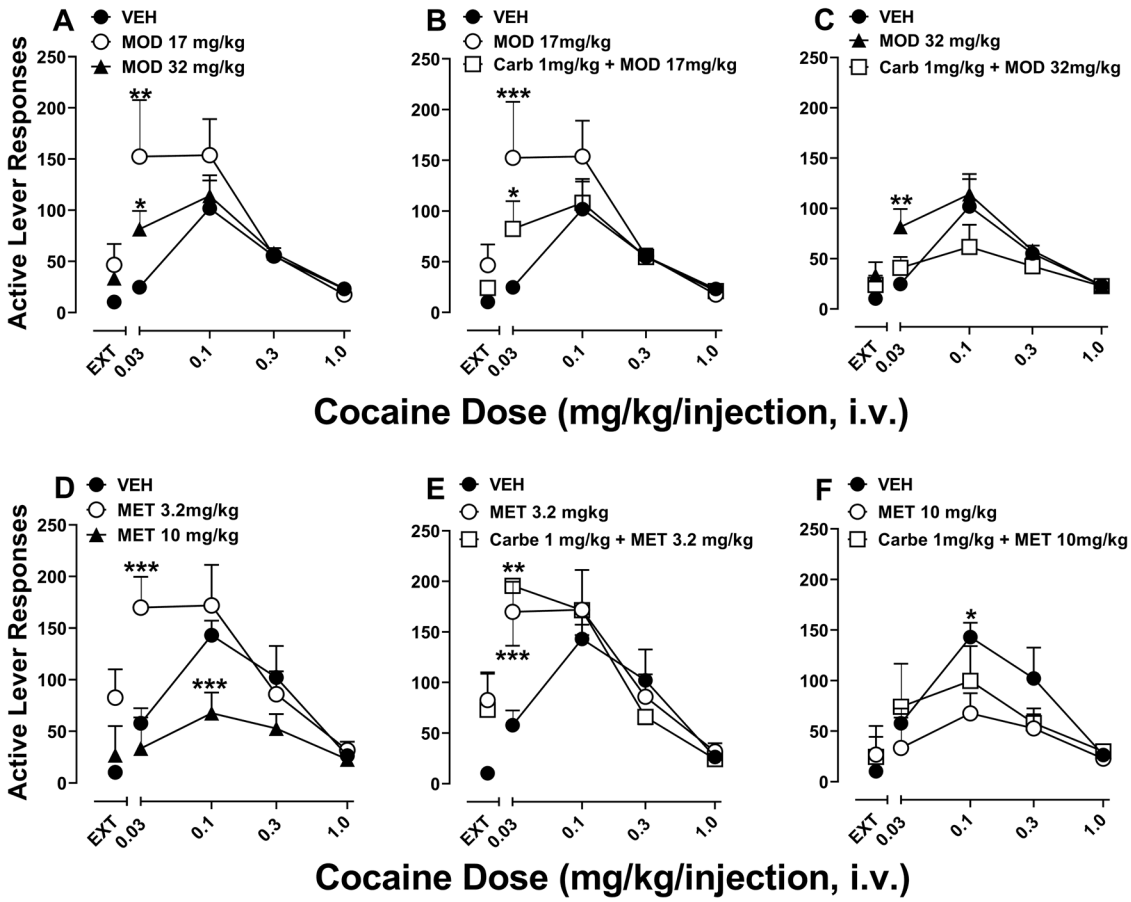

Fig. 5 Inhibition of gap junctions antagonizes modafinil, but not methylphenidate potentiation of cocaine self-administration. Effects of carbenoxolone, a gap junction inhibitor, on modafinil- vs. methylphenidate-induced potentiation of cocaine self-administration. Ordinates: Active lever responses. Abscissae: Cocaine injection dose in milligrams per kilogram per injection. Each point represents the mean, with vertical bars representing S.E.M. ($n = 6$–10). Modafinil or methylphenidate with or without carbenoxolone (1.0 mg/kg, i.p., 5 min prior to each session) was administered i.p. at 5 min before sessions. **a** Replicated effects of modafinil (MOD, 17 and 32 mg/kg; $n = 6$ and 10, respectively) on cocaine self-administration (Vehicle, VEH, $n = 9$). **b** Effects of the lower dose of modafinil (17 mg/kg) in the presence or absence of carbenoxolone (Carben, 1 mg/kg i.p., $n = 10$) on cocaine self-administration. **c** Effects of the higher dose of modafinil (32 mg/kg) in the presence or absence of carbenoxolone (1 mg/kg i.p., $n = 8$) on cocaine self-administration. **d** Replicated effects of methylphenidate (MET, 3.2 and 10 mg/kg; $n = 8$ and 8, respectively) on cocaine self-administration (Vehicle, VEH, $n = 8$). **e** Effects of the lower dose of methylphenidate (3.2 mg/kg) in the presence or absence of carbenoxolone (1 mg/kg i.p., $n = 8$) on cocaine self-administration. **f** Effects of the higher dose of methylphenidate (10 mg/kg) in the presence or absence of carbenoxolone (1 mg/kg i.p., $n = 10$) on cocaine self-administration. The vehicle consists of distilled water including 10% DMSO and 15% Tween 80. EXT: extinction. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. vehicle.

actions at gap junctions, we next compared the effects of modafinil and methylphenidate on cocaine self-administration after pretreatments with the gap junction inhibitor, carbenoxolone [13], in other groups of subjects. As before, pretreatment with modafinil produced significant leftward shifts in the cocaine self-administration dose-effect curve (Fig. 5a), two-way ANOVA dose by treatment interaction, $F_{8,135} = 2.86$, $p < 0.006$. Post-hoc testing revealed that both 17 and 32 mg/kg modafinil increased self-administration of 0.03 mg/kg/injection of cocaine ($p < 0.02$).

Carbenoxolone pretreatment, 1.0 mg/kg i.p., significantly reduced the enhancement of cocaine self-administration behavior produced by 17 mg/kg of modafinil (Fig. 5b). A two-way ANOVA indicated a dose by treatment interaction ($F_{8,135} = 2.42$, $p < 0.01$). At 32 mg/kg of modafinil (Fig. 5c), the two-way ANOVA dose by treatment interaction was not significant ($F_{8,159} = 1.7$, $p = 0.1$). However, post-hoc analyses indicated that rates of response at 0.03 mg/kg/injection of cocaine were significantly increased by modafinil alone compared with vehicle ($p < 0.01$). In contrast, when modafinil was administered with carbenoxolone responding did not differ from vehicle treatment. Carbenoxolone alone did not significantly alter cocaine self-administration, two-way ANOVA, main effect treatment, $F_{1,4} = 1.643$, $p = 0.2$; dose by treatment interaction, $F_{4,104} = 2.3$, $p = 0.06$ (Supplementary Fig. S3).

In contrast to modafinil (Fig. 5b, c), pretreatments with carbenoxolone did not alter methylphenidate-induced enhancement of cocaine self-administration (Fig. 5e, f). As before, pretreatment with methylphenidate produced significant increases in the rate of active lever pressing maintained by cocaine injection (Fig. 5d). A two-way ANOVA indicated a dose by treatment interaction ($F_{8,129} = 2.8$, $p < 0.006$). Post-hoc testing indicated that 3.2 mg/kg methylphenidate increased self-administration responding both under EXT conditions ($p < 0.02$), as well as at 0.03 mg/kg/injection cocaine ($p < 0.001$). In addition, 10 mg/kg methylphenidate significantly decreased responding maintained by 0.1 mg/kg/injection cocaine ($p = 0.002$). Administration of carbenoxolone prior to methylphenidate pretreatment did not significantly alter effects of either dose of methylphenidate on cocaine self-administration (Fig. 5e, f), two-way ANOVA dose by treatment interaction for 3.2 mg/kg methylphenidate ($F_{8,123} = 2.45$, $p < 0.02$), but no significant post-hoc tests; for 10 mg/kg methylphenidate ($F_{8,147} = 1.18$, $p = 0.30$).

DISCUSSION

The present study provides a series of novel and unexpected findings about modafinil and its interactions with cocaine. First, modafinil was not self-administered, even at i.v. doses that increased extracellular NAS DA to levels greater than those elicited by self-administered doses of methylphenidate or cocaine. Second, both modafinil and methylphenidate potentiated the reinforcing effects of cocaine, but at variance with methylphenidate, modafinil did not enhance cocaine-induced stimulation of extracellular DA levels, suggesting a unique mechanism by which modafinil interacts with cocaine unrelated to DAT. Finally, potentiation of cocaine's reinforcing effects by modafinil, but not by methylphenidate, was attenuated by the gap junction blocker, carbenoxolone. Importantly, under these same experimental conditions, carbenoxolone when administered alone did not significantly modify cocaine self-administration.

These findings suggest that modafinil potentiates the self-administration of cocaine through facilitation of electrotonic coupling, a mechanism that has received little attention in the addiction field (see for example: [29, 35, 36]). Our results show that substantial differences exist between modafinil and methylphenidate in terms of abuse potential. As expected, and unlike modafinil, methylphenidate maintained self-administration behavior and significantly increased NAS DA levels at doses comparable to those of cocaine, as described for other species and human subjects [37, 38].

Under our experimental conditions, modafinil when substituted for cocaine, did not maintain self-administration above vehicle levels, even at a dose (10 mg/kg, i.v.) that stimulated extracellular NAS DA to levels greater than those obtained by administration of the doses of cocaine or methylphenidate (0.1 mg/kg i.v.) that were self-administered. This result is consistent with the absence of reports on abuse of modafinil in humans [39]. In our study, both modafinil and methylphenidate pretreatments potentiated the reinforcing effects of cocaine. Such effects, if translatable to human subjects, suggest a potential facilitation of cocaine abuse. However, to our knowledge, consistent reports of concurrent use or abuse of modafinil and psychostimulants have not appeared, and modafinil has been shown to reduce cocaine use in selected populations of cocaine abusers [40, 41].

Administration of methylphenidate at doses that potentiated cocaine self-administration also enhanced the effects of cocaine on NAS DA dialysates. Unexpectedly, when modafinil was injected in combination with cocaine at doses that potentiated cocaine self-administration, there was no enhancement of cocaine-induced stimulation of DA levels. The lack of potentiation of the dopaminergic effects of cocaine by modafinil suggests that a pharmacokinetic interaction did not underly the modafinil potentiation of cocaine self-administration, as that type of interaction would be expected to also alter the effects of cocaine on NAS DA levels. It is of interest to note that in our experiments methylphenidate was approximately five-fold more potent than modafinil in potentiating cocaine self-administration, despite methylphenidate having ~100-fold greater affinity than modafinil for DAT binding [7] and, importantly, for in vivo stimulation of NAS DA levels (present results). These differences in potency suggest that the effects of the two drugs on cocaine self-administration might be due to mechanisms other than those mediated by DAT.

Recent evidence suggests that some actions of modafinil may be mediated by facilitation of electrotonic coupling via effects at gap junctions [13]. In the present study, the gap junction inhibitor, carbenoxolone, selectively reduced the potentiation of cocaine self-administration produced by nontoxic doses of modafinil, but not by methylphenidate. Specifically, carbenoxolone attenuated the potentiation of cocaine self-administration by the 17 mg/kg modafinil dose, and less so at the 32 mg/kg modafinil dose. The attenuation of modafinil's effects by carbenoxolone could be due to enhancement of cocaine self-administration by carbenoxolone alone. However, there was only a modest and non-significant increase in cocaine self-administration when carbenoxolone was administered in the absence of modafinil. Thus, the data collected to date suggest that the potentiation of the self-administration of cocaine by the low, nontoxic modafinil dose is a result of a facilitation of electrotonic coupling which may in turn indirectly enhance the dopaminergic postsynaptic signal elicited by cocaine administration [29, 35] or neuronal ensembles in the NAS [42]. Indeed, it has been shown that both systemic and ventral striatal administration of carbenoxolone selectively blocked oral stereotypies induced by administration of the dopamine agonist, apomorphine [29]. Thus, a facilitation of electrotonic coupling might play a role in specific behaviors mediated by activation of dopamine receptors. The present study is limited by the testing of only one gap junction inhibitor, carbenoxolone, for its effects on the response to modafinil. However, previous studies have shown that the effectiveness of this drug to alter different in vivo and in vitro actions of modafinil has been replicated with administration of other gap junction inhibitors [13, 14, 16]. Those results suggest that carbenoxolone can serve as an initial indicator for assessing the facilitatory effects of modafinil on gap junctions.

The lower potency of modafinil to increase DA levels was accompanied by a limited efficacy (maximal increase of ~250%) as compared with cocaine or methylphenidate (>700% of basal values for both drugs), at doses that did not elicit acute toxicity. Such low potency and efficacy in the maximal stimulation of DA levels produced by modafinil might also have an impact on its lower reinforcing efficacy assessed by self-administration procedures. Indeed, even after delivery of multiple doses and accumulation of a substantial cumulative dose of modafinil, the dialysis data suggest that DA levels would still be substantially low. This contrasts with the effects of cocaine and methylphenidate that show a steeper dose-dependent increase in DA levels, which may facilitate their self-administration as compared with that with modafinil. The present effects are consistent with previous results in mice, in which a modafinil dose-effect curve, at doses devoid of acute toxic effects, showed limited efficacy, a more shallow slope, and clear evidence of a plateau in maximal DA stimulation [6, 43, 44]. Thus, this apparent lower efficacy of modafinil in stimulating DA levels may also play a role in its limited, if any, abuse liability.

In line with differences between modafinil and other psychostimulants, high doses of methylphenidate increased rates of responding during the EXT component prior to cocaine self-administration, an effect often referred to as reinstatement. In contrast, modafinil showed no tendency to produce a similar effect in the present and previous [45] studies, and could actually block cocaine or opioid reinstatement, effects possibly mediated by changes in glutamate levels [12]. These reports suggest critical differences in modafinil actions compared with those of the standard psychomotor stimulant profile, which our data suggest may be due in part to effects at gap junctions. However, an increase in previously extinguished responding was reported [46] in nonhuman primates following modafinil treatment, and an increase in time spent in a compartment previously associated with cocaine effects was obtained in rodents [47]. These and the present findings with modafinil suggest a limited effectiveness to promote relapse to drug use. Similar outcomes were reported for *N*-substituted analogs of benztropine [31] which also blunt psychomotor stimulant effects [4].

Recent clinical studies report positive therapeutic outcomes in a subpopulation of patients with modafinil, but not methylphenidate [8] treatment in cocaine-dependent individuals [40, 41]. Differences in beneficial effects of these drugs could be related to their different interaction with the dopaminergic system and with facilitation of electrotonic coupling. This latter effect has been suggested to play a role in the therapeutic effects of modafinil on sleep disorders and cognitive function [13, 15, 16], which are also

impaired in subjects with substance use disorder [12, 20]. Moreover, modafinil has been reported to improve sleep and cognitive function in psychostimulant-dependent subjects [12, 22, 48, 49]. Thus, though the present study did not directly address how facilitation of gap junction effects of modafinil might be linked to its therapeutic efficacy, it suggests important differences from methylphenidate related to its use as medication for substance use disorder. These results indicated a limited, if any, abuse potential for modafinil, in agreement with the lack of systematic reports of modafinil abuse in humans [12, 39]. Moreover, lack of potentiation of the dopaminergic effects of cocaine, and facilitatory electrotonic coupling effects indicate a unique mechanism of action for modafinil that may be exploited in development of treatment strategies for cocaine use disorders.

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AUTHOR CONTRIBUTIONS

MM, TH, JLK, and GT designed the experiments. MM, TH, JDK, LEC, JPL, MAC, JCQ, CJJ, and GHB carried out the experiments. MM, TH, CJJ, ZXX, AHN, JLK, and GT analyzed the data. AHN provided new agents and analytic tools. MM, TH, JDK, CJJ, ZXX, AHN, JLK, and GT wrote the paper. All authors read and contributed to the final version of the paper.

ADDITIONAL INFORMATION

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