Addiction Biology

ORIGINAL ARTICLE

doi:10.1111/j.1369-1600.2012.00506.x

Modafinil attenuates reinstatement of cocaine seeking: role for cystine–glutamate exchange and metabotropic glutamate receptors

Stephen V. Mahler*, Megan Hensley-Simon*, Pouya Tahsili-Fahadan, Ryan T. LaLumiere, Charles Thomas, Rebecca V. Fallon, Peter W. Kalivas & Gary Aston-Jones

Department of Neurosciences, Medical University of South Carolina, Charleston, SC, USA

ABSTRACT

Modafinil may be useful for treating stimulant abuse, but the mechanisms by which it acts to do so are unknown. Indeed, a primary effect of modafinil is to inhibit dopamine transport, which typically promotes rather than inhibits motivated behavior. Therefore, we examined the role of nucleus accumbens extracellular glutamate and the group II metabotropic glutamate receptor (mGluR2/3) in modafinil effects. One group of rats was trained to self-administer cocaine for 10 days and extinguished, then given priming injections of cocaine to elicit reinstatement. Modafinil (300 mg/kg, intraperitoneal) inhibited reinstated cocaine seeking (but did not alter extinction responding by itself), and this effect was prevented by pre-treatment with bilateral microinjections of the mGluR2/3 antagonist LY-341495 (LY) into nucleus accumbens core. No reversal of modafinil effects was seen after unilateral accumbens core LY, or bilateral LY in the rostral pole of accumbens. Next, we sought to explore effects of modafinil on extracellular glutamate levels in accumbens after chronic cocaine. Separate rats were administered non-contingent cocaine, and after 3 weeks of withdrawal underwent accumbens microdialysis. Modafinil increased extracellular accumbens glutamate in chronic cocaine, but not chronic saline–pre-treated animals. This increase was prevented by reverse dialysis of cystine–glutamate exchange or voltage-dependent calcium channel antagonists. Voltage-dependent sodium channel blockade partly attenuated the increase in glutamate, but mGluR1 blockade did not. We conclude that modafinil increases extracellular glutamate in nucleus accumbens from glial and neuronal sources in cocaine-exposed rats, which may be important for its mGluR2/3-mediated antirelapse properties.

Keywords Microdialysis, nucleus accumbens, self-administration.

Correspondence to: Stephen V. Mahler, Medical University of South Carolina, 173 Ashley Ave, Fourth Floor BSB, Charleston, SC 29425, USA. E-mail: mahler@musc.edu

INTRODUCTION

Modafinil (2-diphenylmethyl-sulfinyl-2 acetamide, Provigil) is the prototype of a class of cognitive-enhancing drugs that is used to treat narcolepsy and other sleep disorders (Minzenberg & Carter 2008). Both clinical (Dackis *et al.* 2003, 2005; Myrick & Anton 2004; Ballon & Feifel 2006; Hart *et al.* 2008; Martinez-Raga, Knecht & Cepeda 2008; Anderson *et al.* 2009) and pre-clinical studies (Reichel & See 2010; Tahsili-Fahadan *et al.* 2010) show that modafinil may also have utility in treating psychostimulant addiction. Despite numerous studies examining the use of modafinil to treat narcolepsy, addiction and other disorders, the cellular mechanisms of action by which this interesting drug exerts its behavioral or clinical effects remain largely unknown.

Modafinil binds to dopamine transporters, and thereby increases extracellular dopamine (Madras *et al.* 2006; Volkow *et al.* 2009; Andersen *et al.* 2010). It has been postulated that this mechanism could permit modafinil to function as a replacement therapy in treating addiction to psychostimulants like cocaine (Karila *et al.* 2008; Schmitt & Reith 2011). However, this dopaminergic action of modafinil would be expected to produce reinforcing and motivational effects, yet most (but not all) studies have reported the opposite in humans and animals (Andersen *et al.* 2010; Reichel & See 2010; Tahsili-Fahadan *et al.* 2010; Young & Geyer 2010;

*Contributed equally as first authors.

Nguyen *et al.* 2011). Therefore, the capacity of modafinil to inhibit drug seeking may not entirely arise from inhibiting dopamine transport.

Over a decade ago, modafinil was shown to increase extracellular glutamate in dorsal striatum, thalamus, hypothalamus and hippocampus *in vivo* (Ferraro *et al.* 1997, 1998, 1999), although the mechanisms of these effects are not clear (Perez de la Mora *et al.* 1999). Many recent studies reveal that extracellular glutamate levels in nucleus accumbens core, in particular, regulate reinstatement of cocaine and heroin seeking, via stimulation of group II metabotropic glutamate receptors (mGluR2/3s) (Baptista, Martin-Fardon & Weiss 2004; Moran *et al.* 2005; Bossert *et al.* 2006; Peters & Kalivas 2006; Xi *et al.* 2010; Moussawi *et al.* 2011). Reinstatement of cocaine seeking is inhibited by increased tone on extrasynaptic mGluR2/3 in accumbens core, but it is not presently known whether modafinil increases extrasynaptic glutamate in this structure. The possibility that modafinil may inhibit drug seeking via this mechanism was recently supported by a report showing that modafinil inhibits reinstatement of extinguished morphine seeking in a conditioned place preference paradigm, and that this effect was prevented by systemic blockade of mGluR2/3 receptors (Tahsili-Fahadan *et al.* 2010).

Here we used a self-administration/reinstatement model of relapse to test the hypothesis that modafinil attenuates cocaine seeking by acting selectively on mGluR2/3s in nucleus accumbens core. Having validated a role for mGluR2/3s in accumbens core in inhibition of reinstatement, we then employed microdialysis to explore whether modafinil increases extracellular glutamate in accumbens, and if so, if this glutamate increase is of synaptic or non-synaptic origin.

MATERIALS AND METHODS

Subjects

All procedures complied with the National Institutes of Health guidelines for care of laboratory animals, and were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee. Male Sprague Dawley rats (250–300 g, Charles River, Wilmington, MA, USA) were used in this study. Rats were housed individually or in pairs on a 12-hour light/dark cycle with food and water *ad libitum*. All rats were acclimated to the vivarium for 7 days prior to surgery.

Drugs

Modafinil [300 mg/kg, intraperitoneal (i.p.); a gift from Cephalon Inc., West Chester, PA, USA] was suspended in 2 ml/kg 0.25% methylcellulose in water. This preparation yielded a suspension of modafinil (unlike dimethyl sulfoxide or cyclodextrine vehicles, which dissolve/cage modafinil molecules), so the mixture was stirred constantly until immediately prior to i.p. injection. This dose of modafinil was previously shown to elevate extracellular glutamate levels in the striatum (Ferraro *et al.* 1998) and to block reinstatement of extinguished morphine place preference (Tahsili-Fahadan *et al.* 2010). Modafinil was given 90 minutes prior to reinstatement or self-administration sessions, close to the peak of modafinil-induced glutamate levels in accumbens (based on microdialysis results described in the following statements). Cocaine (NIDA, Research Triangle Park, NC, USA) was dissolved in 0.9% sterile saline. The mGluR2/3 antagonist LY-341495 (LY; Tocris; 0.2, 1 and 2 μg, Minneapolis, MN, USA) was dissolved in sterile artificial cerebrospinal fluid (aCSF; 0.5 μl). Compounds infused through a dialysis probe included tetrodotoxin (TTX, 3 μM, Sigma-Aldrich, Saint Louis, MO, USA), *w*-conotoxin-GVIA (conotoxin, 10 μM, Tocris), (*S*)-4-carboxyphenylglycine (CPG, 1 μM, Tocris) and (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA, 300 μM, Tocris).

Surgical procedures

Rats were anesthetized with ketamine [87.5 mg/kg, intra muscular (i.m.)], xylazine (5 mg/kg, i.m.) and ketorolac (3 mg/kg, i.p.), placed in a stereotaxic instrument, and bilateral guide cannulae (Plastics One, Roanoke, VA, USA) were implanted over the nucleus accumbens (microdialysis surgical coordinates: anterior-posterior (AP): +1.8 mm, medial-lateral (ML): ±2.5 mm at 6° angle, dorsal-ventral (DV): −5.0 mm; behavioral surgical coordinates: AP: +1.8–2.5 mm, ML: +2.5 at 6° angle, and DV: −5.0 mm). Animals trained to self-administer cocaine were also implanted with chronic indwelling jugular catheters that exited the body via a port between the scapulae. All animals undergoing cocaine self-administration received intravenous (i.v.) cefazolin (10 mg) and heparin (10 U) daily starting 3 days after surgery, and continuing throughout self-administration training (administered after sessions). In all studies, rats were allowed 7 days to fully recover post-surgery before self-administration training or non-contingent cocaine administration commenced.

Self-administration, extinction and reinstatement

Behavioral training

Rats received 10 2-hour cocaine self-administration sessions (>10 cocaine infusions/day, 0.2 mg/50 μl infusion). Pressing the active lever (fixed ratio 1) yielded a 3.6-second cocaine infusion, a 2.9-kHz tone and a light presented above the active lever, followed by a 20-second timeout period. Presses on the inactive lever had no consequences. For animals used to test modafinil effects on

cocaine self-administration, modafinil (300 mg/kg, i.p.) or vehicle was administered 90 minutes prior to testing on days 10 and 11 (counterbalanced order). An additional day of self-administration testing was conducted 48 hours after day 10. To test effects of modafinil and intra-accumbens LY on cocaine-primed reinstatement behavior, animals were extinguished to criterion over at least 7 days (<25 active lever presses for 2 consecutive days).

Reinstatement testing

LY microinjections were administered using injectors (Plastics One; 28 ga) extending 2 mm beyond the guide cannula. The day before testing, sham injections were made to habituate animals to microinjection procedures. On subsequent test days, microinjections were administered 95 minutes prior to each reinstatement test in a 0.5-µl volume over 120 seconds, and injectors were left in place for 1 minute to allow diffusion away from injection sites. Five minutes after microinjections (90 minutes prior to reinstatement tests), animals were injected with modafinil (300 mg/kg, i.p.) or vehicle, and returned to their home cages. Immediately prior to reinstatement testing, animals were injected with cocaine (10 mg/kg, i.p.), then placed in the operant chamber for a 2-hour reinstatement test. Active and inactive lever presses did not result in cue presentations or cocaine. Between cocaine-primed reinstatement sessions, animals received extinction training until they returned to extinction criterion. Reinstatement trials were counterbalanced as follows: vehicle intracranially (i.c.) + vehicle i.p., vehicle i.c. + modafinil i.p. (300 mg/kg), or LY i.c. (0.2, 1, or 2 µg) + modafinil i.p.. Some animals also received LY i.c. (2 µg) + vehicle, i.p.. To test whether modafinil itself altered extinction responding or produced reinstatement behavior, separate groups of animals received modafinil (300 mg/kg) or vehicle 90 minutes prior to saline- or cocaine-primed reinstatement test sessions. These animals received either saline [with modafinil ($n = 14$) or vehicle ($n = 8$) pre-treatment], or cocaine [10 mg/kg; modafinil pre-treatment ($n = 22$), vehicle pre-treatment ($n = 12$)] injections immediately prior to testing.

Non-contingent injections of cocaine

For microdialysis experiments, rats were administered daily non-contingent injections of cocaine or saline according to two protocols. In the first experiment (Fig. 2), cocaine was administered in an ascending daily dosing regimen of 10, 15, 20, 25 and 30 mg/kg, i.p., for 10 days with each dose given on 2 consecutive days. In the second experiment (Fig. 3), rats were administered an injection protocol that produces behavioral sensitization (Pierce *et al*. 1996). On the first day, animals were injected with saline (0.3 ml i.p.) and the next day with cocaine (15 mg/kg, i.p.) or saline. Rats were then administered cocaine (30 mg/kg i.p.) or saline for 5 consecutive days, followed by a final injection of 15 mg/kg, i.p. cocaine or saline. All microdialysis experiments were conducted a minimum of 21 days after the last daily injection of cocaine.

***In vivo* microdialysis procedures**

In-house probe construction procedures and aCSF content are described elsewhere (Baker *et al*. 2003). The night prior to collecting samples, the probes were inserted into the accumbens and perfused with aCSF (0.2 µl/minute). The following morning, the flow rate was increased to 2.0 µl/minute. After 2 hours, six baseline collections were taken in 20-minute intervals. All animals were then administered methylcellulose vehicle (2 ml/kg, i.p.). Sixty minutes later, animals were injected with modafinil (300 mg/kg, i.p.). In the experiment shown in Fig. 3, after vehicle administration, the dialysis probe continued perfusion of aCSF, or aCSF containing TTX (3 µM), conotoxin (10 µM), CPG (1 µM) or AIDA (300 µM). Doses were chosen based on previous dialysis studies (Baker *et al*. 2002; Melendez, Vuthiganon & Kalivas 2005). Dialysis samples were stored at −80°C before being analyzed for glutamate using high-performance liquid chromatography with electrochemical detection, as described elsewhere (Torregrossa & Kalivas 2008).

Histology and statistics

Rats were deeply anesthetized and perfused intracardially with 0.9% saline and fixed with formalin. Sections (40–60 µm thick) were Nissl-stained, and injection/dialysis sites were localized using the Paxinos & Watson atlas (2007). Effects of systemic modafinil on cocaine self-administration were determined with repeated measures ANOVAs on lever pressing and cocaine infusions. Effects of systemic modafinil and i.c. LY on cocaine-primed reinstatement were examined with separate repeated measures analyses of variance (ANOVAs) for each dose of LY in bilateral nucleus accumbens (NAc), bilateral rostral pole and unilateral NAc cannulae groups, because each animal received (i.c./i.p.): vehicle/vehicle, vehicle/modafinil, and LY (0.2, 1 or 2 µg)/modafinil, or vehicle/vehicle and LY (2 µg)/modafinil. Effects of modafinil on reinstatement of cocaine seeking in the absence of cocaine priming injections were examined with a one-way ANOVA, with modafinil versus vehicle and cocaine versus saline priming injection as dependent variables. Microdialysis data were examined using a two-way ANOVA with repeated measures over time. Bonferroni's or Tukey's *post hoc* tests were used for multiple comparisons in all cases, as appropriate.

RESULTS

Modafinil inhibited reinstatement of cocaine seeking, but did not induce reinstatement

Figure 1a–c shows that vehicle–pre-treated rats had robust cocaine-primed reinstatement of active lever pressing, compared with prior extinction training days (animals with bilateral accumbens core cannulae: $t_{13} = 5.3$, $P < 0.001$; animals with cannulae outside accumbens core: $t_{16} = 3.4$, $P < 0.01$). Modafinil (300 mg/kg, i.p., with i.c. vehicle) substantially reduced cocaine-primed reinstatement in all treatment groups (Fig. 1a: $t_{13} = 4.8$, $P < 0.001$; Fig. 1b: $t_{16} = 2.3$, $P < 0.05$; Fig. 1c: $t_{32} = 3.2$, $P < 0.01$). Modafinil pre-treatment

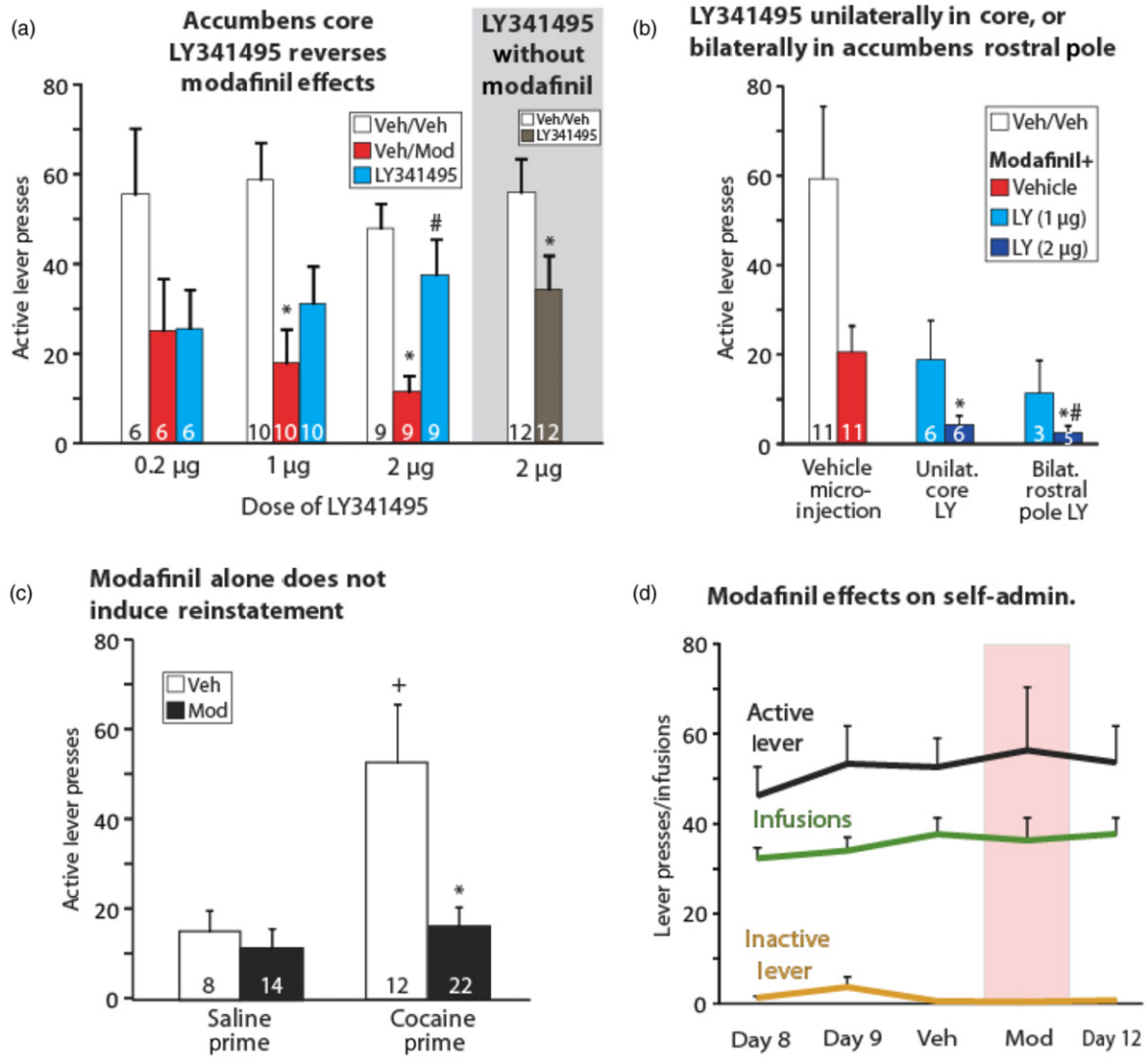

Figure 1 (a) Modafinil (Mod; 300 mg/kg, i.p.) reduced cocaine-primed (10 mg/kg, i.p.) reinstatement (vehicle i.c. + vehicle i.p. = white bars; vehicle i.c. + modafinil i.p. = red bars) that was prevented in a dose-dependent manner by bilateral microinjections of the mGluR2/3 antagonist LY-341495 (LY) into nucleus accumbens core (blue bars; 0.2, 1, 2 µg). The highest dose of LY, when injected in the absence of modafinil pre-treatment (brown bar at right), instead reduced reinstated cocaine seeking. (b) Microinjections of LY (2 µg = dark blue bars, no significant effect of 1 µg = light blue bars) either unilaterally into accumbens core (with contralateral injections into adjacent structures like medial accumbens shell or dorsal striatum; bars at middle), or bilaterally into the far rostral 'pole' of accumbens (bars at right) facilitated modafinil's blunting of reinstated cocaine seeking even further (vehicle i.c. + vehicle i.p. = white bar at right; vehicle i.c. + modafinil i.p. = red bar at right). (c) As expected, a cocaine prime induced reinstatement relative to a saline prime 90 minutes after vehicle injections (white bars; +$P < 0.05$). Similar modafinil pre-treatment before a saline prime did not alter extinction responding or cause reinstatement (black bars, left). As in panels A and B, modafinil-pre-treated animals again reinstated less than vehicle–pre-treated animals after a cocaine prime (right; *$P < 0.01$). (d) Modafinil (300 mg/kg i.p.) during cocaine self-administration did not affect lever pressing or cocaine intake (infusions) compared with i.p. vehicle, prior self-administration days 8 and 9, or subsequent self-administration day 12. *$P < 0.05$ compared with Veh/Veh; #$P < 0.05$ comparing LY with Veh/Mod. Sample sizes of each group are noted inside bars

alone did not significantly alter extinction responding or induce reinstatement of cocaine seeking [Fig. 1c; $F_{(3,52)} = 6.7$, $P = 0.001$; Tukey *post hocs*: veh pre-treatment + saline prime versus modafinil pre-treatment + saline prime: *not significant*; veh pre-treatment + saline prime versus veh pre-treatment + cocaine prime: $P = 0.015$].

Modafinil inhibition of reinstated cocaine seeking was reversed by intra-accumbens LY

Microinjections of LY (2 μg) into accumbens core reversed modafinil-induced inhibition of cocaine seeking. Figure 1a shows that LY (2 μg, i.c.) 5 minutes prior to systemic modafinil yielded reinstatement equivalent to controls [$F_{(2,16)} = 13.9$, $P < 0.001$, veh/veh versus veh/mod: $t_8 = 7.8$, $P < 0.001$; veh/mod versus 2 μg LY/mod: $t_8 = 3.1$, $P < 0.05$; veh/veh versus 2 μg LY + mod: $t_8 = 1.3$, $P = 0.2$; $n = 9$]. The 1-μg dose of LY did not significantly affect modafinil-induced attenuation of reinstatement [$F_{(2,18)} = 13.2$, $P < 0.001$; veh/veh versus veh/mod: $t_9 = 4.3$, $P < 0.01$, veh/mod versus 1 μg LY/mod: $t_9 = 1.7$, $P = 0.12$, veh/veh versus 1 μg LY/mod: $t_9 = 3.9$, $P < 0.01$; $n = 10$], nor did the lowest 0.2-μg dose of LY [$F_{(2,10)} = 2.3$, $P = 0.15$, $n = 6$]. When intra-accumbens LY (2 μg) was administered in the absence of systemic modafinil (i.p. vehicle), the mGluR2/3 antagonist decreased rather than increased reinstated cocaine seeking ($t_{11} = 2.6$, $P < 0.05$).

Prevention of modafinil effects by LY occurred only with bilateral microinjection into the nucleus accumbens core proper, but not when injected into more rostral aspects of accumbens (rostral pole), or unilaterally into accumbens core, with a contralateral injection in the adjacent medial accumbens shell or caudate/putamen. Figure 1b shows that LY (2 μg) failed to reverse modafinil's attenuation of cocaine-primed reinstatement when cannulae were unilaterally outside the accumbens core [in accumbens shell ($n = 2$) or caudate/putamen ($n = 4$); no differences in effects of LY among these structures were observed], or bilaterally in the rostral pole of accumbens ($n = 5$). In fact, LY into rostral accumbens pole plus systemic modafinil suppressed lever pressing even further than modafinil alone [unilateral NAc cannula: $F_{(2,10)} = 5.6$, $P < 0.05$; rostral pole: $F_{(2,8)} = 7.3$, $P < 0.05$].

Modafinil did not affect cocaine self-administration behavior

The average cocaine intake during the 10 training sessions (mean ± standard error of the mean) was 61.7 ± 4.2 mg. Figure 1d shows that modafinil (300 mg/kg) administered 90 minutes prior to self-administration session 10 or 11 did not affect established cocaine self-administration behavior ($n = 9$). The lack of effect of modafinil on self-administration of cocaine indicated that the reduction in reinstated cocaine seeking is not likely due to non-specific sedative effects.

Modafinil elevated extracellular glutamate

In microdialysis experiments, the basal levels of glutamate in each treatment group did not differ significantly, permitting the data to be normalized to percentage of the average of the six baseline samples in each animal [Fig. 2: saline = 22 ± 5 pmol/sample, $n = 10$; cocaine = 21 ± 3, $n = 11$; t(19) = 0.14, $P = 0.889$. Fig. 3: aCSF = 44 ± 7, $n = 12$; CPG = 64 ± 12, $n = 13$; AIDA = 42 ± 12, $n = 8$; TTX = 39 ± 8, $n = 8$; conotoxin = 57 ± 14, $n = 8$; $F(4,48) = 1.10$, $P = 0.368$]. Presumably, probe variability prevented us from identifying the reduction in extracellular glutamate in cocaine subjects that has been

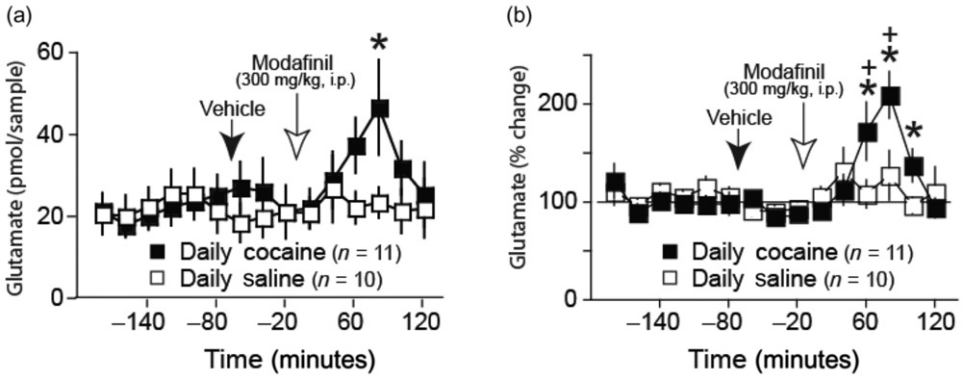

Figure 2 Modafinil (300 mg/kg, i.p.) elicited a delayed increase in extracellular glutamate in the accumbens only in rats pre-treated 3 weeks earlier with daily cocaine injections. After collecting six baseline samples (−180 to −80 minutes), an injection of vehicle was made, and 60 minutes later modafinil was injected and samples collected for another 120 minutes. (a) Data presented a mean ± standard error of the mean pmol/sample. (b) The same data as in panel A shown as percent change from baseline. *$P < 0.05$ compared with baseline within group, +$P < 0.05$ comparing saline with cocaine group

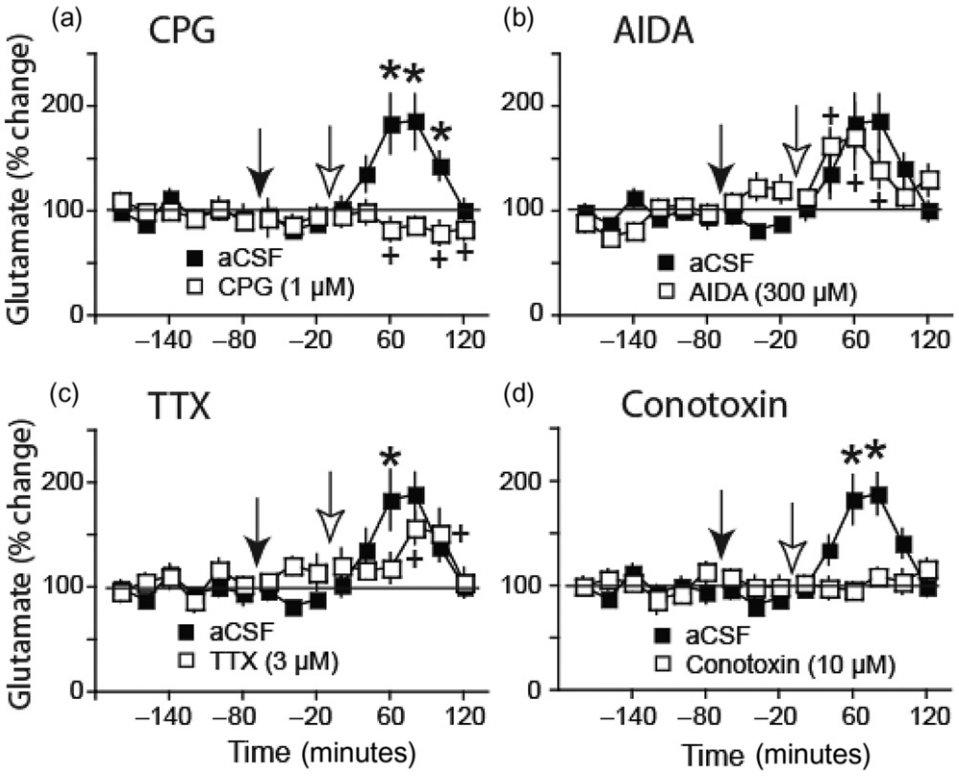

Figure 3 Modafinil increases in glutamate were abolished by (*S*)-4-carboxyphenylglycine (CPG) and conotoxin, and were partly antagonized by tetrodotoxin (TTX). (a) CPG (1 μM, filled arrow; a cystine–glutamate exchange inhibitor) was introduced into the dialysis buffer 60 minutes prior to injecting modafinil (open arrow) and prevented the rise in glutamate, indicating dependence of this effect on cystine–glutamate exchange. (b) (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA) (300 μM; a mGluR1 antagonist) was without effect on modafinil-induced glutamate. (c) TTX (3 μM; a voltage-dependent sodium channel inhibitor) produced a partial reduction in modafinil-induced glutamate, indicating partial mediation of this effect by impulse-dependent glutamate release. (d) Conotoxin [10 μM; a voltage-gated calcium channel (Ca_v) inhibitor] prevented modafinil-mediated increases in glutamate, suggesting dependence of this effect upon Ca_v. The artificial cerebrospinal fluid (aCSF) group is repeated in each panel. *$P < 0.05$ compared with aCSF group, +$P < 0.05$ compared with baseline within group

found previously in no-net-flux dialysis experiments, where interprobe variability does not influence the estimate of glutamate concentrations (Baker *et al*. 2003). In addition, probes were only partially contained within accumbens core, which is the primary site demonstrating reduced extracellular glutamate after withdrawal from chronic cocaine (Pierce *et al*. 1996; Baker *et al*. 2003). Figure 2 shows that 3 weeks after discontinuing daily cocaine injections, modafinil (300 mg/kg, i.p.) increased accumbens levels of extracellular glutamate. Whether evaluated as pmole/sample (Fig. 2a) or percent change from baseline (Fig. 2b), a two-way ANOVA with repeated measures over time revealed significant effects of time [$F(14,14) = 2.55$, $P = 0.002$, Fig. 2a; $F(14,14) = 4.5$, $P < 0.001$, Fig. 2b], and an interaction between time and treatment [$F(14,285) = 2.07$, $P = 0.014$, Fig. 1a; $F(14,285) = 2.4$, $P = 0.003$, Fig. 2b]. The differences between saline and cocaine groups were more robust when evaluated as percent change from baseline probably because of interprobe variability in glutamate recovery. However, both analyses revealed an increase in glutamate that was delayed and showed a peak response at 80 + minutes after modafinil administration. In contrast, when microdialysis was conducted 3 weeks after discontinuing daily saline injections, modafinil produced only a trend towards elevating extracellular glutamate, and the effect of modafinil in the cocaine group was significantly elevated over the saline group between 80 and 100 minutes after injection when the data were analyzed as percent change from baseline. Acute vehicle injection (i.p.) did not alter the levels of glutamate regardless of the pre-treatment group.

Modafinil increased extracellular glutamate via glial and neuronal sources

The fact that modafinil increased glutamate only in the accumbens of animals withdrawn from daily cocaine injections, and not in saline-treated rats, is reminiscent of the glutamate increase produced by N-acetylcysteine injections only in chronic cocaine-treated subjects. N-acetylcysteine increases extracellular glutamate by

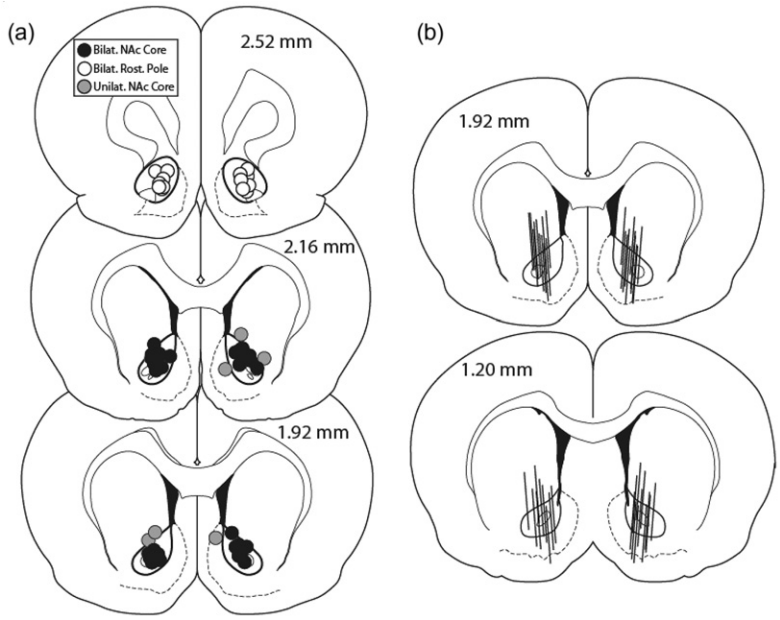

Figure 4 Location of the dialysis probes and microinjection sites in the accumbens. (a) The location of microinjection injection sites were drawn in a coronal view, adapted from Paxinos & Watson (2007) by an individual unaware of the animal's treatment group. Black circles refer to microinjection sites in accumbens core proper, where bilateral injection of LY-341495 (LY) prevented modafinil inhibition of reinstated cocaine seeking. White circles indicate sites in rostral pole of accumbens, where bilateral LY did not reverse modafinil effects. Grey circles indicate injection sites located unilaterally outside accumbens core, in dorsal striatum or medial accumbens shell (contralateral cannulae in these animals were located within accumbens core, represented with black circles). LY failed to decrease modafinil effects in these animals, indicating that bilateral mGluR2/3 antagonism in accumbens core is required for modafinil effects to be reversed by LY. Numbers refer to mm rostral to Bregma. (b) The location of the 2 mm of active membrane in dialysis probes

activating glial cystine–glutamate exchange (Baker *et al.* 2003). Accordingly, we examined whether reverse dialysis of the cystine–glutamate exchange antagonist CPG inhibited modafinil-induced increase in extracellular glutamate. Figure 3a shows that CPG abolished the modafinil increase in glutamate 3 weeks after the last daily cocaine injection [time: $F(14,14) = 3.4$, $P < 0.001$, treatment: $F(1,14) = 19.3$, $P < 0.001$, interaction: $F(14,345) = 5.5$, $P < 0.001$]. The cystine–glutamate exchanger contributes to basal glutamate levels (Baker *et al.* 2002), and accordingly, glutamate dropped below baseline by 120 minutes after introducing CPG into the dialysis buffer.

Although the dose of CPG used was likely below that needed to antagonize mGluR1 receptors (Baker *et al.* 2002), we next determined whether selective blockade of mGluR1 with AIDA affected the modafinil-induced increase in glutamate. Figure 3b shows that AIDA did not prevent modafinil from increasing extracellular glutamate [time: $F(14,14) = 3.5$, $P < 0.001$, interaction: $F(14,270) = 2.3$, $P = 0.006$].

To determine if the effect of modafinil on extracellular glutamate may have depended on voltage-dependent sodium or calcium conductances, TTX or conotoxin, respectively, were infused through the dialysis probe. Figure 3c shows that TTX significantly reduced the capacity of modafinil to increase extracellular glutamate [time: $F(14,14) = 3.1$, $P < 0.001$, interaction: $F(14,270) = 2.2$, $P = 0.007$]. However, TTX inhibited only the rise of glutamate in the sample obtained 60 minutes after injecting modafinil, and the level of glutamate was elevated by modafinil between 60 and 100 minutes after injection. Akin to CPG, Fig. 3d shows that conotoxin prevented the modafinil-induced increase in glutamate [time: $F(14,14) = 13.1$, $P < 0.001$, interaction: $F(14,270) = 12.8$, $P < 0.001$]. However, unlike CPG, conotoxin did not reduce the level of glutamate relative to pre-drug baseline.

Histology

Figure 4a shows the location of microinjection sites in the nucleus accumbens. Microinjections targeted to the accumbens core proper (black dots) were sites where bilateral LY effectively reversed the inhibitory action of modafinil on cocaine seeking. Injections into the rostral pole of accumbens (white dots) did not reverse modafinil effects. Unilateral accumbens core injections also failed to reverse modafinil-induced attenuation of reinstatement. In these 'unilateral accumbens' animals, one injection site was located within accumbens core (black dots), and the contralateral cannula was located outside accumbens core (grey dots in medial accumbens shell and dorsal

striatum). Figure 4b shows the location of dialysis probes in the accumbens for animals used in Fig. 3. The probes possessed 2 mm of active membrane and all were >50% situated in the accumbens core. However, it is important to note that up to 50% of the active membrane of each probe was also partly in the striatum and/or ventral accumbens shell, unlike in previous reports (Pierce *et al.* 1996; Baker *et al.* 2003).

DISCUSSION

The present data show for the first time that modafinil attenuates cocaine-primed reinstatement of cocaine seeking. This effect depended on the capacity of modafinil to increase extracellular glutamate in the accumbens of cocaine-experienced animals, as inhibiting mGlu2/3 receptors bilaterally in accumbens core reversed modafinil effects on reinstatement. In chronic cocaine-treated and withdrawn animals, modafinil promoted the release of glutamate from both glial and neuronal release mechanisms, as blocking cystine–glutamate exchange, voltage-gated calcium channels (Ca_v) or action potentials with TTX reduced the rise in glutamate. These results support a potential role for modafinil in treating addictive disorders, and indicate that these therapeutic effects result in part from elevating extrasynaptic glutamate, thereby stimulating accumbens mGluR2/3s.

Previous clinical work has indicated that modafinil might be useful in treating at least some cases of stimulant abuse in humans (Dackis *et al.* 2003, 2005; Hart *et al.* 2008; Anderson *et al.* 2009). In pre-clinical models, modafinil also reduced reinstatement of methamphetamine and morphine seeking in self-administration and conditioned place preference paradigms, respectively (Reichel & See 2010; Tahsili-Fahadan *et al.* 2010). Modafinil is known to interfere with dopamine transporters, and although this action may not necessarily be similar to the transporter inhibition by cocaine and other rewarding drugs (Schmitt & Reith 2011; Loland *et al.* 2012), modafinil does increase dopamine levels in accumbens. Facilitation of accumbens dopamine has been linked to the motivational and rewarding effects of modafinil in pre-clinical models, including its ability to reinstate drug-seeking behaviors in some paradigms (Bernardi *et al.* 2009; Zolkowska *et al.* 2009; Andersen *et al.* 2010; Cao *et al.* 2010; Spencer *et al.* 2010; Young & Geyer 2010; Nguyen *et al.* 2011; Schmitt & Reith 2011).

Of course, increased motivation and reward is unlikely to mediate the capacity of modafinil to reduce reinstated drug seeking, and we show here that modafinil inhibition of cocaine seeking instead depends on the stimulation of mGlu2/3s in the accumbens core. mGluR2/3s are located presynaptically in accumbens core, and respond to extrasynaptic glutamate by inhibiting synaptic glutamate release there (Moussawi & Kalivas 2010), reducing reinstatement behavior (Moussawi *et al.* 2011). Taken together with the literature, our findings support the likelihood that antirelapse properties of modafinil are due (at least in part) to an increase in extrasynaptic glutamate levels in accumbens, and subsequent stimulation of mGluR2/3 receptors.

Previous reports have shown that modafinil can increase extrasynaptic glutamate in other brain areas (Ferraro *et al.* 1997, 1998, 1999). Here, we found increases in nucleus accumbens glutamate in cocaine-experienced, but not naïve, animals. This argues that cocaine-induced adaptations interact with modafinil effects on accumbens glutamate. Chronic cocaine downregulates cystine–glutamate exchange as well as glial glutamate transport via glutamate transporter-1 (GLT-1) in the accumbens, and this glutamate dysregulation has been strongly linked to reinstatement of cocaine seeking (Kalivas 2009). Downregulated GLT-1 mediates the increase in glutamate overflow measured by microdialysis in the accumbens during cocaine-induced reinstatement (Knackstedt, Melendez & Kalivas 2010), so it seems probable that downregulated GLT-1 also contributes to the augmented increases in glutamate by acute modafinil in cocaine-experienced rats.

Although we did not determine the molecular binding site for modafinil in these studies, the rise in extracellular glutamate we observed involves cellular processes akin to what has previously been reported for N-acetylcysteine, which also inhibits cocaine-primed reinstatement (Moran *et al.* 2005; Xi *et al.* 2010). Like N-acetylcysteine (Baker *et al.* 2003), the modafinil-induced increases in extracellular glutamate require activity of cystine–glutamate exchange, as indicated by our finding that CPG blocked modafinil-induced glutamate increases. The cystine–glutamate exchanger is largely glial, and catalyzes the 1:1 stoichiometric exchange of extracellular cystine for intracellular glutamate (McBean 2002). Whether modafinil acts directly on the exchanger to increase activity, or like N-acetylcysteine indirectly increases its activity remains to be determined (Kupchik *et al.* 2012). However, indirect regulation appears likely given that the increases in glutamate by modafinil were entirely dependent upon cystine–glutamate exchange (CPG blockade) and Ca_v conductance (conotoxin blockade), and partly depended on action potential conduction (partial blockade by TTX). It is interesting that the increase in glutamate by modafinil occurred >60 minutes after injection, which supports the idea that the accumulation of glutamate may not depend directly on synaptic release (because this would be a more rapid event), and therefore may be a function of accumulation because of reduced glutamate uptake (see statements mentioned earlier). Regardless of the mechanism, the timeframe of

glutamate accumulation in the extracellular space corresponds nicely to the blockade of reinstated behavior by modafinil that was administered 90 minutes prior to initiating cocaine-induced reinstatement, further supporting the contention that increased accumbens glutamate contributes to modafinil's suppression of cocaine seeking.

In contrast to bilateral accumbens core, mGluR2/3 antagonism in the rostral accumbens 'pole' did not reverse modafinil's inhibition of cocaine seeking, nor did unilateral antagonism in core. Both of these argue for anatomical specificity of LY effects. We found that bilateral LY injections in accumbens core were required to reverse modafinil effects on reinstatement: no restoration of reinstatement was seen when LY was injected unilaterally in accumbens core, and contralaterally into other nearby structures. As unilateral accumbens mGluR2/3 blockade likely spared effects of modafinil-induced extracellular glutamate at these receptors in accumbens in one hemisphere, no reversal of modafinil reinstatement effects were observed. In addition, LY significantly potentiated the inhibitory effect of modafinil when administered in the rostral pole of accumbens. Rostro-caudal differences in the functional and anatomical organization of the accumbens have been observed previously (Vaccarino 1994; Reynolds & Berridge 2003; Pecina & Berridge 2005; Mahler, Smith & Berridge 2007; Gill & Grace 2011; Richard & Berridge 2011). While differences in connectivity offer one possible explanation, it is also possible that cocaine-induced changes in glutamate homeostasis contributing to reinstated cocaine seeking may not be as robust in the far rostral accumbens. For example, some effects of chronic cocaine differ between the core and the shell, and rostral pole of accumbens shares anatomical features with accumbens shell (Zahm & Brog 1992; Zahm & Heimer 1993; Pierce *et al.* 1996; Wolf 2010). It is not known if similar distinctions in the neuroadaptations produced by repeated cocaine exist between rostral pole and more caudal accumbens core that might contribute to the differential effects of LY observed here.

Interestingly, we found that LY microinjections into accumbens in the absence of modafinil, or into rostral pole even in the presence of modafinil, attenuated reinstatement behavior even below modafinil levels. Crucially, this demonstrates that mGlu2/3 antagonism in accumbens core does not simply increase cocaine seeking, but that it specifically prevents modafinil-induced effects, presumably mediated by extrasynaptic glutamate. Although a previous report did not find similar inhibition of reinstatement by intra-accumbens LY (Moussawi *et al.* 2011), that earlier study involved microinjections of LY immediately before cue-induced reinstatement sessions, instead of 95 minutes prior to cocaine-primed reinstatement as we did here (to block modafinil effects on glutamate levels that rise post-modafinil, but prior to reinstatement testing; Fig. 2). Potentially, this suggests that mGlu2/3 antagonism causes persistent compensatory effects long after acute administration, or suggests a difference between the roles of mGluR2/3 in cue-primed and in cocaine-primed reinstatement. This issue requires future examination.

It is important to note that the cocaine treatment paradigms used here were different in behavioral and microdialysis experiments. Reinstatement effects were examined in animals allowed to self-administer cocaine, as is required for this model of relapse in addiction. However, microdialysis data were obtained using a non-contingent cocaine/withdrawal model, as this has been shown to produce differences in glutamate between cocaine-exposed and non-exposed animals (Swanson *et al.* 2001; Moussawi *et al.* 2011). Although there is extensive overlap among cocaine effects on glutamate homeostasis between the contingent and non-contingent cocaine exposure paradigms (Kalivas 2009; Steketee & Kalivas 2011), some distinctions have been noted, especially with regard to the release of glutamate by an acute cocaine challenge. For example, in rats withdrawn from self-administered cocaine, acute cocaine-induced accumbens glutamate increases are entirely TTX-dependent (McFarland, Lapish & Kalivas 2003), but only initial glutamate release was TTX-dependent in rats withdrawn from chronic non-contingent cocaine (Pierce *et al.* 1996). Additionally, in rats that received chronic non-contingent yoked i.v. cocaine, acute cocaine did not cause accumbens glutamate release (McFarland *et al.* 2003), although accumbens glutamate release did result after acute cocaine was administered to animals withdrawn from a sensitizing regimen of non-contingent i.p. cocaine (Pierce *et al.* 1996). Here, we showed that in animals which self-administered cocaine, modafinil reduced reinstatement in an accumbens mGluR2/3-dependent manner, and that in animals exposed to chronic, non-contingent cocaine (but not saline-exposed animals), modafinil increased extrasynaptic accumbens glutamate, which primarily acts upon presynaptic mGluR2/3s (Moussawi & Kalivas 2010). Although it seems significant that modafinil blocked reinstatement behavior 90 minutes after injection (a time point where modafinil-induced accumbens glutamate increases were near maximum), we cannot conclude that identical mechanisms exist for modafinil-induced glutamate increases in animals trained to self-administer cocaine. However, it seems probable that similar actions of modafinil underlie both effects.

It is also worth noting that the effects observed here are unlikely to have been caused by generalized states such as sedation or anxiety. First, while modafinil is a stimulant and induces wakefulness, unlike conventional

stimulants it does not affect locomotion at 300 mg/kg or lower doses in rats; therefore it is unlikely to have reduced reinstatement by suppressing locomotion here (Edgar & Seidel 1997). Second, modafinil failed to affect cocaine self-administration behavior (Fig. 1d), which a drug that non-specifically sedated animals would be expected to have done. Third, modafinil (admittedly at lower doses than used here) failed to affect an elevated plus maze measure of anxiety in mice (Simon, Panissaud & Costentin 1994). Finally, intra-accumbens injections of LY failed to affect cue-induced reinstatement behavior in previous reports, suggesting a lack of profound locomotor or anxiety effects (Moussawi *et al.* 2011; Kupchik *et al.* 2012).

In summary, modafinil inhibits cocaine-primed reinstatement of cocaine seeking, and this behavioral effect likely involves a novel mechanism by which modafinil increases extrasynaptic glutamate in nucleus accumbens core, and thereby activates mGluR2/3s in this structure. Importantly, modafinil-induced suppression of relapse was blocked by accumbens core pre-treatment with an mGluR2/3 antagonist. Although dose- and time course-dependency of modafinil effects remain to be fully explored, the present data suggest a causal role for elevated extrasynaptic glutamate, and concomitant activation of mGluR2/3s, in the ability of modafinil to decrease cocaine seeking (among other mechanisms through which this complex drug acts). Together, these results show that modafinil may have the potential as a pharmacotherapy for treating relapse to cocaine use, and point to a novel mechanism of action for this poorly understood, but clinically useful drug.

Acknowledgements

This research was supported in part by NIH grants DA015369 (PWK), DA003906 (PWK), DA026692 (SVM) and DA06214 (GAJ).

Authors Contribution

SVM: conceived experiments, conducted experiments, wrote paper, analyzed data, MH-S: conducted experiments, wrote paper, RL: conceived experiments, conducted experiments, PTF: conceived experiments, conducted experiments, CT: conducted experiments, RVF: conducted experiments, PWK: conceived experiments, analyzed data, wrote paper, GA-J: conceived experiments, wrote paper.

References

Andersen ML, Kessler E, Murnane KS, McClung JC, Tufik S, Howell LL (2010) Dopamine transporter-related effects of modafinil in rhesus monkeys. Psychopharmacology 210:439–448.

Anderson AL, Reid MS, Li SH, Holmes T, Shemanski L, Slee A, Smith EV, Kahn R, Chiang N, Vocci F, Ciraulo D, Dackis C, Roache JD, Salloum IM, Somoza E, Urschel HC 3rd, Elkashef AM (2009) Modafinil for the treatment of cocaine dependence. Drug Alcohol Depend 104:133–139.

Baker DA, McFarland K, Lake RW, Shen H, Tang XC, Toda S, Kalivas PW (2003) Neuroadaptations in cystine–glutamate exchange underlie cocaine relapse. Nat Neurosci 6:743–749.

Baker DA, Xi ZX, Shen H, Swanson CJ, Kalivas PW (2002) The origin and neuronal function of in vivo nonsynaptic glutamate. J Neurosci 22:9134–9141.

Ballon JS, Feifel D (2006) A systematic review of modafinil: potential clinical uses and mechanisms of action. J Clin Psychiatry 67:554–566.

Baptista MA, Martin-Fardon R, Weiss F (2004) Preferential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on conditioned reinstatement versus primary reinforcement: comparison between cocaine and a potent conventional reinforcer. J Neurosci 24:4723–4727.

Bernardi RE, Lewis JR, Lattal KM, Berger SP (2009) Modafinil reinstates a cocaine conditioned place preference following extinction in rats. Behav Brain Res 204:250–253.

Bossert JM, Gray SM, Lu L, Shaham Y (2006) Activation of group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. Neuropsychopharmacology 31:2197–2209.

Cao J, Prisinzano TE, Okunola OM, Kopajtic T, Shook M, Katz JL, Newman AH (2010) Structure–activity relationships at the monoamine transporters for a novel series of modafinil (2-[(diphenylmethyl)sulfinyl]acetamide) analogues. ACS Med Chem Lett 2:48–52.

Dackis CA, Kampman KM, Lynch KG, Pettinati HM, O'Brien CP (2005) A double-blind, placebo-controlled trial of modafinil for cocaine dependence. Neuropsychopharmacology 30:205–211.

Dackis CA, Lynch KG, Yu E, Samaha FF, Kampman KM, Cornish JW, Rowan A, Poole S, White L, O'Brien CP (2003) Modafinil and cocaine: a double-blind, placebo-controlled drug interaction study. Drug Alcohol Depend 70:29–37.

Edgar DM, Seidel WF (1997) Modafinil induces wakefulness without intensifying motor activity or subsequent rebound hypersomnolence in the rat. J Pharmacol Exp Ther 283:757–769.

Ferraro L, Antonelli T, O'Connor WT, Tanganelli S, Rambert F, Fuxe K (1997) The antinarcoleptic drug modafinil increases glutamate release in thalamic areas and hippocampus. Neuroreport 8:2883–2887.

Ferraro L, Antonelli T, O'Connor WT, Tanganelli S, Rambert FA, Fuxe K (1998) The effects of modafinil on striatal, pallidal and nigral GABA and glutamate release in the conscious rat: evidence for a preferential inhibition of striato-pallidal GABA transmission. Neurosci Lett 253:135–138.

Ferraro L, Antonelli T, Tanganelli S, O'Connor WT, Perez de la Mora M, Mendez-Franco J, Rambert FA, Fuxe K (1999) The vigilance promoting drug modafinil increases extracellular glutamate levels in the medial preoptic area and the posterior hypothalamus of the conscious rat: prevention by local GABAA receptor blockade. Neuropsychopharmacology 20:346–356.

Gill KM, Grace AA (2011) Heterogeneous processing of amygdala and hippocampal inputs in the rostral and caudal subregions of the nucleus accumbens. Int J Neuropsychopharmacol 14:1301–1314.

Hart CL, Haney M, Vosburg SK, Rubin E, Foltin RW (2008) Smoked cocaine self-administration is decreased by modafinil. Neuropsychopharmacology 33:761–768.

Kalivas PW (2009) The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci 10:561–572.

Karila L, Gorelick D, Weinstein A, Noble F, Benyamina A, Coscas S, Blecha L, Lowenstein W, Martinot JL, Reynaud M, Lepine JP (2008) New treatments for cocaine dependence: a focused review. Int J Neuropsychopharmacol 11:425–438.

Knackstedt LA, Melendez RI, Kalivas PW (2010) Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. Biol Psychiatry 67:81–84.

Kupchik YM, Moussawi K, Tang XC, Wang X, Kalivas BC, Kolokithas R, Ogburn KB, Kalivas PW (2012) The effect of N-acetylcysteine in the nucleus accumbens on neurotransmission and relapse to cocaine. Biol Psychiatry 71:978–986.

Loland CJ, Mereu M, Okunola OM, Cao J, Prisinzano TE, Mazier S, Kopajtic T, Shi L, Katz JL, Tanda G, Newman AH (2012) R-modafinil (armodafinil): a unique dopamine uptake inhibitor and potential medication for psychostimulant abuse. Biol Psychiatry 72:405–413.

Madras BK, Xie Z, Lin Z, Jassen A, Panas H, Lynch L, Johnson R, Livni E, Spencer TJ, Bonab AA, Miller GM, Fischman AJ (2006) Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. J Pharmacol Exp Ther 319:561–569.

Mahler SV, Smith KS, Berridge KC (2007) Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. Neuropsychopharmacology 32:2267–2278.

Martinez-Raga J, Knecht C, Cepeda S (2008) Modafinil: a useful medication for cocaine addiction? Review of the evidence from neuropharmacological, experimental and clinical studies. Curr Drug Abuse Rev 1:213–221.

McBean GJ (2002) Cerebral cystine uptake: a tale of two transporters. Trends Pharmacol Sci 23:299–302.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 23:3531–3537.

Melendez RI, Vuthiganon J, Kalivas PW (2005) Regulation of extracellular glutamate in the prefrontal cortex: focus on the cystine glutamate exchanger and group I metabotropic glutamate receptors. J Pharmacol Exp Ther 314:139–147.

Minzenberg MJ, Carter CS (2008) Modafinil: a review of neurochemical actions and effects on cognition. Neuropsychopharmacology 33:1477–1502.

Moran MM, McFarland K, Melendez RI, Kalivas PW, Seamans JK (2005) Cystine/glutamate exchange regulates metabotropic glutamate receptor presynaptic inhibition of excitatory transmission and vulnerability to cocaine seeking. J Neurosci 25:6389–6393.

Moussawi K, Kalivas PW (2010) Group II metabotropic glutamate receptors (mGlu2/3) in drug addiction. Eur J Pharmacol 639:115–122.

Moussawi K, Zhou W, Shen H, Reichel CM, See RE, Carr DB, Kalivas PW (2011) Reversing cocaine-induced synaptic potentiation provides enduring protection from relapse. Proc Natl Acad Sci U S A 108:385–390.

Myrick H, Anton R (2004) Recent advances in the pharmacotherapy of alcoholism. Curr Psychiatry Rep 6:332–338.

Nguyen TL, Tian YH, You IJ, Lee SY, Jang CG (2011) Modafinil-induced conditioned place preference via dopaminergic system in mice. Synapse 65:733–741.

Paxinos G, Watson C (2007) The Rat Brain in Stereotaxic Coordinates, 6th edn. Boston, MA: Academic Press/Elsevier.

Pecina S, Berridge KC (2005) Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? J Neurosci 25:11777–11786.

Perez de la Mora M, Aguilar-Garcia A, Ramon-Frias T, Ramirez-Ramirez R, Mendez-Franco J, Rambert F, Fuxe K (1999) Effects of the vigilance promoting drug modafinil on the synthesis of GABA and glutamate in slices of rat hypothalamus. Neurosci Lett 259:181–185.

Peters J, Kalivas PW (2006) The group II metabotropic glutamate receptor agonist, LY379268, inhibits both cocaine- and food-seeking behavior in rats. Psychopharmacology 186:143–149.

Pierce RC, Bell K, Duffy P, Kalivas PW (1996) Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci 16:1550–1560.

Reichel CM, See RE (2010) Modafinil effects on reinstatement of methamphetamine seeking in a rat model of relapse. Psychopharmacology 210:337–346.

Reynolds SM, Berridge KC (2003) Glutamate motivational ensembles in nucleus accumbens: rostrocaudal shell gradients of fear and feeding. Eur J Neurosci 17:2187–2200.

Richard JM, Berridge KC (2011) Nucleus accumbens dopamine/glutamate interaction switches modes to generate desire versus dread: D(1) alone for appetitive eating but D(1) and D(2) together for fear. J Neurosci 31:12866–12879.

Schmitt KC, Reith ME (2011) The atypical stimulant and nootropic modafinil interacts with the dopamine transporter in a different manner than classical cocaine-like inhibitors. PLoS ONE 6:e25790.

Simon P, Panissaud C, Costentin J (1994) The stimulant effect of modafinil on wakefulness is not associated with an increase in anxiety in mice. A comparison with dexamphetamine. Psychopharmacology 114:597–600.

Spencer TJ, Madras BK, Bonab AA, Dougherty DD, Clarke A, Mirto T, Martin J, Fischman AJ (2010) A positron emission tomography study examining the dopaminergic activity of armodafinil in adults using [(1)(1)C]altropane and [(1)(1)C]raclopride. Biol Psychiatry 68:964–970.

Steketee JD, Kalivas PW (2011) Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. Pharmacol Rev 63:348–365.

Swanson CJ, Baker DA, Carson D, Worley PF, Kalivas PW (2001) Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for Homer. J Neurosci 21:9043–9052.

Tahsili-Fahadan P, Carr GV, Harris GC, Aston-Jones G (2010) Modafinil blocks reinstatement of extinguished opiate-seeking in rats: mediation by a glutamate mechanism. Neuropsychopharmacology 35:2203–2210.

Torregrossa MM, Kalivas PW (2008) Neurotensin in the ventral pallidum increases extracellular gamma-aminobutyric acid and differentially affects cue- and cocaine-primed reinstatement. J Pharmacol Exp Ther 325:556–566.

Vaccarino FJ (1994) Nucleus accumbens dopamine-CCK interactions in psychostimulant reward and related behaviors. Neurosci Biobehav Rev 18:207–214.

Volkow ND, Fowler JS, Logan J, Alexoff D, Zhu W, Telang F, Wang GJ, Jayne M, Hooker JM, Wong C, Hubbard B, Carter P, Warner D, King P, Shea C, Xu Y, Muench L, Apelskog-Torres K (2009)

Effects of modafinil on dopamine and dopamine transporters in the male human brain: clinical implications. JAMA 301:1148–1154.

Wolf ME (2010) The Bermuda Triangle of cocaine-induced neuroadaptations. Trends Neurosci 33:391–398.

Xi ZX, Li X, Peng XQ, Li J, Chun L, Gardner EL, Thomas AG, Slusher BS, Ashby CR, Jr (2010) Inhibition of NAALADase by 2-PMPA attenuates cocaine-induced relapse in rats: a NAAG-mGluR2/3-mediated mechanism. J Neurochem 112:564–576.

Young JW, Geyer MA (2010) Action of modafinil—increased motivation via the dopamine transporter inhibition and D1 receptors? Biol Psychiatry 67:784–787.

Zahm DS, Brog JS (1992) On the significance of subterritories in the 'accumbens' part of the rat ventral striatum. Neuroscience 50:751–767.

Zahm DS, Heimer L (1993) Specificity in the efferent projections of the nucleus accumbens in the rat: comparison of the rostral pole projection patterns with those of the core and shell. J Comp Neurol 327:220–232.

Zolkowska D, Jain R, Rothman RB, Partilla JS, Roth BL, Setola V, Prisinzano TE, Baumann MH (2009) Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. J Pharmacol Exp Ther 329:738–746.

