Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Dopamine D_1 and D_2 Receptor Family Contributions to Modafinil-Induced Wakefulness

Jared W. Young
Department of Psychiatry, University of California, San Diego, La Jolla, California 92093-0804
Review of Qu et al. (http://www.jneurosci.org/cgi/content/full/28/34/8462)

Modafinil (2-[(diphenylmethyl) sulfinyl] acetamide; United States brand name Provigil) is a wake-promoting agent first prescribed in France to treat narcolepsy-associated somnolence in the 1990s. Modafinil is currently prescribed in the United States for narcolepsy-associated somnolence, shift-work sleep disorder, and obstructive sleep apnea syndrome (Minzenberg and Carter, 2008), and it is being investigated for treating cognitive dysfunction in schizophrenia, depression, and attention deficit hyperactivity disorder (Minzenberg and Carter, 2008). Modafinil is considered safe, because subjects taking modafinil do not experience amphetamine-like withdrawal symptoms during discontinuation. Side effects of modafinil have been observed, however, including anxiety (4%), depression (4%), dyskinesia (2%), and psychosis [1 case (Wu et al., 2008)]. Understanding the precise mechanism(s) of action for modafinil may enable the development of more selective compounds having fewer side effects.

Studies performed *in vivo* and *in vitro* have suggested several possible mechanisms for the action of modafinil, including inhibiting dopamine and norepinephrine transporters and increasing dopamine, serotonin, glutamate, and histamine release (Minzenberg and Carter, 2008). While it was suggested that effective doses of modafinil are insufficient to inhibit the dopamine transporter (DAT) and hence do not act via this mechanism (Mignot et al., 1994), a recent positron emission tomography study in monkeys indicated 50% binding of modafinil to the DAT at lower plasma concentrations than are induced by therapeutic doses (Madras et al., 2006). Moreover, mice lacking the DAT do not exhibit modafinil-induced wakefulness (Wisor et al., 2001). DAT knock-out (KO) mice exhibit altered dopamine D_1 and D_2 receptors, however, as well as other compensatory mechanisms such as norepinephrine abnormalities. Thus, the mechanism of modafinil-induced wakefulness requires further investigation.

Qu et al. (2008) combined pharmacological and genetic techniques to investigate the contribution of D_1 and D_2 receptors to modafinil-induced wakefulness. The authors used male D_2 receptor KO mice, as well as the D_1- and D_2-family antagonists SCH23390 and raclopride, respectively, to investigate possible dopaminergic mechanisms for modafinil-induced wakefulness. Mice were equipped with EEG and electromyogram electrodes in the cortex and both trapezius muscles respectively for polysomnographic recordings. Sleep–wakefulness states were recorded for 48 h, comparing baseline and experimental days. Polygraphic recordings were scored by 10 s epochs as wakefulness, rapid-eye-movement (REM), and non-REM (NREM) sleep according to published criteria.

The authors first demonstrated a long-lasting, dose-dependent increase in wakefulness after modafinil administration [Qu et al. (2008), their Fig. 1*A–D* (http://www.jneurosci.org/cgi/content/full/28/34/8462/F1)], in accordance with other publications. Mice exhibited increased awake time, as well as reduced NREM and REM sleep [Qu et al. (2008), their Fig. 2*A* (http://www.jneurosci.org/cgi/content/full/28/34/8462/F2)]. Both SCH23390 and raclopride fully blocked low-dose modafinil-induced increases in wakefulness [Qu et al. (2008), their Fig. 1*E*,*I* (http://www.jneurosci.org/cgi/content/full/28/34/8462/F1)] and attenuated the effects of modafinil at higher doses [Qu et al. (2008), their Fig. 1*F–H*,*J–L* (http://www.jneurosci.org/cgi/content/full/28/34/8462/F1)]. While the use of low doses of SCH23390 and raclopride increased the likelihood of specifically targeting D_1 and D_2 receptor families, respectively, neither drug is wholly selective within these families. Thus, the effects observed could also reflect antagonizing D_5 or $D_{3/4}$ receptors, respectively. To address this confound, the authors investigated wakefulness effects of modafinil in D_2 receptor KO mice. While low doses of modafinil increased wakefulness in wild-type mice [Qu et al. (2008), their Fig. 6*A* (http://www.jneurosci.org/cgi/content/full/28/34/

Received Dec. 8, 2008; revised Jan. 7, 2009; accepted Jan. 7, 2009.

This work was supported by National Institutes of Health Grant R01-MH071916 and the Stein Institute for Research on Aging. I thank Drs. Mark Geyer, Victoria Risbrough, Susan Powell, and Andrew Grottick for their helpful comments.

Correspondence should be addressed to Dr. Jared W. Young, Department of Psychiatry, University of California, San Diego, 9500 Gilman Drive MC 0804, La Jolla, CA 92093-0804. E-mail: jaredyoung@ucsd.edu.

DOI:10.1523/JNEUROSCI.5843-08.2009

 0270-6474/09/292663-03$15.00/0

8462/F6)], this effect was absent in KO mice [Qu et al. (2008), their Fig. 6*C* (http://www.jneurosci.org/cgi/content/full/28/34/8462/F6)]. Thus, higher doses of modafinil were required to induce wakefulness in D_2 KO mice [Qu et al. (2008), their Fig. 6*C* (http://www.jneurosci.org/cgi/content/full/28/34/8462/F6)]. These data support the authors' conclusions from initial studies that the D_2 receptor contributes to modafinil-induced wakefulness. Also consistent with their initial studies, the authors observed that the D_1 receptor antagonist SCH23390 attenuated modafinil-induced wakefulness in wild-type mice [Qu et al. (2008), their Fig. 6*B* (http://www.jneurosci.org/cgi/content/full/28/34/8462/F6)]. Modafinil-induced wakefulness was completely antagonized, however, by SCH23390 in D_2 receptor KO mice [Qu et al. (2008), their Fig. 6*D* (http://www.jneurosci.org/cgi/content/full/28/34/8462/F6)]. The authors conclude that these data support a D_2 and D_1 receptor interaction for modafinil-induced wakefulness. Thus, the authors contribute to evidence for $D_{1/2}$ receptor involvement in sleep/wake profiles.

While this combined genetic and pharmacological approach provides striking evidence for D_2 receptor and D_1 family involvement in modafinil-induced increases in wakefulness, further evidence may be required. For example, the authors' interpretation of SCH23390- and raclopride-induced attenuation of modafinil-induced increase in wakefulness may be premature. Although the pharmacological evidence was compelling, the authors did not demonstrate whether the SCH23390 or raclopride reduction in modafinil-induced wakefulness was an additive or competitive effect, as they did not examine the effects of these drugs alone on wakefulness. Previous reports suggest that SCH23390 reduces wakefulness in rats by increasing REM and NREM sleep (Ongini et al., 1993), while the D_2 antagonist increases sleep time in mice (Cohen et al., 1997), opposite to the effects of modafinil in mice reported by Qu et al. (2008). Thus, it cannot be concluded that the opposing effects of SCH23390 and raclopride on modafinil-induced wakefulness are not a result of competing orthogonal systems.

In addition, the authors argued that modafinil acts directly at D_1 and D_2 receptors, despite reporting no binding data to support this theory. Alternatively, modafinil may increase extracellular dopamine via DAT inhibition (Madras et al., 2006), with SCH23390 or raclopride blocking its effects on wakefulness by competitively blocking the availability of dopamine to activate these receptors. [^{11}C]raclopride binding is reduced in sleep-deprived humans, putatively via competition with increased endogenous extracellular dopamine after sleep deprivation (Volkow et al., 2008). Thus, mediation of modafinil-induced wakefulness via a D_1/D_2 receptor as opposed to DAT requires further evidence.

The interpretation of D_1-selective SCH23390 effects on modafinil-induced wakefulness must also be qualified. As discussed above, SCH23390 is not selective for the D_1 receptor, since it also binds to the D_5 receptor in mice and has behavioral effects in D1 KO mice (Centonze et al., 2003) at doses used in the study by Qu et al. (2008). Thus, a role for the D_5 receptor cannot be discounted. The reduced effects of modafinil on D_2 receptor KO mice provide evidence that the observed raclopride effects were likely to have been mediated by the D_2 receptor. Thus, greater evidence for a D_1 receptor involvement for modafinil-induced increases in wakefulness could be generated by assessing the effects of modafinil in D_1 KO mice, in combination with raclopride. These data would provide evidence similar to that generated by D_2 KO mice and strengthen the argument for D_1 receptor selectivity in the effects of modafinil. These findings would be particularly important given the interaction of D_5 receptors with ADHD, their localization on cholinergic neurons that are important for attentional performance, and the putative attention-enhancing effects of modafinil (Minzenberg and Carter, 2008). It would also be informative to administer raclopride in efficacious (high)-dose modafinil-pretreated D_2 KO mice, to further demonstrate D_2 selectivity of raclopride.

Modafinil does not induce wakefulness in mice lacking the DAT (Wisor et al., 2001). Qu et al. (2008) caution against the interpretation that the DAT is therefore required for modafinil-induced wakefulness because DAT KO mice exhibit abnormal D_1 and D_2 receptor regulation, which may mediate the reduced efficacy of modafinil in these mice. Moreover, Qu et al. (2008) indicate that the nomifensine- and modafinil-evoked current–voltage relationships differ on rat brain slices (Korotkova et al., 2007), suggesting a different mechanism of action than DAT selectivity. Nomifensine is, however, threefold to fourfold more selective to the norepinephrine transporter than DAT (Tatsumi et al., 1997), while modafinil binds threefold to fourfold more selectively to the DAT at clinically relevant doses (Madras et al., 2006). GBR12909 is also more selective to the DAT, exhibits a similar behavioral profile to modafinil, and was also ineffective in DAT KO mice (Wisor et al., 2001), suggesting that modafinil-induced DAT inhibition remains a viable hypothesis for the effects of modafinil. Moreover, the reduced effectiveness of modafinil in D_2 KO mice observed in the study by Qu et al. (2008), could be interpreted as a result of the 50% reduction in functional DAT levels in these mice (Dickinson et al., 1999).

The pharmacological studies by Qu et al. (2008) provide support for D_1 and D_2 receptor family involvement in wakefulness in mice. Moreover, the authors' combined genetic and pharmacological study provides evidence that the D_2 receptor is important in the arousal effects of modafinil. The authors' approach to investigating the mechanism of modafinil-induced wakefulness is an effective way to selectively examine receptor contributions to this effect. This approach could therefore be used to investigate the mechanism of modafinil-induced effects in other areas, such as locomotor activity or cognitive performance as these behaviors may be mediated by distinct mechanisms (Minzenberg and Carter, 2008). Increased knowledge of the mechanisms of the effects of modafinil may allow increasing selectivity for these effects, or the generation of drugs with reduced side effects. Furthermore, Qu et al. (2008) provide thoughtful and incisive interpretations of genetic and pharmacological manipulations when using this combined genetic and pharmacological approach. Care must therefore be taken when interpreting the need for both the D_1 and D_2 receptors in mediating the modafinil-induced increases in wakefulness. D_5 receptor contributions to modafinil-induced wakefulness cannot as yet be discounted, nor can the contribution of the DAT. However, the study by Qu et al. (2008) provides a platform from which the contributions of these receptors can be further investigated.

References

Centonze D, Grande C, Saulle E, Martin AB, Gubellini P, Pavón N, Pisani A, Bernardi G, Moratalla R, Calabresi P (2003) Distinct roles of D_1 and D_5 dopamine receptors in motor activity and striatal synaptic plasticity. J Neurosci 23:8506–8512.

Cohen C, Perrault G, Sanger DJ (1997) Evidence for the involvement of dopamine receptors in

ethanol-induced hyperactivity in mice. Neuropharmacology 36:1099–1108.

Dickinson SD, Sabeti J, Larson GA, Giardina K, Rubinstein M, Kelly MA, Grandy DK, Low MJ, Gerhardt GA, Zahniser NR (1999) Dopamine D2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. J Neurochem 72:148–156.

Korotkova TM, Klyuch BP, Ponomarenko AA, Lin JS, Haas HL, Sergeeva OA (2007) Modafinil inhibits rat midbrain dopaminergic neurons through D2-like receptors. Neuropharmacology 52:626–633.

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.

Mignot E, Nishino S, Guilleminault C, Dement WC (1994) Modafinil binds to the dopamine uptake carrier site with low affinity. Sleep 17:436–437.

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

Ongini E, Bonizzoni E, Ferri N, Milani S, Trampus M (1993) Differential effects of dopamine D-1 and D-2 receptor antagonist antipsychotics on sleep-wake patterns in the rat. J Pharmacol Exp Ther 266:726–731.

Qu WM, Huang ZL, Xu XH, Matsumoto N, Urade Y (2008) Dopaminergic D_1 and D_2 receptors are essential for the arousal effect of modafinil. J Neurosci 28:8462–8469.

Tatsumi M, Groshan K, Blakely RD, Richelson E (1997) Pharmacological profile of antidepressants and related compounds at human monoamine transporters. Eur J Pharmacol 340:249–258.

Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Wong C, Ma J, Pradhan K, Tomasi D, Thanos PK, Ferré S, Jayne M (2008) Sleep deprivation decreases binding of [^{11}C]raclopride to dopamine D_2/D_3 receptors in the human brain. J Neurosci 28:8454–8461.

Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM (2001) Dopaminergic role in stimulant-induced wakefulness. J Neurosci 21:1787–1794.

Wu P, Jones S, Ryan CJ, Michail D, Robinson TD (2008) Modafinil-induced psychosis. Intern Med J 38:677–678.