Modafinil and circadian rhythms in Syrian hamsters: Assessment of the
chronobiotic potential of a novel alerting compound

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Running Title: Modafinil and Circadian Rhythms

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Number of Text Pages: 17
Number of Tables: 1
Number of Figures: 7
Number of References: 40
Number of Words in Abstract: 245
Number of Words in Introduction: 468
Number of Words in Discussion: 1472

Abbreviations used: LD = light-dark, ZT = zeitgeber time, DD = constant darkness, LL = constant light, W = wakefulness, SWS = slow-wave sleep, PS = paradoxical sleep.

Recommended Section: Behavioral Pharmacology
Abstract

In Syrian hamsters, behavioral procedures for inducing arousal (e.g., running in a novel wheel or gentle handling) can shift circadian rhythms when applied during the usual sleep period (‘subjective day’) and can attenuate phase shifts to light during the active period (‘subjective night’). This raises the possibility that drugs that affect behavioral state may have ‘chronobiotic’ potential. We characterized the effects of modafinil, an atypical alerting compound, on circadian rhythms in male Syrian hamsters. EEG recordings and video observations confirmed that modafinil dose-dependently increases wakefulness at the expense of slow-wave and paradoxical sleep with no increase in locomotor activity per unit time awake. Despite inducing arousal, modafinil at these doses (150 or 300 mg/kg), administered in the subjective day or early or late in the subjective night, did not perturb circadian phase. Modafinil (300 mg/kg) also had no effect on phase shifts to light exposure either early or late in the night and did not alter the size of phase shifts induced by running in a novel wheel for 3 h during the mid-day. Modafinil (300 mg/kg) did, however, decrease by ~50% the amount of novel wheel-stimulated running, moving leftward the dose-response relation between wheel revolutions and shift magnitude. These results indicate that, in Syrian hamsters, modafinil alone has no significant chronobiotic efficacy. Nevertheless, this agent may increase the sensitivity of the circadian pacemaker to non-photic stimuli, and may thus have some potential as a tool for promoting clock resetting in combination with behavioral strategies.
Introduction

Convergent evidence indicates that the hypothalamic suprachiasmatic nucleus is the locus of the mammalian circadian clock driving daily rhythms of behavior and physiology (Klein et al., 1991). While daily light-dark (LD) cycles are the most powerful cue (‘zeitgeber’) for synchronizing this endogenous pacemaker to local time, non-photic stimuli can also exert considerable influence on circadian timing (Mistlberger and Skene, 2004, 2005). In Syrian hamsters, for example, behavioral arousal during the usual sleep period (‘subjective day’ in nocturnal animals), stimulated by running in a novel wheel, social interactions, saline injection, or gentle handling, can induce large phase advance shifts of circadian rhythms (Mrosovsky, 1996a; Mistlberger et al., 2000). Stimulated running and short-term sleep deprivation have also been reported to attenuate phase shifts in response to photic stimuli (Ralph and Mrosovsky, 1992; Mistlberger et al, 1997; Mistlberger and Antle, 1998; Challet et al, 2001).

Given this evidence for regulation of circadian timing by arousal, it is reasonable to suspect that pharmaceutical agents capable of influencing behavioral state may have similar effects. Such ‘chronobiotics’ could have potential therapeutic applications in the treatment of circadian sleep disorders, jet lag, and shift work malaise. While several benzodiazepine hypnotics have been shown to shift circadian rhythms in Syrian hamsters and squirrel monkeys (Mrosovsky, 1996a; Mistlberger et al, 1991), there are no reports yet of circadian clock resetting by stimulants. Previous studies, however, have suggested that pharmacologically induced arousal may be insufficient. Both caffeine and the anxiogenic α2 noradrenergic agonist yohimbine potently stimulate arousal when administered to Syrian hamsters in the mid-subjective day but do not induce phase shifts.
Here we characterize the effects of the atypical stimulant modafinil on circadian rhythms in male Syrian hamsters and examine its interaction with photic and non-photic zeitgebers.

Modafinil is a unique wake-promoting pharmaceutical that is structurally unrelated to the classic psychomotor stimulants. This agent has been reported to prolong wakefulness in several mammalian species, including humans, with little, if any, rebound sleep (McClellan and Spencer, 1998; Bonnet et al., 2005). Modafinil’s pharmacological and behavioral profiles appear quite distinct from that of other stimulants (Akaoka et al., 1991; Engber et al., 1998; Scammell et al., 2000). Unlike amphetamine, modafinil does not produce stereotypical movement (Simon et al., 1994), nor does it increase locomotor activity above levels expected for a normal waking animal (Edgar and Seidel, 1997). Furthermore, modafinil appears to exhibit a low potential for abuse (Schwartz, 2005). Clinical studies have demonstrated its efficacy in the treatment of excessive daytime sleepiness associated with narcolepsy and a variety of other conditions (2000; Schwartz, 2005). Given its clinical efficacy, reported low incidence of side effects, and apparent low abuse liability, modafinil has become an increasingly popular treatment and, therefore, it is pertinent that the chronobiotic properties of this agent be assessed.
Methods

Animals

Young male Syrian hamsters (60-170g, Charles River, Montreal, Quebec, Canada) were individually housed in polypropylene cages (47 x 26 x 20 cm) with wire mesh bottoms. The cages were equipped with 17.5 cm diameter running wheels and daily rhythms of locomotor activity were continuously monitored via microswitches interfaced with a computer. The subjects were kept under a 14:10 LD (~350 lx:0 lx) cycle with food and water provided ad libitum. All manipulations were approved by the university animal care committee and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as outlined by the U.S. National Institutes of Health.

Drugs

Modafinil (2-[(diphenylmethyl)sulfinyl]acetamide) was generously supplied by Cephalon, Inc. (West Chester, PA.). The drug was suspended in a sterile solution of 0.25% methylcellulose (Sigma-Aldrich Canada Ltd.) immediately prior to i.p. administration. Injections of sterile 0.25% methylcellulose served as the vehicle control.

EEG Recordings and Behavioral Observations

To mimic other behavioral arousal procedures known to induce large phase shifts of circadian wheel running activity rhythms, pilot EEG recordings and behavioral observations were carried out to select a dose that would produce virtually complete wakefulness over a 3 h period. These recordings were performed as described previously (Mistlberger et al., 2003). Briefly, under sodium pentobarbital (50 mg/kg) anesthesia and
using standard stereotaxic techniques, stainless steel screws and subcutaneous electrodes were implanted into the skull and nuchal muscle, respectively. The electrodes were connected to a plastic headcap that was subsequently cemented to the skull using dental acrylic. Following 7 days of recovery, the animals were habituated to a recording cable in an electrically shielded recording chamber for a period of 3 days. Hamsters were then injected with vehicle (n = 4), 100 mg/kg (n = 2), 150 mg/kg (n = 1), 200 mg/kg (n = 1) or 300 mg/kg (n = 6) modafinil at zeitgeber time (ZT) 6 and recorded for 12 h. In standard chronobiology notation, time within a LD cycle is referred to as ZT (there are 24 zeitgeber hours in a solar day), and ZT12 is defined as lights-off. ZT6 is thus 6 h before lights-off, the middle of the rest phase of the daily rest-activity cycle in nocturnal rodents. EEG and EMG signals were amplified (Grass model 79D, Grass Instruments), digitized at a sampling rate of 250 Hz, and stored on a computer using AcqKnowledge software (Biopac, Goleta, CA). Behavioral state was scored in 10s epochs as wakefulness (W), slow-wave sleep (SWS), paradoxical sleep (PS), transitional sleep (TS), or low amplitude sleep (LS) using unfiltered lateral EEG, lateral EEG filtered for delta waves (1-3 Hz), midline EEG filtered for theta (5-15 Hz), and EMG (high pass filtered at 50 Hz), as described previously (Mistlberger et al., 2003). Each epoch was classified as whichever state filled the majority of the time.

A second group of hamsters (n = 10) were administered vehicle or 300 mg/kg modafinil in counterbalanced order and videotaped in their home cages using an overhead camera. The injections were given at ZT6 and video recordings were made until ZT9. An observer blind to the experimental conditions later recorded the amount of time each animal engaged in active wake (arousal with ambulation), quiet wake (arousal without
ambulation, head up, eyes open), behavioral sleep (quiescent, sleep posture, eyes closed), grooming, feeding, and drinking. A switch from one behavioral category to another was only deemed to occur following a 10 s occurrence of the new behavior. Locomotor activity also was quantified via image analysis using the methods of Antle and Mistlberger (2000). Briefly, the path traveled by each animal was traced onto acetate transparencies, digitized, and loaded into Image J (NIH). The total area of the path line was calculated and divided by the average line width, thus giving an approximation of the total distance traveled during the 3 h period. Both the EEG recordings and the behavioral observations were carried out under a 14:10 LD schedule.

Effects of Modafinil on Circadian Phase When Administered During the Light Period

To determine if modafinil can mimic the phase shifting effects of behavioral arousal procedures, hamsters (n = 20) stably entrained to LD were administered vehicle or modafinil (150mg/kg or 300mg/kg) at ZT6. The lights were then turned off, and left off for 4 days. The injections were given in a counterbalanced order and the hamsters were re-entrained to LD for at least 7 days between treatments. Phase shifts were measured as outlined below.

Effects of Modafinil on Circadian Phase Following One Night of LL and its Interaction with Shifts to Novel Wheel Confinement

Phase shifts induced by behavioral arousal are substantially potentiated by brief (1-3 days) exposure to constant light (LL) (Knoch et al., 2004). Therefore, we reasoned that any phase shifts in response to modafinil administration would likely also be
amplified and more easily detected by this procedure. Accordingly, following one night of LL, hamsters (n = 10) were left undisturbed in their home cage or administered vehicle or 300mg/kg modafinil at ZT4 in counterbalanced order. The room lights were turned out at ZT6 in all conditions and remained out for 4 days.

Caffeine has been reported to attenuate shifts in response to running in a novel wheel (Antle et al., 2001). We therefore sought to determine how modafinil might interact with other non-photic stimuli. Following one night of LL, vehicle or 300mg/kg modafinil was administered at ZT4 in combination with confinement to a novel wheel at ZT6. The latter manipulation involved placing the hamsters in a novel 33 cm diameter Wahmann running wheel for 3 h. This procedure stimulates running in most hamsters, and induces large (2 h or more) phase advance shifts in those hamsters that run more or less continuously (Mrosovsky, 1996a). The room lights were turned out during wheel confinement, and remained off for 4 days after the animals were returned to their home cages.

Effects of Modafinil on Phase Shifts to Photic Stimuli

Novel wheel-induced running and short-term sleep deprivation can attenuate phase shifts in response to light (Ralph and Mrosovsky, 1992; Mistlberger et al, 1997; Mistlberger and Antle, 1998; Challet et al, 2001). Therefore, we assessed whether modafinil-induced arousal might have a similar effect. Hamsters (n = 10) were injected with 300 mg/kg modafinil or vehicle at ZT6 or ZT12 with or without a 15 min light pulse (~190 lx) beginning at ZT13 (i.e., 1 h after lights-off). A second group of animals (n = 10) were administered 300 mg/kg modafinil or vehicle at ZT17 with or without exposure
to a 15 min light pulse at ZT18. For the latter condition, the injections were given under dim red light (DDred, ~ 1 lx).

For the light pulse experiments described above, the room lights went out at ZT12 as scheduled on the manipulation day and then remained off for 4 or 7 days. The conditions were counterbalanced for order and separated by at least 14 days, resulting in a minimum of 30 days between successive drug administrations.

Data Analysis

Phase shifts were measured by comparing the time of spontaneous activity onset on day 2, 3 or 6 of DD following the manipulation with the average time of activity onset during the 3 days prior to the manipulation day (the so-called Aschoff Type II procedure; Mrosovsky, 1996b). A computer algorithm was used to identify the onset of the main period of daily wheel running. Phase shift and activity measures were evaluated by within-subjects ANOVA with post hoc Bonferroni comparisons, Tukey multiple comparisons, or paired t-tests where appropriate. Means are presented +/- the standard error of the mean.
Results

Modafinil Induces Arousal When Administered During the Mid-day

The exploratory EEG recordings showed that modafinil, administered during the mid-light (i.e., sleep) period, dose-dependently increased W at the expense of SWS and PS, as compared to vehicle alone (Fig. 1a). At 300 mg/kg, modafinil produced virtually continuous wakefulness for 6 h and this dosage was therefore selected for the subsequent phase shift experiments. More detailed examination at 300 mg/kg indicated elevated W and decreased SWS, at least for the remainder of the light period, as compared to vehicle injection alone (Fig. 1b-c). PS appeared almost completely suppressed over the 12 h recording period (Fig. 1d).

Behavioral observations confirmed increased arousal over a 3 h period following drug administration (Table 1). Modafinil (300 mg/kg) significantly increased the percentage of total wakefulness ($t_{(8)} = 8.740, p < 0.0001$) and quiet wakefulness ($t_{(8)} = 9.317, p < 0.0001$), and significantly decreased the percentage of behavioral sleep ($t_{(8)} = 8.717, p < 0.0001$) and grooming ($t_{(8)} = 2.649, p < 0.05$), as compared to vehicle alone. Drug administration, however, did not affect the percentage of active wakefulness ($t_{(8)} = 1.125, p > 0.05$) and only marginally increased the total linear distance traveled within the home cage ($22.8 +/- 6.2m$ vs $9.5 +/- 2.3m$; $t_{(8)} = 2.214, p = 0.058$). The amount of activity per minute awake did not differ between modafinil and vehicle ($0.17 +/- 0.02 m/min$ vs $0.18 +/- m/min$; $t_{(8)} = 0.12, p = 0.9$). One outlier was excluded from this analysis for a lack of response to the drug (likely due to a misplaced injection).

Modafinil Does Not Shift Circadian Phase When Administered Mid-day
Modafinil, administered at ZT6, did not induce phase shifts significantly different from the vehicle control injections, at doses of 150 mg/kg ($t_{(8)} = 1.631$, $p = 0.1416$) or 300 mg/kg ($t_{(8)} = 0.8082$, n.s.; Figs. 2a-c & 3).

*Modafinil Does Not Alter Circadian Phase or Shifts in Response to Wheel Confinement Following a Day of LL*

After 1 day in LL, injections of vehicle or of modafinil (300 mg/kg) at ZT4, followed by DD, resulted in phase shifts of 91 +/- 11 min and 97 +/- 24 min, respectively, that were not significantly different from each other ($t_{(9)} = 0.1990$, n.s.) or from the no-injection control condition (95 +/- 21 min; $t_{(9)} = 0.1539$, n.s. vs. vehicle alone; Figs. 2d-f & 3). A 3 h bout of running stimulated by confinement to a novel wheel from ZT6-9, following treatment with either vehicle or modafinil (300 mg/kg) at ZT4, induced large phase advance shifts of 255 +/- 38 min and 210 +/- 39 min, respectively. The difference between drug and vehicle conditions was again not significant ($t_{(9)} = 1.678$, $p > 0.05$; Figs. 2g-h & 3).

The large phase advance shifts apparent in the control, drug and wheel confinement conditions following a night of LL could be secondary to a shorter circadian period. Nineteen hamsters in total were released into DD in the no-injection control condition following entrainment to LD and following a night of LL. Regression lines were fit to activity onsets during each day of DD to obtain an estimate of the circadian period following the initial shift. The circadian period did not differ between lighting conditions ($23.7 +/- .33$ h vs $23.65 +/- .22$ h, in the LD vs LL conditions, respectively; $t_{(18)} =0.65$, $p=.521$).
Modafinil Suppresses Wheel Running Activity When Administered Mid-day Following One Night of LL

Inspection of the activity charts revealed that modafinil suppressed spontaneous wheel running activity, both during novel wheel confinement and during the following night in the home cage. To quantify this effect, the mean number of revolutions over an 18 h period following administration was compared across the DD, vehicle, and 300 mg/kg conditions with or without wheel confinement from ZT6-9. Also, to assess the time course of any drug effect, the 18 h period following drug administration was divided into six 3 h blocks (ZT6-9, ZT9-12, ZT12-15, ZT15-18, ZT18-21, and ZT21-24), and the mean number of wheel revolutions was compared across these time blocks.

The overall within two-way ANOVA revealed a significant effect of condition ($F_{(4,36)} = 6.259, p < 0.001$), time of day ($F_{(6,54)} = 13.132, p < 0.0001$), and a significant interaction ($F_{(24,216)} = 9.509, p < 0.0001$). By comparison with the vehicle control conditions, modafinil injection at ZT4 was associated with a 51.5% decrease in 18 h cumulative wheel revolutions ($t_{(9)} = 3.560, p < 0.01$), while modafinil combined with wheel confinement from ZT6-9 was associated with a 55.4% decrease ($t_{(9)} = 3.039, p < 0.05$; Fig. 4). The number of wheel revolutions during wheel confinement was decreased by 53.3% following modafinil administration by comparison with vehicle ($t_{(9)} = 4.891, p < 0.001$; Fig. 4). Modafinil also significantly suppressed wheel running by 64.6% in the home cage from ZT12-15, by comparison with the vehicle injection condition ($t_{(9)} = 4.316, p < 0.001$; Fig. 4).
Given the significantly reduced level of running during wheel confinement in the modafinil treatment condition, it bears repeating that the phase shifts induced by wheel confinement did not differ between vehicle control and modafinil conditions (Fig. 3c). A scatterplot clearly shows large phase advance shifts associated with lower levels of novel wheel-induced running in the modafinil condition (Fig. 5).

**Modafinil Does Not Perturb Circadian Phase When Administered During the Night and Does Not Alter Light-Induced Shifts**

A 15 min light pulse at ZT13 induced a significant phase delay shift in both the vehicle ($t_{(9)} = 3.813, p < 0.01$) and drug ($t_{(9)} = 4.381, p < 0.001$) conditions (Figs. 6a-e & 7a). The drug and vehicle control conditions did not differ significantly ($t_{(9)} = 1.238, \text{n.s.}$). Similar phase delays were obtained for 15 min light pulses that were preceded by injections of modafinil or vehicle at ZT6, rather than ZT12 (Figs. 6f-g & 7b). Again there was no difference between drug and vehicle conditions ($q_{(5,45)} = 1.156, p > 0.05$).

At ZT18, a 15 min light pulse elicited a significant phase advance shift in both the vehicle ($t_{(9)} = 3.472, p < 0.01$) and drug conditions ($t_{(9)} = 5.510, p < 0.001$; Figs. 6i-l & 7c). Differences between the drug and vehicle control conditions were again not significant ($t_{(9)} = 2.301, p > 0.05$).
Discussion

In agreement with studies of other mammalian species (Edgar and Seidel, 1997; McClellan and Spencer, 1998), modafinil administered to Syrian hamsters during the normal sleep period dose-dependently increased wakefulness at the expense of SWS and PS but only marginally increased locomotor activity. Despite a strong induction of arousal, modafinil, at these doses, administered in the light or the dark period, did not induce significant phase shifts by comparison with vehicle control injections. Modafinil also did not induce phase shifts after a day in LL, a treatment that greatly potentiates phase shifts in response to at least some behavioral arousal procedures (Knoch et al., 2004). These results, in combination with the recent finding that modafinil does not eliminate excessive sleepiness during the night-shift (suggesting no significant circadian adaptation to night work; Czeisler et al., 2005), indicate that this agent has little, if any, resetting action on the mammalian circadian pacemaker. Modafinil also had no effect on phase shifts to sub-saturating light pulses presented either early or late in the night, suggesting that this compound is unlikely to interfere with photic entrainment when used therapeutically.

The absence of phase shifts in response to modafinil is concordant with our observations that two other wake-promoting drugs, caffeine and yohimbine, also do not induce phase shifts in Syrian hamsters when administered during the mid subjective-day (Antle et al., 2001; Webb and Mistlberger, unpublished results). Given that behavioral manipulations capable of shifting circadian rhythms strongly stimulate arousal, it seems surprising that these agents do not have similar clock resetting effects. These results, however, do build on evidence from recent behavioral studies indicating that arousal, per
se, is not sufficient to induce phase shifts. Physical restraint (with intermittent compressed air stimulation) or confinement to a small pedestal over water, procedures that induce continuous waking and elevated cortisol, do not perturb circadian phase (Mistlberger et al., 2003). Arousal states are heterogeneous, and the constellation of neural and endocrine correlates unique to each of these states appears to have differential effects on the circadian pacemaker. Which of these correlates is critical for phase shifting has not been fully resolved, and it is conceivable that some correlates promote shifting, while others block shifting.

Given that restraint and pedestal confinement prevent locomotion and do not induce shifts, neural and endocrine correlates of activity are of special interest as critical clock resetting stimuli. High intensity activity (‘exercise’) is clearly not necessary for clock resetting, given that arousal enforced by the sleep deprivation procedure of gentle handling can induce large phase shifts despite minimal stimulation of locomotor activity (Antle and Mistlberger, 2000). However, hamsters subjected to this procedure are free to move about in their cages, and accumulate ~80 m of linear distance traveled in 3 h in standard size cages (47 x 26 x 20 cm). This is much less activity than is accumulated by hamsters confined to a novel wheel, which may run several kilometers or more in 3 h. Nonetheless, a low level of forward locomotion sustained over 3 h may be both necessary and sufficient to induce clock resetting. In the present study, modafinil potently stimulated arousal but only marginally increased locomotor activity over a 3 h period, by comparison with the vehicle control condition. The linear distance traveled averaged only ~20 m, or about one quarter the distance measured in hamsters sleep deprived by gentle handling (Antle and Mistlberger, 2000). The predominant behavior displayed by hamsters
during modafinil-induced arousal in the usual sleep period can be described as ‘restless
fidgeting’, with relatively little forward locomotion; the subjective impression is of an
animal that wants to sleep but cannot. A working hypothesis, therefore, is that sensori-
motor correlates of forward locomotion are necessary for clock resetting to arousal, and
that these correlates are not sufficiently present in the arousal state induced by modafinil.

An alternative perspective is that behavioral stress procedures (e.g., confinement
to a restraint tube or a pedestal) and alerting compounds such as modafinil, caffeine and
yohimbine have in common neural correlates that block non-photic phase shifts. If so,
then modafinil would be predicted to inhibit phase shifts induced by running in a novel
wheel, a property already demonstrated for caffeine (Antle et al, 2001). Several studies
have reported a sigmoidal relationship between the magnitude of running-induced phase
shifts and the number of wheel revolutions generated during wheel confinement, with
small advance shifts (~30 min) typically occurring below 4000 wheel revolutions in 3 h
and maximal advances (≥ 2 h) above 5000 revolutions (Janik and Mrosovsky, 1993;
Bobrzynska and Mrosovsky, 1998). Unexpectedly, in the current study, phase shifts
following wheel confinement were not decreased despite a modafinil-induced 50% reduction in activity during the procedure, and large advances were evident at well under
3000 revolutions. This novel finding distinguishes modafinil from caffeine, and weighs
against the idea that modafinil activates neural pathways that inhibit phase resetting
correlates of arousal or locomotor activity.

This result instead appears to suggest that modafinil may reduce the threshold for
shifts induced by running in a novel wheel, thus shifting leftward the dose-response
relation between wheel revolutions and shift magnitude. Alternatively, it may be that LL
alone increased the sensitivity of the circadian pacemaker to the phase resetting correlates of stimulated activity, thus permitting normal size shifts despite the lower levels of running in the modafinil condition. One obvious prediction of this hypothesis is that the dose-response relation between running levels and shift magnitude should also be shifted left in the vehicle control condition. In the current study, none of the vehicle treated hamsters ran at intermediate levels, thus there are no data by which to directly evaluate this prediction. Given the potential value of a compound that might potentiate the phase shifting effects of exercise, this issue warrants further attention.

Modafinil administration had pronounced effects on home cage running activity. It is important to note, however, that despite a modafinil-induced decrease of wheel running, particularly during the early night, ambulation was not absent following drug administration. Indeed, our behavioral observations, in agreement with previous investigations of other species (Simon et al., 1995; Edgar and Seidel, 1997), revealed no change in the level of activity per minute awake in the home cage following modafinil treatment, as compared to vehicle. In Syrian hamsters, amphetamine also has been reported to inhibit wheel running at doses that increase or do not affect locomotor activity in an open field (Della Maggiore and Ralph, 2000). Therefore, it is worth highlighting that, in this species, the methodology used to assess pharmacological effects on locomotor activity may impact the experimental outcome.

The cellular actions of modafinil are not yet fully elucidated, although changes in dopaminergic and noradrenergic transmission have been implicated (de Saint Hilaire et al., 2001; Saper and Scammell, 2004). Most recently, it has been suggested that modafinil may influence behavior through a blockade of dopamine reuptake followed by
subsequent dopaminergic stimulation of α1 adrenergic receptors (Wisor and Eriksson, 2005). Increased glutamatergic and histaminergic transmission, and decreased GABAergic transmission have also been reported in distinct brain regions following modafinil treatment (Ferraro et al., 1996; Ferraro et al., 1999; Ishizuka et al., 2003). Interestingly, modafinil has been shown to increase the release of cortical 5-HT both in vitro and in vivo (Ferraro et al., 2000; de Saint Hilaire et al., 2001), with differential efficacy across brain areas. Non-photic shifts can be induced by NPY, 5-HT and GABA agonists, and can be blocked by glutamate agonists (Morin and Allen, In Press). Data on how modafinil affects transmission at these synapses within the circadian system are lacking.

In addition to its efficacy for the treatment of excessive daytime sleepiness, modafinil has been reported to enhance cognitive performance in both sleep-deprived (Bonnet et al., 2005) and normal volunteers (e.g., Baranski et al., 2004) and may have some utility in the treatment of a variety of psychological disorders (Rugino and Samsock, 2003; Ninan et al., 2004). It is likely, therefore, that utilization of this compound will only increase in the coming years. In summary, the current study suggests that acute administration of modafinil, despite potent stimulation of arousal, has little effect on the circadian pacemaker and is unlikely to perturb circadian phase or alter photic entrainment in individuals using this compound therapeutically. This agent, however, is administered chronically in the clinical environment and further study is required to rule out the possibility of long-term effects on circadian timing. The present results also suggest that modafinil may modulate the relationship between phase shifts and locomotor activity and may thus have some potential as a tool for promoting clock
resetting in combination with exercise or other behavioral arousal procedures. Further, the current data add support to the idea that arousal, per se, is not sufficient to induce phase shifts of a non-photic nature and demonstrate that different states of arousal can have divergent effects on the mammalian circadian clock.
Acknowledgments

We thank Liam Yeung, Dana Martin, James Handel, and Glenn Yamakawa for technical assistance.
References


Footnotes

Supported by operating grants (R.E.M.) and a Postgraduate Scholarship (I.C.W.) from the National Science and Engineering Research Council of Canada.

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Legends for Figures

Fig 1. Effects of vehicle and modafinil on behavioral state following administration at ZT6. (A) Effects of increasing dosages of modafinil on the percentage of behavioral state over a 6 h period following administration. (B) Effects on wakefulness, (C) slow-wave sleep, and (D) paradoxical sleep over a 12 h period following administration. Data shown as mean +/- SEM where appropriate. The shading indicates the dark period of the LD cycle.

Fig 2. Wheel running activity records of representative hamsters illustrating phase shifts in response to (A) vehicle, (B) 150 mg/kg modafinil, or (C) 300 mg/kg modafinil administered at ZT6. Also shown are phase shifts in response to (D) one night of constant light [LL] followed by constant darkness, (E) vehicle, (F) or 300 mg/kg modafinil administration at ZT4, and (G) LL followed by vehicle (H) or 300mg/kg modafinil administration at ZT4 with novel wheel confinement from ZT6-9. Each horizontal line represents a 24 h period with wheel revolutions plotted in 10 min bins from left to right. Wheel running is indicated by vertical deflections and shading marks the dark period of the LD cycle. The circle and diamond symbols represent vehicle and drug injections, respectively, and the ‘v’ markers designate the beginning and end of novel wheel confinement.

Fig 3. Mean phase shifts in response to vehicle, 150 mg/kg modafinil, and 300 mg/kg modafinil administered at ZT6. Also illustrated are shifts in response to one night of constant light [LL] followed by constant darkness [DD], vehicle [Veh], or 300 mg/kg
modafinil administration at ZT4 with and without novel wheel confinement [WC] from ZT6-9. Data shown as mean +/- SEM. ** = conditions significantly different at p < 0.001.

Fig 4. Mean wheel revolutions over six 3 h periods following one night of constant light [LL] and subsequent exposure to constant darkness [DD], vehicle [Veh], or 300 mg/kg modafinil administration at ZT4, with or without novel wheel confinement [WC] from ZT6-9. Data shown as mean +/- SEM. ** = significantly different from appropriate vehicle or drug alone control condition at p < 0.001. ††† = significantly different from Veh + WC condition at p < 0.001.

Fig 5. Scatter plot of resultant phase shifts versus the number of wheel revolutions stimulated by novel wheel confinement [WC] from ZT6-9 following one night of constant light and the administration of vehicle [Veh] or 300 mg/kg modafinil at ZT4.

Fig 6. Wheel running activity records of representative hamsters illustrating phase shifts in response to (A) constant darkness, (B) vehicle, or (C) 300 mg/kg modafinil administered at ZT12. Also shown are shifts in response to (D) a 15 min light pulse [LP] at ZT13 preceded by vehicle, or (E) 300 mg/kg modafinil administered at ZT12 and (F) a 15 min LP at ZT13 preceded by vehicle, or (G) 300 mg/kg modafinil administration at ZT6. The last five panels illustrate phase shifts in response to (H) constant darkness, (I) vehicle or (J) 300 mg/kg modafinil administration at ZT17 and (K) a 15 min LP at ZT18 preceded by vehicle or (L) 300 mg/kg modafinil at ZT17. The circle, diamond, and
square symbols represent vehicle injections, drug injections, and light pulses, respectively.

Fig 7. Mean phase shifts in response to constant darkness [DD], vehicle [Veh] or 300 mg/kg modafinil, with or without exposure to a 15 min light pulse [LP]. (A) DD, veh or modafinil administered at ZT12 with or without a LP at ZT13, (B) DD, veh or modafinil administered at ZT6 with a LP at ZT13. (C) DD, veh or modafinil administered at ZT17 with or without a LP at ZT18. Data shown as mean +/- SEM. * = conditions significantly different at p < 0.05; ** = conditions significantly different at p < 0.01; *** = conditions significantly different at p < 0.001.
Table 1. Effects of vehicle and modafinil on behavior following administration at ZT6.

The mean percentage of time spent engaged in various behaviors in the home cage over a 3 h period following modafinil or vehicle administration at ZT6. Data shown as mean (± SEM).

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<tr>
<th>Behavior</th>
<th>Vehicle</th>
<th>Modafinil</th>
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<tr>
<td>Active Wake</td>
<td>3.1 (1.8)</td>
<td>4.6 (1.3)</td>
</tr>
<tr>
<td>Quiet Wake</td>
<td>15.1 (3.3)</td>
<td>80.7 (4.4)a</td>
</tr>
<tr>
<td>Behavioral Sleep</td>
<td>70.3 (4.2)</td>
<td>9.7 (3.6)a</td>
</tr>
<tr>
<td>Grooming</td>
<td>6.4 (1.0)</td>
<td>2.9 (0.8)b</td>
</tr>
<tr>
<td>Feeding</td>
<td>4.6 (1.0)</td>
<td>2.0 (0.7)</td>
</tr>
<tr>
<td>Drinking</td>
<td>0.4 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>Total Wake</td>
<td>29.6 (4.2)</td>
<td>90.4 (3.5)a</td>
</tr>
</tbody>
</table>

a = conditions significantly different at p < 0.001, b = conditions significantly different at p < 0.05.
Fig. 1

A) % Behavioral State vs. Dose (mg/kg)

B) % W vs. Time of Day (ZT)

C) % SWS vs. Time of Day (ZT)

D) % PS vs. Time of Day (ZT)
Fig. 2

Time of Day (ZT)

A

B

C

D

E

F

G

H
Fig. 4

The bar chart displays the wheel revolutions at different times of the day (ZT) for different groups:
- DD
- Veh
- 300
- Veh+WC
- 300+WC

The chart indicates significant differences in wheel revolutions across the time periods, with peaks at 12-15 ZT and 15-18 ZT for the DD group, and 9-12 ZT for the Veh+WC group.