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Armodafinil, the R-enantiomer of modafinil: Wake-promoting effects and pharmacokinetic profile in the rat

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Abstract

Modafinil reduces the excessive sleepiness associated with narcolepsy, obstructive sleep apnea/hypopnea syndrome, and shift work sleep disorder. In rats, modafinil promotes dose-dependent increases in wake duration. The wake-promoting activity of the R-enantiomer of modafinil (armodafinil) was evaluated in WKY rats and compared to the classical stimulant, D-methamphetamine. Electroencephalographic and electromyographic signals were assessed via a tethered cranial implant. Body temperature and locomotor activity were assessed by telemetry via intraperitoneal implant. Rats ($n=60$, 12 per group) were subjected to one of five parallel treatments: armodafinil at 30, 100 and 300 mg/kg i.p.; D-methamphetamine, 1 mg/kg i.p. and vehicle. Armodafinil and D-methamphetamine increased time spent awake relative to vehicle. Armodafinil-evoked increases in wake duration were dose-dependent and proportional to plasma compound exposure. Induction of wakefulness by D-methamphetamine was associated with an approximately two-fold increase in locomotor activity during the 2-h period immediately following administration relative to vehicle. D-methamphetamine also increased body temperature over the same time interval. The dose of armodafinil (100 mg/kg, i.p.) that was closest to D-methamphetamine in its wake-promoting efficacy did not produce changes in either body temperature or the intensity of locomotor activity relative to vehicle. Acute rebound hypersomnolence, characterized by increases in non-rapid eye movement sleep (NREMS) as a percentage of time and NREMS bout duration and by a decreased frequency of brief awakenings following sleep deprivation, occurred following D-methamphetamine—but not armodafinil-induced wake in this rat model which has been shown to be predictive of human drug responses.

Keywords: Modafinil; Armodafinil; Methamphetamine; Rebound hypersomnolence

1. Introduction

Modafinil is among the most widely used and most effective pharmaceutical agents for the treatment of excessive sleepiness caused by narcolepsy, shift work sleep disorder and obstructive sleep apnea. Despite considerable research, the mechanism of action for modafinil remains unclear, but is likely to involve the selective potentiation of catecholaminergic signaling in the CNS (Duteil et al., 1990; Lin et al., 1992; Madras et al., 2006; Nishino et al., 1998; Sebban et al., 1999; Stone et al., 2002a,b; Wisor et al., 2001). Unlike amphetamines and related central nervous system stimulants, which have been used historically for the treatment of excessive sleepiness, modafinil has minimal abuse potential (Myrick et al., 2004). Comparison of the pharmacokinetic profiles of equivalent doses of modafinil and its R-enantiomer, armodafinil, in humans revealed that armodafinil sustains higher plasma concentrations 6–14-h post-administration than that of racemic modafinil which translated to longer maintenance of wakefulness (Dinges et al., 2006). In a pharmacologically naïve state, sleep need increases in proportion to the duration of wake (Borbely and Achermann, 2000; Franken et al., 1991). Whether this relationship is maintained after pharmacological manipulation of sleep timing

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is uncertain. The relationship between the pharmacokinetic disposition of a wake-promoting compound and the duration of its wake-promoting efficacy provides information that is critical to this issue. One purpose of the current study was to assess, in a pre-clinical model for sleep/wake therapeutics, the wake-promoting efficacy of armodafinil and its pharmacokinetic profile in parallel, and thereby determine the degree to which wake-promoting effects are proportional to circulating levels of armodafinil.

To assess the wake-promoting efficacy of armodafinil, its effects on sleep/wake patterns were examined in rats, an experimental model in which modafinil has been shown to induce sustained wake without increasing locomotor activity (LMA) or elevating body temperature (Edgar and Seidel, 1997). The present data largely replicate these previous findings on the wake-promoting effects of modafinil and demonstrate that armodafinil can promote dose-dependent increases in wake duration in the rat that are related to plasma exposure of the compound.

2. Materials and methods

2.1. Animals

Male WKY rats (Taconic; 8–10 weeks at the time of surgery), were anesthetized with isoflurane (Abbott Laboratories; North Chicago, IL; 3% in medical grade oxygen) and surgically prepared with a cranial implant for measurement of electroencephalographic (EEG) and electromyographic (EMG) potentials, and with an intraperitoneal (i.p.) transmitter for measurement of body temperature (Tb) and LMA (see Edgar and Seidel, 1997 for details). Following recovery, rats were housed in isolation under a 12-h light/12-h dark cycle in individual compartments of a stainless steel cabinet. Experiments were conducted at 23+1°C ambient temperature. Animals had unrestricted access to food and water throughout experimentation. These experiments were approved by institutional animal care and use committees.

2.2. Data collection

Data were processed in 10-s epochs by the SCORE™ data acquisition and analysis system (Edgar and Seidel, 1997; Edgar et al., 1991). The EEG was sampled at 100 Hz frequency and bandpass filtered at 1–30 Hz. The EMG was bandpass filtered at 10–100 Hz and integrated across 10-s epochs. LMA was recorded as a binary variable, and Tb as a single measurement, within each 10-s epoch. Each 10-s epoch was classified as wake, rapid eye movement sleep (REMS) or non-REMS (NREMS) by a pattern matching algorithm that assessed the degree of similarity of data to template epochs chosen separately for each animal by an experimenter blinded to the treatment groups.

EEG data were subjected to Hartley's modified fast Fourier transform in 10-s epochs. EEG data from NREMS epochs not marked as artifact were subjected to further analysis. Average delta power (i.e., power in the 1–4 Hz range) was normalized to the mean of delta power in all NREMS epochs in 24 h immediately prior to drug administration.

2.3. Compound administration

Armodafinil (Cephalon, Inc., West Chester, PA, lot # P040308) was suspended, and D-methamphetamine (METH; Sigma-RBI, St. Louis, MO) was dissolved, in sterile 0.5% methylcellulose/0.2% Tween-80™ vehicle and injected i.p. in volumes ranging from 0.8 to 1.4 ml. Injection volumes varied with animal weight, as injections were delivered from a stock solution made for each treatment group. Rats were randomly divided into parallel treatment groups and were subjected to a 60-h recording session in a light/dark 12:12 cycle. The recording session consisted of a 30-h baseline followed by one of five parallel treatments (armodafinil 30 mg/kg, 100 mg/kg and 300 mg/kg i.p.; D-methamphetamine 1 mg/kg or vehicle i.p.) administered 5 h into the light phase of the LD12:12 cycle (in accordance with previous studies on wake-promoting effects of racemic modafinil in rats; Edgar and Seidel, 1997). Injection was followed by 30 h of post-injection data collection. Samples sizes (n) were 12 per group. LMA data were unavailable for 2 animals in the armodafinil 100 mg/kg group and 2 in the armodafinil 30 mg/kg group due to equipment failure.

2.4. Data analysis and statistics

While data were collected over a 60-h session, only the data from the 24-h period immediately preceding treatment and the 24-h period immediately after treatment were used to perform inferential statistics. These two time intervals were selected as they were equivalent in duration and aligned in circadian phase. The principal variables collected were: NREMS, REMS, and total sleep (sum of NREMS and REMS) as a percentage of time; LMA and body temperature (average hourly deviation from the 24-h mean baseline). LMA "intensity" was defined as the number of epochs per minute of wake time in which LMA was detected during the first 2 h after treatment. Treatment groups were compared by independent measures ANOVA. In the presence of a significant main effect, Student's t-test was performed to assess differences between active treatment groups and vehicle controls.

The magnitude and time course of drug-induced waking effects were assessed as both acute and cumulative variables. Acute waking effects were assessed by averaging dependent variables across the first 2 h post-treatment and subjecting these values to ANOVA. The net cumulative waking effects of treatments were assessed by measurement of cumulative wake surplus (Edgar and Seidel, 1997). This variable was calculated by subtracting the number of minutes of sleep during each post-treatment hour from the analogous hour of the baseline day and adding these values serially. The net wake-promoting efficacy of any given treatment was defined by the maximum cumulative wake surplus for that treatment in 24 h immediately subsequent to treatment. Analogously, the time course of sleep loss was assessed by calculating the NREMS and REMS deficit. These variables were calculated by subtracting the number of minutes

of NREMS (or REMS) during each baseline hour from the analogous time of day on the post-treatment day and adding these values serially.

To determine whether the occurrence or magnitude of rebound hypersomnolence was dependent on the compound used to promote wake, acute changes in variables known to be modulated in proportion to sleep loss (e.g., wake duration) during sleep deprivation (Borbely and Achermann, 2004; Franken et al., 2001, 1998; Tobler et al., 1983; Wisor et al., 2002) were assessed. Sleep deprivation results in a constellation of changes in the timing of subsequent sleep episodes and in the EEG events that occur during those sleep episodes. These changes have been described as sleep recovery (Easton et al., 2004), sleep homeostasis (Tobler et al., 1983) or rebound hypersomnolence (Edgar and Seidel, 1997). Among the acute changes occurring after sleep deprivation are increases in sleep as a percentage of time and the duration of individual sleep bouts and a decrease in the frequency of brief awakenings from sleep episodes (Borbely and Achermann, 2004; Franken et al., 2001, 1991; Tobler et al., 1983; Wisor et al., 2002). These changes occur acutely, e.g., in the time interval immediately subsequent to the onset of sleep (Franken et al., 2001, 1998), as the effects of sleep loss on the timing of subsequent sleep are transient in nature. To control for the time sensitive nature of these variables, the onset of consolidated sleep (i.e., the first occurrence of 2 continuous minutes of sleep after injection; a 2-min 'sleep bout'), was determined, and sleep loss-dependent variables (NREMS bout duration and number of brief awakenings) were measured in the first hour of sleep subsequent to that time point. Edgar and Seidel (1997) demonstrated that NREM sleep was increased 4–7 h after D-methamphetamine (1 mg/kg, i.p.) administration, e.g., during the last 4 h of lights-on in the LD12:12 cycle on the day of treatment. In accordance with that observation, NREMS was assessed as the percentage of time during the remainder of the daily light phase of the LD12:12-h cycle (e.g., hours 1–7) after administration of equally wake-promoting doses of D-methamphetamine (1 mg/kg, i.p.) and armodafinil (100 mg/kg, i.p.).

2.5. Pharmacokinetics of armodafinil

Armodafinil was formulated in 0.5% methylcellulose and 0.2% Tween-80™ and administered to male WKY rats ($n=5$/group) at a dose volume of 5 ml/kg i.p. Blood samples (250 μl) were drawn from a lateral tail vein starting at 30 min post-armodafinil administration into heparinized collection tubes at the appropriate sampling times and placed on wet ice until centrifuged to separate plasma. Immediately following the transfer of each plasma sample, *N, N*-dimethylformamide (DMF; 10 μl/100 μl plasma) was added to each tube which was then gently mixed. The plasma was frozen on dry ice and stored at approximately −20 °C pending analysis. Samples were prepared for analysis by the addition of two volumes of cold acetonitrile containing an internal standard. The samples were mixed on a vortex and centrifuged. The supernatant was removed, placed into an auto sampler vial and the amount of armodafinil present in the samples analyzed by liquid chromatography/tandem mass spectrometry. The concentration of armodafinil in the samples was quantified against a rat plasma standard curve made via serial dilution in a concentration range from 5 to 20,000 ng/ml. Samples containing concentrations greater than 5% above the top of the standard curve were diluted 1:10 with acetonitrile and reanalyzed.

The plasma concentration data for all rats were entered into Excel spreadsheets in preparation for pharmacokinetic analysis. Pharmacokinetic parameters were estimated for each rat by non-compartmental analysis (Gibaldi and Perrier, 1982) of the plasma concentration vs. time data using WinNonlin software (Professional Version 4.0.1, Pharsight Corporation, Palo Alto, CA, 1997).

3. Results

3.1. Pharmacokinetic profile of armodafinil

The plasma concentration of armodafinil was determined for each dose in order to establish the pharmacokinetic (PK) and pharmacodynamic (PD) relationship of this agent. Dose related increases in the maximal plasma level (C_{max}), the time to C_{max} (t_{max}), the overall plasma exposure (AUC) and the plasma half-life ($t_{1/2}$) of armodafinil occurred following administration of armodafinil (Table 1).

3.2. Wake-promoting effect of armodafinil

Under baseline conditions in the 24-h interval immediately prior to treatment, rats exhibited time of day-dependent modulation of all sleep–wake variables studied in baseline conditions (NREMS as a percentage of time, REMS as a percentage of time, total sleep as a percentage of time, wake as a percentage of time, the average and longest hourly NREMS bout durations, raw and normalized body temperature, raw and normalized LMA, normalized NREMS EEG delta power or wake with LMA as a percentage of total wake) as assessed by repeated measures ANOVA (all ANOVAs $P<0.05$, data not shown). Of the 13 variables assayed none was significantly affected by treatment group assignment under baseline conditions (all ANOVAs $P\geq 0.1$). These data demonstrated that the rats assigned to each treatment group were derived from a homogeneous population with respect to baseline sleep and thus allowed the direct comparison of post-treatment data across groups.

Armodafinil administration during the daily rest phase (5 h after lights-on) increased wake time relative to vehicle at all

Table 1
Pharmacokinetic values for armodafinil

	Mean ± S.E.M.		
	30 mg/kg	100 mg/kg	300 mg/kg
C_{max}, μg/ml	9.66 ± 2.47	32.18 ± 4.12	79.02 ± 21.31
t_{max}, h	0.40 ± 0.10	0.70 ± 0.12	2.40 ± 0.93
AUC_{0-t}, μg h/ml	7.46 ± 2.24	53.18 ± 8.39	269.47 ± 62.09
$AUC_{0-\infty}$, μg h/ml	7.47 ± 2.25	53.26 ± 8.30	345.95 ± 50.49
$t_{1/2}$, h	0.33 ± 0.08	0.50 ± 0.06	2.63 ± 0.72

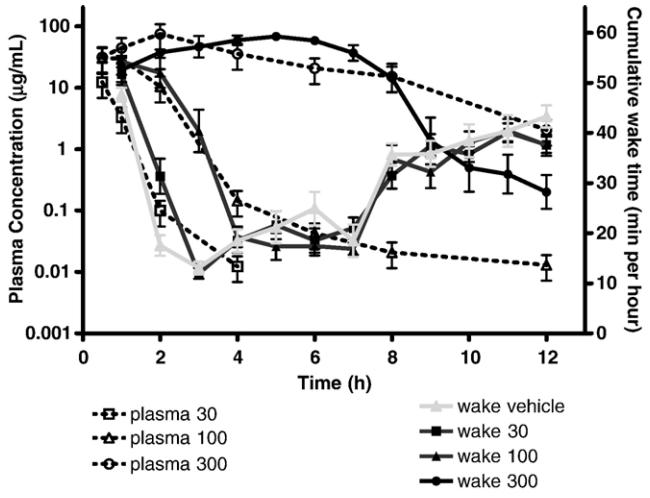

Fig. 1. Pharmacokinetic (left) and wake-promoting profiles (right) of three doses of armodafinil (30, 100 and 300 mg/kg, i.p.). Wake as a percentage of time was significantly increased by all three doses (see Table 2).

doses tested (Fig. 1), resulting in significant changes in sleep/wake variables (Table 2). During the first 2 h after injection, wake, NREMS and REMS as a percentage of time and wake bout duration were affected by treatment group ($P<0.001$; Table 2). Relating the overall plasma exposure to the cumulative wake time associated with each dose of armodafinil administered in the rat revealed a direct PK/PD relationship (Fig. 1). The increase in plasma concentration of armodafinil between 30 mg/kg, i.p. vs. 100 mg/kg, i.p. was relatively dose-proportional, as were the increases in the wake; however between 100 and 300 mg/kg, i.p. a greater than dose-proportional increase in plasma concentration, as well as wake, was observed. Overall, significant wake activity was associated with plasma levels at and above approximately 1 µg/ml (Table 1; Fig. 1).

3.3. Net wake-promoting effect — comparison to D-methamphetamine

At 1 mg/kg, i.p., D-methamphetamine produced changes in sleep/wake variables. Planned contrasts by ANOVA indicated significant effects of D-methamphetamine on wake, NREMS, REMS as a percentage of time and on average wake bout duration ($P \leq 0.029$; Table 2). To determine which of the three doses of armodafinil most closely matched D-methamphetamine (1 mg/kg, i.p.) in wake-promoting efficacy, the maximum hourly cumulative wake surplus produced by compound administration over the 24-h post-treatment relative to pre-treatment baseline was measured. By this measure, the two highest doses of armodafinil increased wake time relative to vehicle, as did the single dose of D-methamphetamine tested (Fig. 2A). ANOVA indicated a significant main effect for treatment on maximal cumulative wake surplus as well as significant main effects of

Table 2
Sleep/wake states, locomotor activity and temperature in the first 2 h after treatment

	n	Wake [min/h]		NREM [min/h]		REM [min/h]		Wake bouts [min/h]		Body temp [°C]	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Vehicle	12	25.3	1.8	30.0	1.4	4.7	0.6	1.8	0.2	−0.11	0.06
R-MOD030	12	36.7	2.4	20.4	2.1	2.8	0.6	5.8	1.1	0.14	0.05
R-MOD100	12	53.3	2.0	6.0	1.7	0.7	0.3	34.1	11.9	−0.10	0.13
R-MOD300	12	57.1	2.4	2.3	1.9	0.6	0.5	92.7	12.8	−0.78	0.13
METH01	12	53.8	3.7	5.1	2.9	1.1	0.8	49.3	13.3	0.32	0.10

	n	LMA [epochs/h]		LMA intensity	
		Mean	S.E.	Mean	S.E.
Vehicle	12	68	8	2.1	0.2
R-MOD030	10	134	12	3.0	0.2
R-MOD100	10	151	15	2.3	0.3
R-MOD300	12	133	25	2.4	0.5
METH01	12	207	26	3.6	0.4

	ANOVA main effect across treatment groups		Significant constrasts ($P<0.05$)				
			vs. vehicle				Comparable doses
			R-MOD			METH	
	F	P	30	100	300	1	METH01 vs. R-MOD100
Wake	28.93	<0.001	<0.001	<0.001	<0.001	<0.001	–
NREM sleep	33.49	<0.001	<0.001	<0.001	<0.001	<0.001	–
REM sleep	9	<0.001	0.014	<0.001	<0.001	<0.001	–
Wake bouts	7.27	<0.001	–	0.015	0.001	0.029	–
Tb	14.1	<0.001	0.006	–	<0.001	0.003	0.019
LMA	5.54	0.001	<0.001	0.001	0.023	<0.001	–
LMA intensity	3.36	0.018	0.002	–	–	0.002	0.01

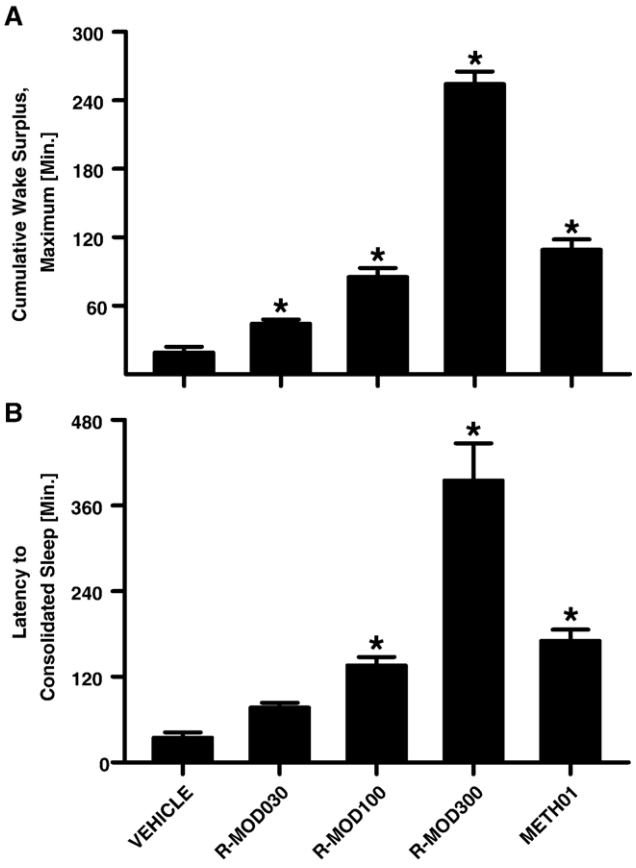

Fig. 2. Assessment of dose equivalency for armodafinil vs. D-methamphetamine. A. Maximum cumulative wake surplus for treatment groups, the accumulation of wake in excess of baseline values, in 24 h after treatment. B. Latency to consolidated sleep subsequent to administration. Treatment groups in A as specified in B. *$P<0.05$ vs. vehicle; *t*-test with Bonferroni correction.

armodafinil at 100 and 300 mg/kg, i.p. relative to vehicle in planned contrasts by ANOVA (Table 3; Fig. 2A). Armodafinil at 100 mg/kg, i.p. produced a maximal wake surplus of 85±8 min over 3 h immediately after treatment and most closely approximated the effect of D-methamphetamine (108±9 min) in this measure (Fig. 2A). Planned contrasts by ANOVA indicated that NREMS time, REMS time, wake time and wake bout duration did not differ significantly between the D-methamphetamine 1 mg/kg, i.p. and armodafinil 100 mg/kg, i.p. groups during the first 2 h after administration (Table 2).

ANOVA indicated a significant effect of treatment on maximum NREMS deficit ($P<0.001$), as well as significant effects for all compound treatments relative to vehicle in planned contrasts (Table 3). This deficit was attained at 2, 3, 8 and 4 h after drug administration for armodafinil 30, 100 and 300 mg/kg, i.p. and D-methamphetamine, respectively. The maximum NREMS deficit did not differ between D-methamphetamine and armodafinil at 100 mg/kg, i.p. in planned contrasts. ANOVA indicated a significant effect of treatment group on cumulative REMS deficit (Table 3). A cumulative REMS deficit was accrued by all compound treatment groups relative to vehicle ($P\leq0.001$, planned contrasts) with the exception of the armodafinil 30 mg/kg, i.p. group (Table 3).

3.4. Acute effects of drug administration on body temperature (Tb) and locomotor activity

During the first 2 h after drug administration, net LMA and the percent wake time with LMA were affected by treatment group ($P\leq0.018$). Planned contrasts by ANOVA indicated significant effects of D-methamphetamine and of all three doses of armodafinil on net LMA ($P\leq0.023$; Table 2). To determine whether compound administration increased the intensity of locomotor activation during wake *per se*, as opposed to simply increasing wake time, the number of epochs/min of wake time in which LMA was detected during the first 2 h after injection ("locomotor intensity"; Table 2) were measured. Planned contrasts by ANOVA indicated significant effects of D-methamphetamine and of armodafinil 30 mg/kg, i.p. on locomotor intensity ($P\leq0.004$; Table 2). Armodafinil at 100 or 300 mg/kg, i.p. did not increase locomotor intensity relative to vehicle injection. Thus, although both armodafinil treatments increased wake time, they did so without inducing behavioral activation.

During the first 2 h after drug administration body temperature was significantly affected by treatment group ($P\leq0.001$; Table 2). Planned contrast by ANOVA indicated a significant effect of D-methamphetamine on temperature ($P=0.003$; Table 2). Armodafinil had dose-dependent, albeit non-consistent effects on body temperature. Planned contrast by ANOVA indicated a significant elevation of temperature (albeit to a much smaller degree than D-methamphetamine) at 30 mg/kg, i.p. ($P=0.006$), no effect at 100 mg/kg, i.p. and a decrease in body temperature at 300 mg/kg, i.p. armodafinil ($P<0.001$; Table 2). Planned contrast of D-methamphetamine vs. armodafinil 100 mg/kg, i.p. by ANOVA indicated a significant difference in body temperature (Tb) change between these groups during the first 2 h after administration ($P=0.019$).

Table 3
Maximum cumulative wake surplus and NREMS and REMS deficits

	n	Wake surplus [min]		NREMS deficit		REMS deficit	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
Vehicle	12	10	9	−11	6	−2	4
R-MOD030	12	44	4	−36	4	−9	2
R-MOD100	12	85	8	−67	7	−18	2
R-MOD300	12	254	11	−198	10	−54	5
METH01	12	108	9	−84	6	−5	2
ANOVA	*F*	P		F	P	F	P
Main effect	88.86	<0.001		115.23	<0.001	38.11	<0.001
R-MOD030–vehicle	3.96	0.056		16.49	<0.001	1.87	–
R-MOD100–vehicle	13.41	0.001		44.85	<0.001	22.3	<0.001
R-MOD300–vehicle	223.32	<0.001		290.21	<0.001	86.56	<0.001
R-MOD100–METH01	7.7	0.011		3.3	–	2.44	–
METH01–vehicle	32.05	<0.001		105.84	<0.001	24.94	<0.001

Thus, armodafinil did not elevate Tb at a dose comparable in wake-promoting efficacy to 1 mg/kg, i.p. D-methamphetamine.

3.5. Acute changes in sleep after wake-promoting effects

The time interval between injection and of consolidated sleep ('latency to consolidated sleep') was determined individually for each rat, and was treatment-dependent (ANOVA F 30.70; df 4, 55; $P<0.001$; Fig. 2B). Armodafinil dose-dependently increased the latency to onset of consolidated sleep; D-methamphetamine increased the latency to the onset of consolidated sleep to a degree that was not different from that of armodafinil at 100 mg/kg, i.p. (Fig. 2B). Furthermore, the duration of NREMS suppression by 100 mg/kg, i.p. armodafinil (Fig. 3A) and D-methamphetamine (Fig. 3B) was approximately equivalent. Comparisons by ANOVA indicated a robust treatment (vehicle vs. armodafinil at 100 mg/kg, i.p. vs. D-methamphetamine)×time (hours 1–7) interaction (F 22.48; df 12,198; $P<0.001$) in NREMS timing (Fig. 3C). NREMS as a percentage of time was lower in both the 100 mg/kg, i.p. armodafinil and D-methamphetamine treatment groups relative to vehicle during the first 3 h after administration (Fig. 3C). However, during hours 5 and 6 after administration, NREM sleep as a percentage of time was significantly greater than vehicle in the D-methamphetamine treatment group (Fig. 3C) but not in the 100 mg/kg, i.p. armodafinil treatment group. In contrast, during the same time period, delta power as a percentage of total NREMS EEG power was greater than

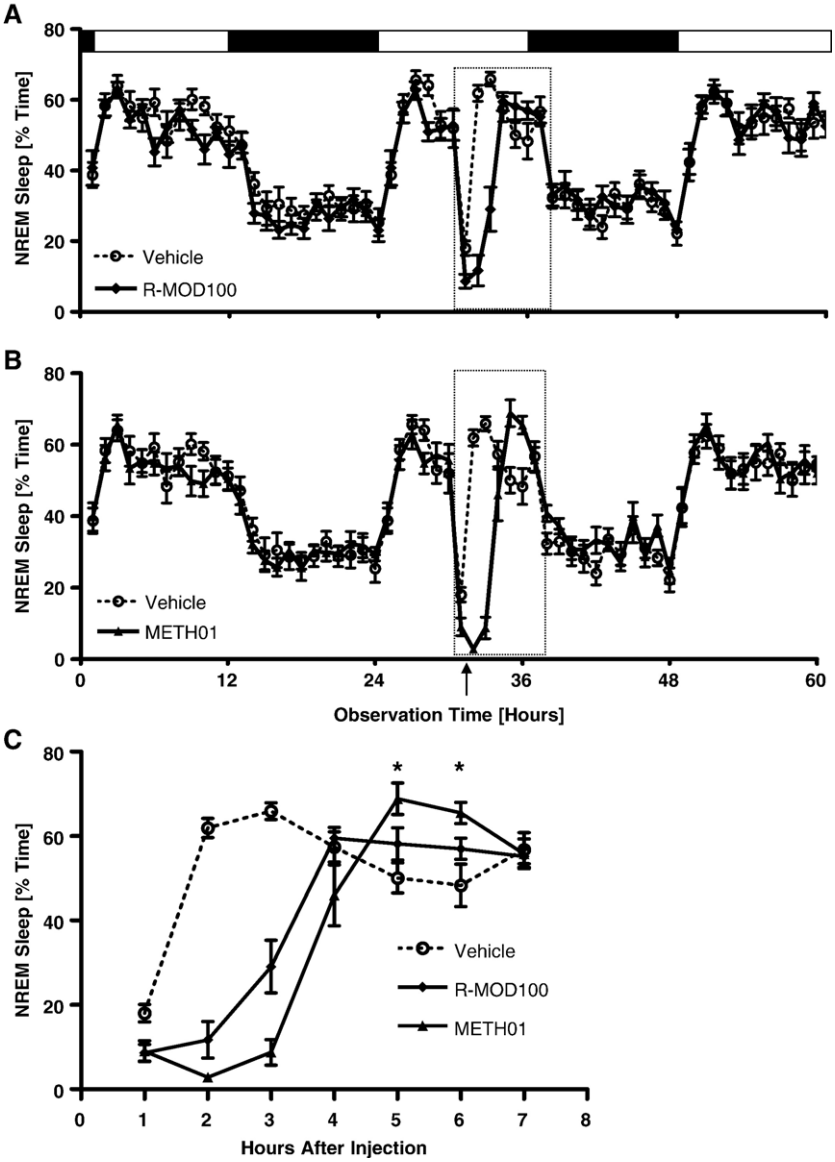

Fig. 3. A, B. NREMS as a percentage of time during the entire 60-h recording session in the armodafinil 100 mg/kg, i.p. treatment group (R-MOD100) with wake surplus equivalent to D-methamphetamine-treated rats (1 mg/kg, i.p.; METH01). Data from vehicle-treated rats are plotted in both panels for comparison. Inset boxes contain data plotted in C. Arrow indicates the time of injection. C. Data from 7 h immediately subsequent to injection. #$P<0.05$, armodafinil 100 mg/kg, i.p. (R-MOD100) vs. vehicle and D-methamphetamine 1 mg/kg, i.p. (METH01) vs. vehicle; t-test with Bonferroni correction. *$P<0.05$, D-methamphetamine vs. vehicle; t-test with Bonferroni correction.

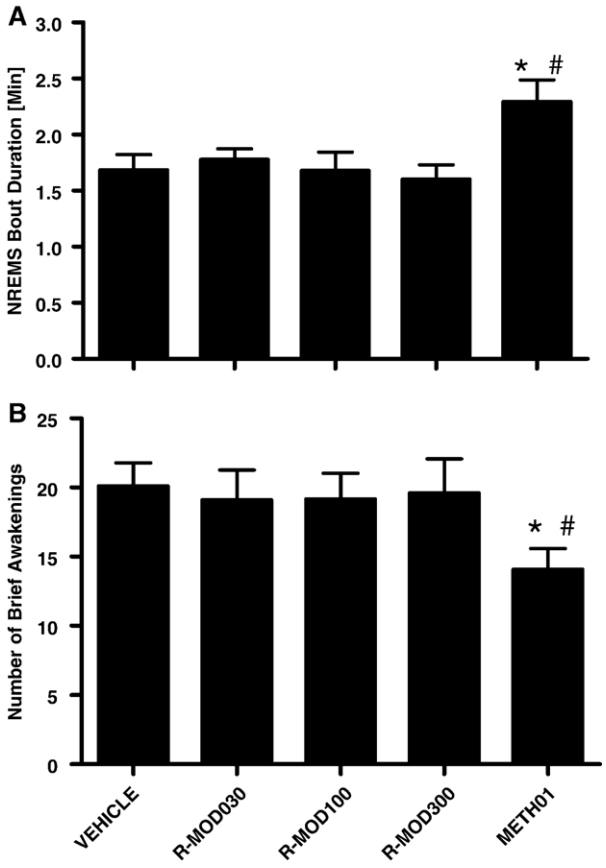

Fig. 4. Effects of treatments on sleep consolidation. A) NREMS bout duration. B) Number of brief awakenings from sleep (i.e., wake episodes <30 s). Treatment groups in A are as specified in B. #$P<0.05$, *t*-test with Bonferroni correction, D-methamphetamine 1 mg/kg, i.p. (METH01) vs. armodafinil (100 mg/kg, i.p.; R-MOD100). *$P<0.05$ vs. vehicle; *t*-test with Bonferroni correction.

vehicle in rats treated with 100 mg/kg armodafinil i.p., but not in D-methamphetamine-treated rats (Bonferroni corrected *t*-test; data not shown). The magnitude of REMS recovery was inconsistent, and did not differ among groups (data not shown).

The average NREMS bout duration, a measure of NREMS consolidation that is typically increased by sleep deprivation, was treatment-dependent (ANOVA F 30.70; df 4, 55; $P<0.001$). Armodafinil did not alter NREMS bout duration relative to vehicle at any dose tested, while D-methamphetamine resulted in increased NREMS bout duration relative to both vehicle and armodafinil (100 mg/kg, i.p.; Fig. 4A). Even at the higher dose of 300 mg/kg, i.p. of armodafinil (Fig. 4A), the NREMS bout duration was not different from vehicle. The number of brief awakenings (NBA) from sleep, which are typically suppressed during sleep subsequent to sleep deprivation, was not altered by armodafinil relative to vehicle at any dose tested (Fig. 4B). D-methamphetamine decreased NBA relative to both vehicle and armodafinil (100 mg/ kg, i.p.; Fig. 4B).

4. Discussion

Armodafinil administered i.p. to WKY rats during the rest phase of the LD12:12-h cycle increased wake time and wake consolidation in a non-linear, dose-dependent manner that was not accompanied by an increase in LMA intensity. Armodafinil (100 mg/kg, i.p.), a dose equivalent in wake-promoting efficacy to D-methamphetamine (1 mg/kg, i.p.), did not acutely induce rebound hypersomnolence or an increase in Tb. In comparing the physiological and behavioral effects of these two wake-promoting compounds, it is important to compare doses that are equal with respect to the magnitude and duration of their wake-promoting effects, as the magnitude of compensatory changes in sleep EEG and timing is proportional to prior sleep loss (Borbely and Achermann, 2004; Franken et al., 2001). Furthermore, responses to sleep deprivation may be modulated by circadian phase-dependent suppression of the compensatory response to sleep loss (Edgar et al., 1991; Kas and Edgar, 1999; Klerman et al., 1999). As a result, the compensatory responses to two wake-promoting treatments administered at the same phase of the LD12:12 cycle might differ if the offsets of their wake-promoting effects occur at different phases of the circadian cycle. The wake-promoting efficacy of D-methamphetamine (1 mg/kg, i.p.) was approximately equal to that of armodafinil administered at 100 mg/kg, i.p.. Indeed, when D-methamphetamine and armodafinil (100 mg/kg, i.p.) were statistically compared, the maximum NREMS deficit and wake, NREMS and REMS as a percentage of the first 2 h after administration were not different between these two treatment groups. Consequently, D-methamphetamine and armodafinil (100 mg/kg, i.p.) were considered to be equal in efficacy.

At doses with approximately equal wake-promoting effects, the two compounds differed in their effects on physiological and behavioral parameters. D-methamphetamine increased Tb and the intensity of locomotor activity during wake. By contrast, armodafinil (100 mg/kg, i.p.) was without effect on either body temperature or LMA intensity. Neither of these variables differed between the armodafinil (100 mg/kg, i.p.) and vehicle groups in the first 2 h after treatment, despite the robust wake-promoting effect of armodafinil (100 mg/kg, i.p.). Similarly, the highest dose (300 mg/kg, i.p.) of armodafinil examined, which had a more extended wake-promoting effect than D-methamphetamine, did not increase LMA intensity above that observed in the vehicle-treated group. The lowest dose of armodafinil did significantly increase LMA intensity relative to vehicle administration. The reason for this dose-specific increase in LMA intensity is not clear. It is possible that higher doses of armodafinil have an LMA-suppressing effect despite their wake-promoting effect: the total number of epochs of LMA during the first 2 h after administration was nearly identical in the armodafinil 30 mg/kg and 300 mg/kg groups despite drastic differences in time spent awake (Table 2).

Armodafinil (100 mg/kg, i.p.) and D-methamphetamine were further distinguished by differences in their acute effects on sleep subsequent to treatment. The frequency of brief awakenings from sleep decreased, and both NREMS as a percentage of time and NREMS bout duration increased subsequent to D-methamphetamine administration relative to vehicle control. These changes resemble those that occur after sleep deprivation (Borbely and Achermann, 2004; Franken et al., 2001, 1998, 1991; Tobler et al., 1983; Wisor et al., 2002)

and thus constitute rebound hypersomnolence in response to D-methamphetamine (Edgar and Seidel, 1997). By contrast, following armodafinil-induced wake, at either 100 or 300 mg/kg, i.p., NBA, NREMS as a percentage of time and NREMS bout duration were not different from vehicle control values. These observations collectively demonstrate a lack of acute rebound hypersomnolence after armodafinil (100 mg/kg i.p.)-induced waking.

In summary, acute rebound hypersomnolence similar to that induced by sleep deprivation – characterized by increases in NREMS as a percentage of time and NREMS bout duration and a decrease in the frequency of brief awakenings from sleep – occurred in the hours immediately after the cessation of D-methamphetamine-induced wake. An equally efficacious dose of armodafinil did not induce rebound hypersomnolence, but did result in an increase in NREMS EEG power during the same time interval after administration. The data in the present study on armodafinil support clinical data that armodafinil (Roth et al., 2005), like modafinil (Czeisler et al., 2005; US Modafinil in Narcolepsy Multicenter Study Group, 2000, 1998; Schwartz et al., 2003), may be efficacious in the treatment of disorders of excessive sleepiness in humans.

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