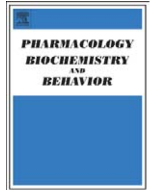
Pharmacology, Biochemistry and Behavior 92 (2009) 549–557

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh



Armodafinil promotes wakefulness and activates Fos in rat brain

Elaine M. Fiocchi [1], Yin-Guo Lin, Lisa Aimone, John A. Gruner, Dorothy G. Flood *

Discovery Research, Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380, USA

ARTICLE INFO

Article history:
Received 22 October 2007
Received in revised form 13 February 2009
Accepted 18 February 2009
Available online 26 February 2009

Keywords:
Sleep
Waking
Narcolepsy
Fos
Immediate early gene
Immunohistochemistry
Armodafinil
Rat

ABSTRACT

Modafinil increases waking and labeling of Fos, a marker of neuronal activation. In the present study, armodafinil, the *R*-enantiomer of racemic modafinil, was administered to rats at 30 or 100 mg/kg i.p. about 5 h after lights on (circadian time 5 and near the midpoint of the sleep phase of the sleep:wake cycle) to assess its effects on sleep/wake activity and Fos activation. Armodafinil at 100 mg/kg increased wakefulness for 2 h, while 30 mg/kg armodafinil only briefly increased wakefulness. Armodafinil (30 and 100 mg/kg) also increased latencies to the onset of sleep and motor activity. Armodafinil had differential effects in increasing neuronal Fos immunolabeling 2 h after administration. Armodafinil at 100 mg/kg increased numbers of Fos-labeled neurons in striatum and anterior cingulate cortex, without affecting nucleus accumbens. Armodafinil at 30 mg/kg only increased numbers of light Fos-labeled neurons in the anterior cingulate cortex. In brainstem arousal centers, 100 mg/kg armodafinil increased numbers of Fos-labeled neurons in the tuberomammillary nucleus, pedunculopontine tegmentum, laterodorsal tegmentum, locus coeruleus, and dorsal raphe nucleus. Fos activation of these brainstem arousal centers, as well as of the cortex and striatum, is consistent with the observed arousal effects of armodafinil.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Armodafinil is the *R*-enantiomer of racemic modafinil, 2-[(diphenylmethyl)sulfinyl]acetamide. Modafinil is wake promoting in a number of species (Bastuji and Jouvet, 1988; Hermant et al., 1991; Kopp et al., 2002; Lagarde and Milhaud, 1990; Lin et al., 1992; Shelton et al., 1995; Touret et al., 1995) and is useful for the treatment of excessive sleepiness associated with narcolepsy, obstructive sleep apnea/hypopnea syndrome, and shift work sleep disorder (Czeisler et al., 2005; Pack et al., 2001; US Modafinil in Narcolepsy Multicenter Study Group, 1998, 2000). Likewise, armodafinil is wake promoting in humans and rats (Harsh et al., 2006; Hirshkowitz et al., 2007; Roth et al., 2005, 2006; Wisor et al., 2006). On a comparative basis, 200 mg armodafinil showed higher plasma concentrations, improved wakefulness, and longer sustained attention than 200 mg modafinil in a study of acutely sleep-deprived healthy human volunteers (Dinges et al., 2006).

The immediate early gene c-*fos* and its encoded protein, the transcription factor Fos, are expressed in brain regions that are physiologically activated by a variety of stimuli and, therefore, are useful as markers of functional activity in the brain (Sagar et al., 1988). Furthermore, the brain activation associated with the waking phase of the sleep/wake cycle strongly induces c-*fos* expression in many brain regions (Cirelli and Tononi, 2000). Study of Fos activation in animals is useful for ascertaining the neuroanatomical substrates involved in the wake-promoting effects of compounds such as modafinil. The patterns of waking-associated neuronal activation induced by modafinil have been described in rat (Engber et al., 1998; Scammell et al., 2000), cat (Lin et al., 1996), and mouse (Willie et al., 2005).

In the present study, wake promotion and the pattern of Fos neuronal activation induced by armodafinil were investigated at two doses, 30 and 100 mg/kg i.p. in the Sprague Dawley rat. An additional study was performed to establish the pharmacokinetic profile of armodafinil in this rat strain. The pharmacokinetic profile and wake-promoting effects of armodafinil were similar in the WKY rat (Wisor et al., 2006) and Sprague Dawley rat. In the Sprague Dawley rat, many of the brain regions activated by modafinil in a previous study (Scammell et al., 2000) were also activated by armodafinil in the present study. However, some brainstem regions associated with waking that were not activated by modafinil in previous studies were activated in the present study by armodafinil.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were provided food and water ad libitum. Lights were on between 0700 and 1900 h. Surgical procedures were performed with sodium pentobarbital general anesthesia (45 mg/kg i.p.) and 2% lidocaine local anesthesia. All animal procedures

* Corresponding author. Tel.: +1 610 738 6249; fax: +1 610 344 0065.
E-mail address: dflood@cephalon.com (D.G. Flood).
[1] Retired.

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.02.006

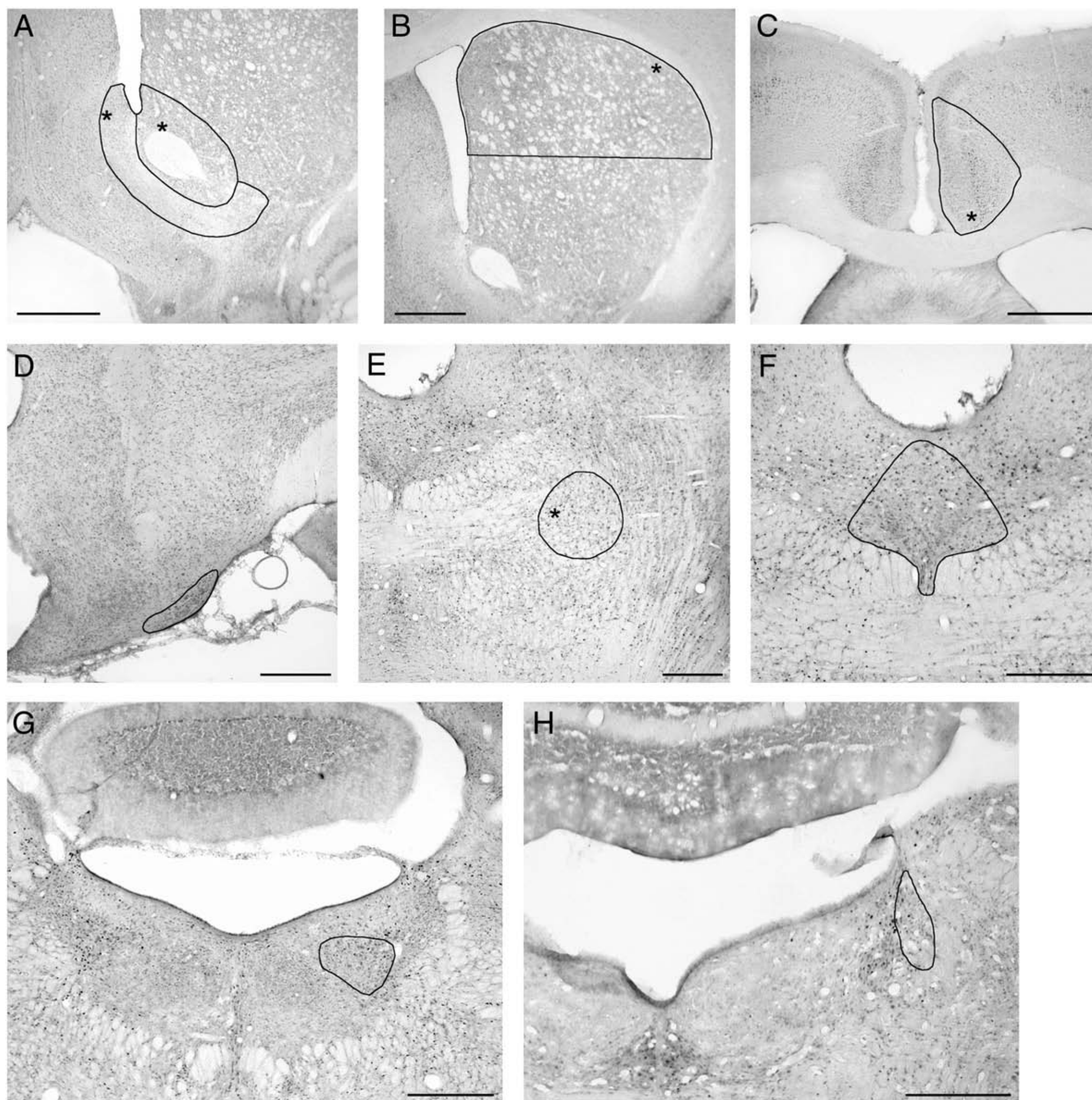


Fig. 1. Low power photomicrographs of Fos labeling, showing the boundaries used for cell counting, as defined by Paxinos and Watson (1986, 2005). Asterisks represent the locations of the high power photomicrographs in Figs. 4 and 5, when only a portion of the brain region is shown. A. Nucleus accumbens, core (upper area) and shell (lower area). B. Striatum. C. Anterior cingulate cortex, on both sides of the midline. D. TMN. E. PPTg. F. DR, on both sides of the midline. G. LDTg. H. LC. Scale bars in A–C are 1000 µm and in D–H are 500 µm.

were approved by the Cephalon, Inc. Institutional Animal Care and Use Committee and were in accordance with the guidelines in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Suspensions of armodafinil (lot # P040308, Cephalon, Inc., West Chester, PA) were prepared fresh daily at 5 mL/kg in 0.5% methylcellulose (Methocel® A15 Premium LV, Dow Chemical, Midland, MI) and 0.2% Tween 80 (Fisher Scientific, Pittsburgh, PA) in water. Armodafinil was resuspended just prior to dispensing and injection.

2.3. Pharmacokinetics of armodafinil

Group-housed rats (average weight 330 g) were administered 30 or 100 mg/kg i.p. armodafinil at 1200 h. Tail vein blood samples of 250 µL were drawn at 0.25–8 h postinjection. Plasma samples (100 µL) were mixed with 10 µL of *N*, *N*-dimethylformamide and 200 µL of acetonitrile and centrifuged. The supernatant was analyzed for armodafinil concentration by high performance liquid chromatography and mass spectrometry and compared to a rat plasma standard curve in the range of 5–20 000 ng/mL. The detection limit of the assay was 50 ng/mL. Pharmacokinetic parameters were derived from the plasma concentration versus time data for each rat by non-compartmental analysis (Gibaldi and Perrier, 1982), using WinNonlin software (Professional Version 4.0.1, Pharsight Corp., Palo Alto, CA).

2.4. Waking activity, motor activity, and body temperature

Pair-housed rats were implanted with electrodes at least 2 weeks prior to the recording sessions. Electroencephalographic (EEG) electrodes were stainless steel screws implanted in the cranium: 2 over frontal cortex (+3.0 mm AP from bregma, ±2.0 mm ML) and 2 over occipital cortex (−4.0 mm AP from bregma, ±2.0 mm ML). Two electromyographic (EMG) electrodes were positioned in the nuchal muscles. A temperature/activity transmitter (TA10TA-FA, Data Sciences International, St. Paul, MN) was implanted in the peritoneal cavity.

One day prior to the recording session, rats were placed singly into Nalgene containers (31×31×31 cm) with wire-mesh tops, within sound attenuation cabinets (Med Associates, Inc., St. Albans, VT). Rats were left undisturbed except for dosing at 5 h after lights on, until 24 h postinjection. Each rat received vehicle or armodafinil (30 or 100 mg/kg i.p.) in 2 recording sessions that were separated by 2 weeks. Rats were returned to their home cages between sessions.

Cortical EEG activity was recorded differentially between the left frontal and right occipital electrodes, with the right frontal electrode serving as the ground. Amplified EEG and EMG signals were digitized at 128 samples per second using ICELUS sleep scoring software (M. Opp, U. Michigan, Ann Arbor, MI) operating under Labview 5.1 (National Instruments, Austin, TX) data acquisition software. Body temperature and motor activity were recorded every 5 min using Artquest software (Data Sciences International). Data were recorded for 25 h, starting 1 h prior to dosing.

EEG and EMG records were scored in 6-s epochs as wake, slow wave sleep (SWS), or rapid eye movement sleep (REMS) stages according to standard criteria (Edgar and Seidel, 1997; Opp and Krueger, 1994) using a semi-automated computer algorithm. Average wake, SWS, and REMS times were determined for 30-min periods relative to the time of dosing. Cumulative waking, SWS, and REMS times were calculated for the first 2 h after dosing. Cumulative wake surplus was calculated relative to the mean cumulative wake activity of the vehicle group by serially adding the difference between the cumulative wake time for each animal and the corresponding mean value for the vehicle group. The maximal cumulative wake surplus value for each animal in the first 2 h postinjection was then determined. Latencies to the onset of 2 min uninterrupted SWS and 1 min uninterrupted REMS were also determined. Motor activity was expressed as the total activity (arbitrary units) in the first 2 h postinjection. Motor intensity was defined as motor activity/total minutes awake over the first 2 h postinjection (Edgar and Seidel, 1997). Average body temperature was calculated for the first 2 h postinjection.

2.5. Fos activation by armodafinil

Since Fos can be activated by prolonged waking, rats were implanted with indwelling i.p. catheters to permit administration of armodafinil or vehicle with a minimum of disturbance. Catheters were externalized through a skin incision between the scapulae. Following surgery, rats were pair-housed in their home cages in a dedicated sleep-study room for one week prior to dosing. Catheters were flushed daily with 1.5 mL of heparinized saline to habituate the rats to dosing.

Pairs of rats were dosed at 1115, 1200, or 1245 h with vehicle or armodafinil (30 or 100 mg/kg) through i.p. catheters. The treatment order was varied daily. Two h later, the postinjection time used in most Fos studies with modafinil (Engber et al., 1998; Chemelli et al., 1999; Scammell et al., 2000; Willie et al., 2005), rats were deeply anesthetized with sodium pentobarbital (75 mg/kg via i.p. catheter) and blood was collected by cardiac puncture and prepared for analysis of armodafinil concentration. Rats were perfused with phosphate buffered 4% paraformaldehyde/0.4% picric acid. Following postfixation and cryoprotection, brains were coronally sectioned at 40 µm.

Table 1
Mean (±SEM) pharmacokinetic values for armodafinil administered i.p.

	30 mg/kg	100 mg/kg
C_{max} (µg/mL)	9.05±1.88	30.96±4.50
t_{max} (h)	0.25±0.00	0.30±0.05
AUC_{0-t} (µg h/mL)	4.09±0.84	25.49±2.79
$AUC_{0-\infty}$(µg h/mL)	4.42±0.91	25.90±2.75
$t_{1/2}$ (h)	0.23±0.02	0.31±0.05

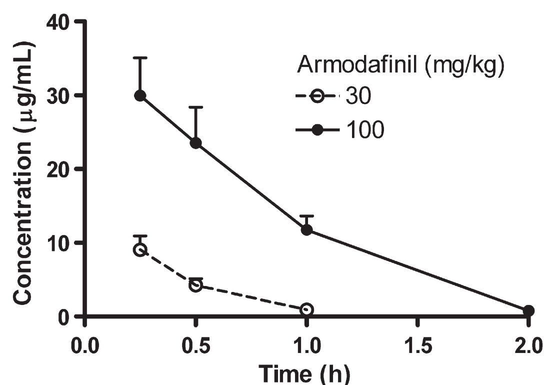


Fig. 2. Plasma concentrations of armodafinil after administration of either 30 or 100 mg/kg i.p. at 0 h. Number of subjects per group = 5. Error bars are standard error of the mean.

Free-floating sections were incubated overnight at 4 °C with a rabbit polyclonal antiserum against the N-terminus of Fos (Anti-c-*fos*, Ab-2, Calbiochem, La Jolla, CA) at 1:400. Sections were incubated in biotinylated goat anti-rabbit IgG and in peroxidase-conjugated avidin biotin complex (VectaStain ABC Elite, Vector Laboratories, Burlingame, CA). The final reaction product was visualized with 0.04% 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO).

Numbers of Fos-labeled neurons were counted in 9 brain regions delineated using stereotaxic atlases (Paxinos and Watson, 1986, 2005): nucleus accumbens shell, nucleus accumbens core, striatum, anterior cingulate cortex, tuberomammillary nucleus (TMN), pedunculopontine tegmentum (PPTg), laterodorsal tegmentum (LDTg), locus coeruleus (LC), and dorsal raphe nucleus (DR) (Fig. 1). Brain regions were selected because they were studied previously following treatment with modafinil (Engber et al., 1998; Scammell et al., 2000) and/or because of their involvement in arousal (for review, Jones, 2003; España and Scammell, 2004). A minimum of 5 counts were performed in each rat, utilizing both sides of the brain on non-consecutive tissue sections representing the central portion of the rostral–caudal extent of each brain region. Following delineation of the brain region at low power (Fig. 1), neurons were counted using a 20× objective, if the intensity of the nuclear Fos labeling was above background and either dark or light (CAST2, Olympus Danmark A/S, Albertslund, Denmark). Nuclei of dark neurons contained a dark brown to black reaction product in either a uniformly dispersed or granular pattern. When the reaction product was granular, the majority of the nucleus was dark. Nuclei of light neurons contained a light brown or tan reaction product, sometimes with a few clusters of darker reaction product. Level of staining intensity was inferred to represent level of protein present and therefore level of Fos activation. Area of the counting field was estimated by point counting and was used to calculate neuronal density (number/mm^2) for light and dark Fos-labeled neurons. Section thicknesses and areas of light and dark nuclei were measured under oil immersion with a 60× objective. Nuclear diameters for circles of equivalent areas were calculated and a correction factor (Abercrombie, 1946) was applied to the neuronal densities.

High power photomicrographs showing comparative staining represented the average appearance of Fos labeling in each treatment group. All photomicrographs were oriented to represent the right side of the brain, with dorsal at the top and the midline at the lefthand side, unless otherwise indicated.

2.6. Statistics

Treatment groups were compared by ANOVAs using SigmaStat 3.05 Network (SPSS, Inc., Chicago, IL). When the overall ANOVAs were

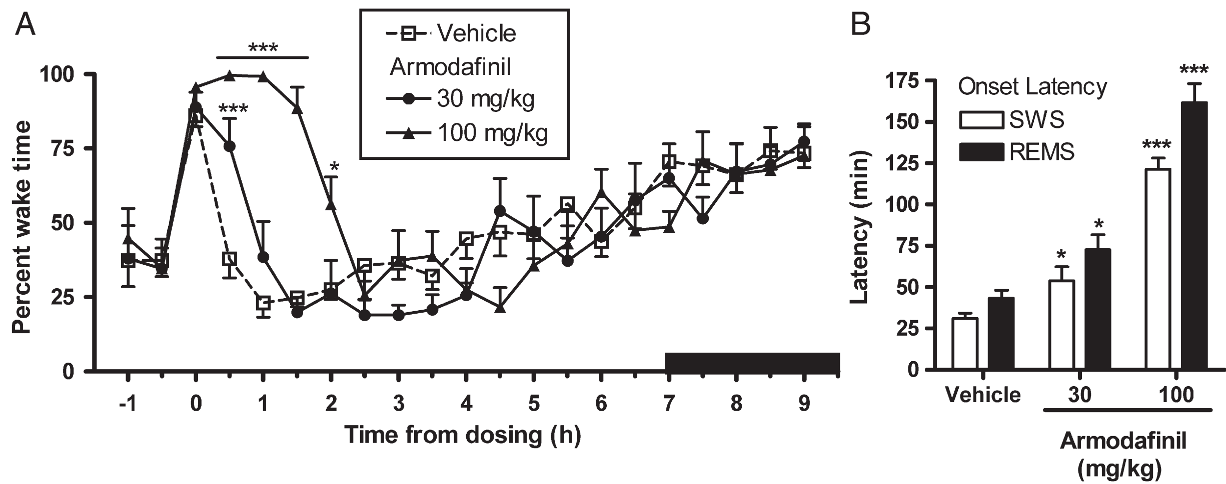


Fig. 3. Sleep/wake activity after administration of armodafinil (30 or 100 mg/kg) at 0 h. A. Black bar beginning at 7 h indicates the dark phase of the diurnal light cycle. B. Latency in minutes to the onset of the first bout of SWS and REMS. Asterisks represent significant post hoc differences from the vehicle-treated group: *$P<0.05$ and ***$P<0.001$. Numbers of subjects are given in Table 2. Error bars are standard error of the mean.

significant at $P\leq0.05$, post hoc Bonferroni t tests compared the armodafinil groups with the vehicle group.

3. Results

3.1. Pharmacokinetic profile for armodafinil

Plasma concentrations of armodafinil are shown for 0.25–2 h (Fig. 2). Armodafinil was no longer detectable in plasma at 2 h for the 30-mg/kg dose and at 4 h for the 100-mg/kg dose. The derived pharmacokinetic parameters showed dose-related increases in maximum plasma level (C_{max}), overall plasma exposure to the last detectable time point (AUC_{0-t}), and overall plasma exposure extrapolated to infinity ($AUC_{0-\infty}$) (Table 1). Time to C_{max} (t_{max}) and plasma half-life ($t_{1/2}$) were similar for the 30- and 100-mg/kg doses of armodafinil (Table 1).

At 2 h postinjection in the pharmacokinetic study, plasma levels averaged 0.77 ± 0.25 μg/mL (range of 0.25 to 1.48, $n=5$) for the 100-mg/kg armodafinil group. Plasma was not taken from rats in the sleep study since sampling would have disrupted the normal sleep/wake architecture. In the Fos study, plasma levels of armodafinil 2 h postinjection averaged 0.19 ± 0.05 μg/mL (range of 0.10 to 0.38, $n=5$) for the 30-mg/kg armodafinil group and 3.69.0 ± 0.81 μg/mL (range of 2.15 to 6.66, $n=5$) for the 100-mg/kg armodafinil group. The plasma levels were higher in the Fos study animals than in the pharmacokinetic study animals but were similar to the levels observed in WKY rats (Wisor et al., 2006).

3.2. Wake-promoting effect of armodafinil

Percent wake time did not differ among the treatment groups for the 1-h period prior to dosing (Fig. 3A). For 100 mg/kg armodafinil, percent wake time was greater than vehicle for each 0.5-h time point between 0.5 and 2 h postinjection. For 30 mg/kg armodafinil, only the 0.5-h time point was greater than vehicle. At both the 30- and 100-mg/kg doses, 2-h cumulative wake time was increased by armodafinil (F[2, 26] = 52.434, $P<0.001$) (Table 2). The maximal cumulative wake surplus was increased for both 30 and 100 mg/kg armodafinil ($P<0.01$) (Table 2). Percent wake time did not differ among the treatment groups for the period 2.5–24 h (Fig. 3A).

Armodafinil produced corresponding reductions in SWS (F[2, 26] = 42.435, $P<0.001$) and REMS (F[2, 26] = 35.416, $P<0.001$) for the 2-h postinjection period (Table 2). By post hoc comparison, only the 100-mg/kg dose produced a significant reduction in SWS and REMS. Latencies to the onset of SWS and REMS were increased by armodafinil (SWS — F[2, 26] = 65.334, $P<0.001$; REMS — F[2, 26] = 58.762, $P<0.001$) (Fig. 3B). Both 30 mg/kg ($P<0.05$) and 100 mg/kg ($P<0.001$) were effective in delaying the onset of sleep.

Body temperature was not significantly altered by armodafinil (Table 2). Motor activity was increased by armodafinil (F[2, 23] = 10.959, $P<0.001$) at both 30 mg/kg ($P<0.01$) and 100 mg/kg ($P<0.001$) (Table 2). Motor intensity (motor activity normalized to wake time) was increased by armodafinil (F[2, 23] = 5.791, $P=0.009$) (Table 2). The increase was significant at the 30- but not the 100-mg/kg dose.

3.3. Fos activation by armodafinil

Nuclear diameters did not differ among treatment groups for either light or dark Fos-labeled neurons but were different across brain regions (light — F[8, 75] = 53.768, $P<0.001$ and dark — F[8, 75] = 91.823, $P<0.001$). Armodafinil at 100 mg/kg induced an increase in numbers of Fos-labeled neurons in many brain regions (Figs. 4 and 5). Armodafinil at 30 mg/kg caused minimal activation; and Fos labeling was generally comparable to that in rats treated with vehicle (Figs. 4 and 5). Light Fos-labeled neurons were more prevalent than dark Fos-labeled neurons in all treatment groups and brain regions. In most brain regions, the pattern of changes across the treatments in

Table 2
Mean (± SEM) parameters for the first 2 h after treatment[a].

	Vehicle	Armodafinil (mg/kg)	
		30	100
Number of subjects	11–13[b]	7–8[b]	8
Cumulative wake time (min)	51.5 ± 4.2	66.9 ± 6.0*	114.9±2.2***
Maximal cumulative wake surplus (min)[c]	–	22.1 ± 5.2**	78.8 ± 9.5***
SWS time (min)	55.4 ± 3.8	43.5 ± 5.1	4.5 ± 2.2***
REMS time (min)	13.1 ± 0.9	9.6 ± 1.6	0.6 ± 0.4***
Body temperature (°C)	37.2 ± 0.1	37.2 ± 0.1	37.0 ± 0.1
Total motor activity	7.0 ± 0.8	22.4 ± 5.0**	25.9 ± 4.0***
Motor intensity[d]	13.7 ± 1.4	34.3 ± 7.8**	22.8 ± 3.9

[a] Significant differences by post hoc tests for the armodafinil groups from the vehicle group are denoted by asterisks. *$P<0.05$, **$P<0.01$, and ***$P<0.001$.
[b] Body temperature and motor data were not available from some rats due to equipment failure. Body temperature: $n=12$ (vehicle) and 7 (30 mg/kg). Total motor activity and motor intensity: $n=11$ (vehicle) and 7 (30 mg/kg).
[c] For this measure, H_0: Mean = 0 was tested using Student's t tests.
[d] Motor intensity = (total motor activity over 2 h) / (wake time over 2 h) × 100.

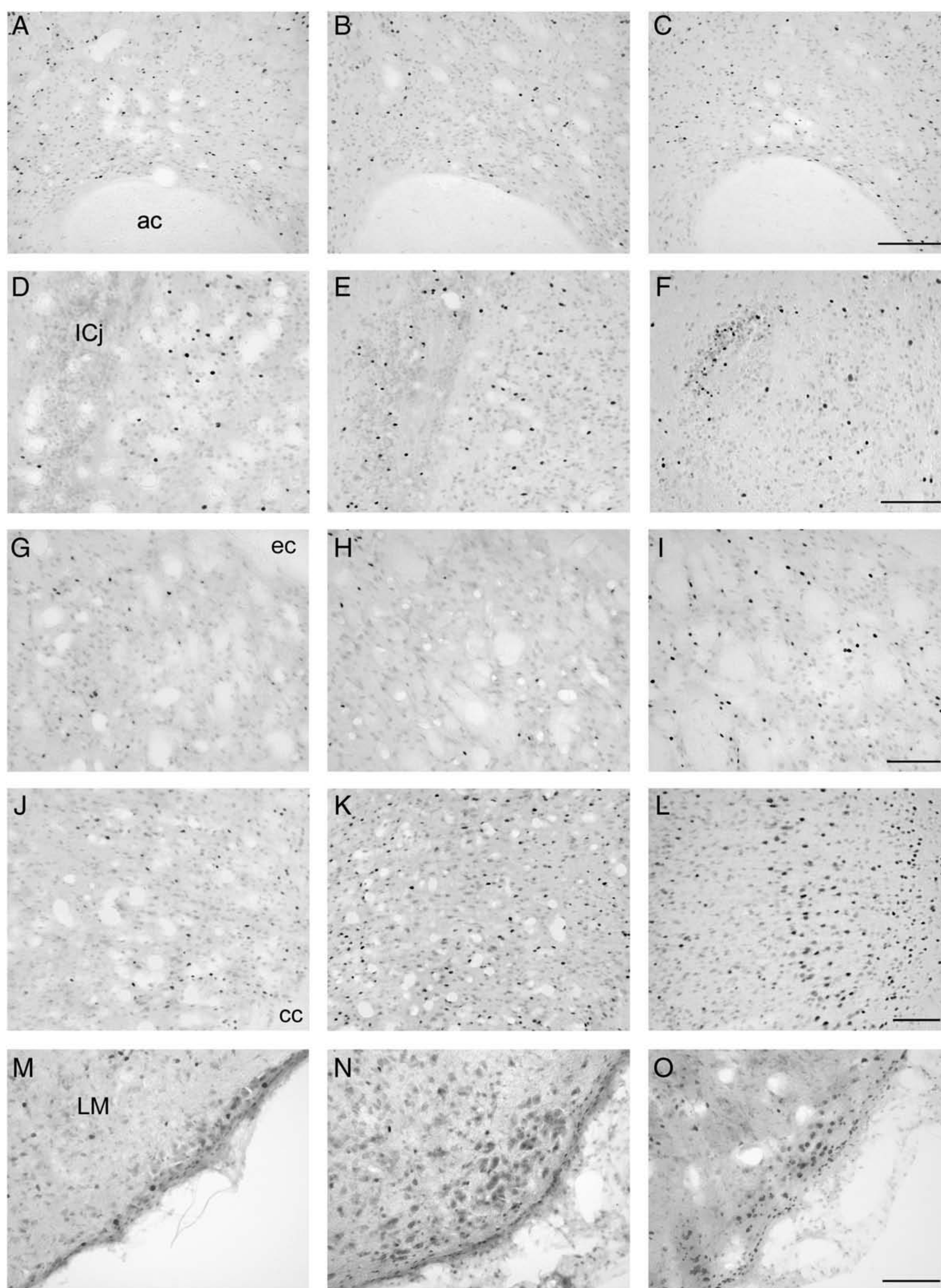


Fig. 4. Representative photomicrographs of Fos labeling in forebrain regions after treatment with vehicle (A, D, G, J, M), 30 mg/kg armodafinil (B, E, H, K, N), or 100 mg/kg armodafinil (C, F, I, L, O). A–C. Nucleus accumbens, core, illustrating the portion dorsal to the anterior commissure (ac). D–F. Medial portion of the nucleus accumbens, shell (right) and an island of Calleja (ICj) where the background level of staining is darker and the neuronal density is higher (left). In D and E, a major island is seen and in F, a minor island is shown. Within the nucleus accumbens, shell, the medial portion had the highest number of Fos-labeled neurons. G–I. Dorsolateral striatum, just below and medial to the adjacent white matter of the external capsule (ec). J–L. Anterior cingulate cortex (area 24a′), adjacent to the corpus callosum (cc). M–O. The histaminergic TMN of the posterior hypothalamus, adjacent to the lateral mammillary nucleus (LM). For each brain region, similar brain levels and locations are illustrated. Scale bars (100 μm) for each brain region are shown on C, F, I, L, and O.

numbers of light Fos-labeled neurons mirrored the pattern of changes observed for the numbers of dark Fos-labeled neurons. Thus, only changes in numbers of light Fos-labeled neurons that differed from changes in dark-labeled neurons will be described below. Mean densities of dark Fos-labeled neurons were the average of 3–5 rats per group and are presented in Table 3.

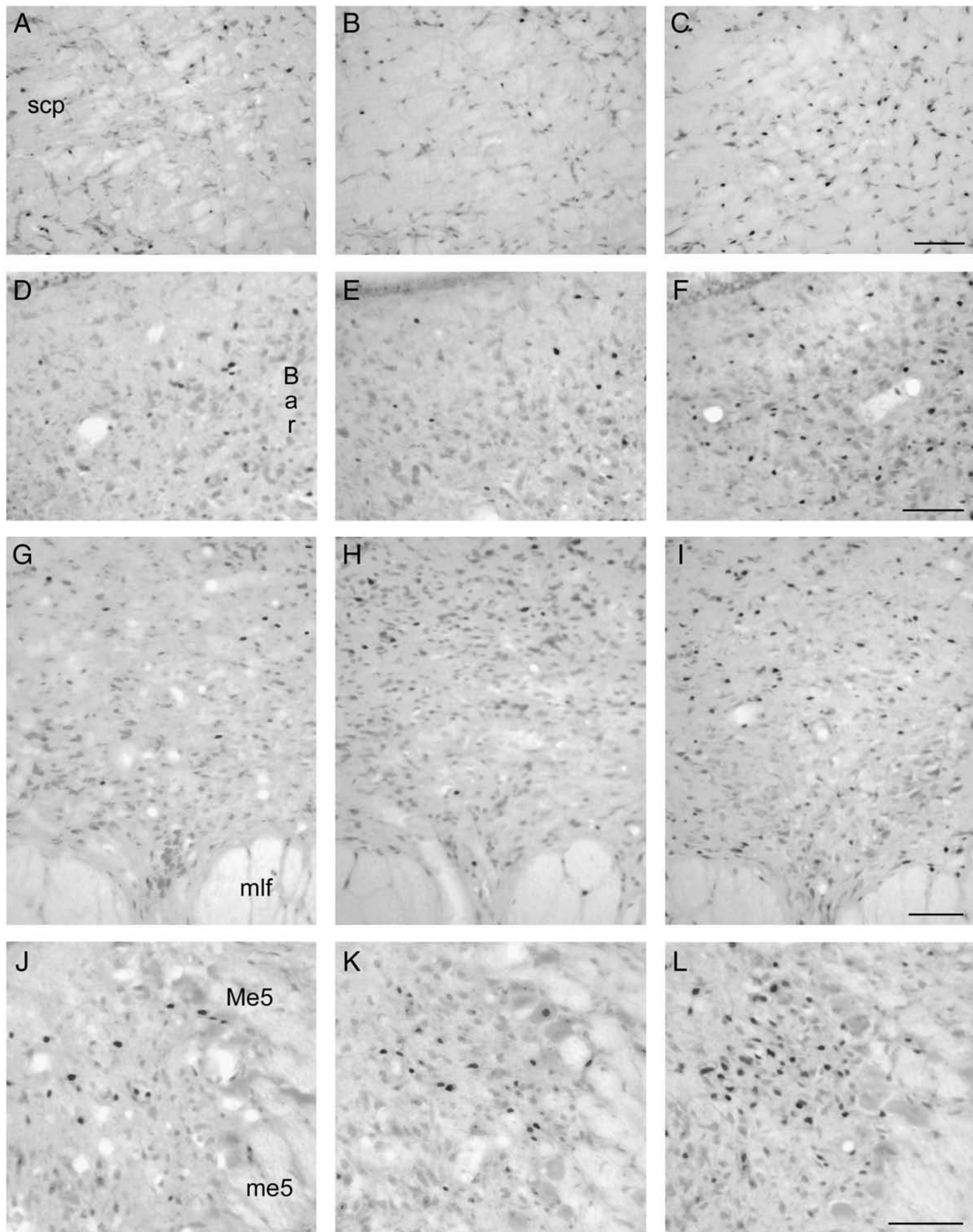


Fig. 5. Representative photomicrographs of Fos labeling in brainstem nuclei after treatment with vehicle (A, D, G, J), 30 mg/kg armodafinil (B, E, H, K), or 100 mg/kg armodafinil (C, F, I, L). A–C. PPTg, lateral to the superior cerebellar peduncle (scp) in the midbrain. D–F. LDTg in the rostral pons, where neurons were counted. Lateral is Barrington's nucleus (Bar) and in the upper lefthand corner is the 4th ventricle. G–I. The dorsal and ventral DR in the midbrain, dorsal to the medial longitudinal fasciculus (mlf). Right and left DR are shown, on either side of the midline. J–L. The LC in the pons, located medial to the mesencephalic nucleus of cranial nerve V (Me5) and its tract (me5). Fos labeling predominated in the dorsal portion of the LC in all treatment groups. For each brain region, similar brain levels and locations are illustrated. Scale bars (100 µm) for each brain region are shown on C, F, I, and L.

The overall numerical density of Fos-labeled neurons was higher in the nucleus accumbens core (Fig. 4A–C) than in the nucleus accumbens shell (Fig. 4D–F). In nucleus accumbens, core and shell, there were no changes in Fos labeling at either 30 or 100 mg/kg armodafinil. Medial and ventral to the nucleus accumbens, major and minor islands of Calleja were markedly activated by modafinil in rats and mice (Scammell et al., 2000; Willie et al., 2005). In vehicle-treated rats, a few neurons were darkly Fos-labeled in the islands of Calleja (Fig. 4D). Treatment with either 30 or 100 mg/kg armodafinil markedly increased numbers of dark Fos-labeled neurons in the islands (Fig. 4E–F). This represented one of the few brain regions in which 30 mg/kg armodafinil induced a marked increase in numbers of dark Fos-labeled neurons.

In the striatum, there was a dorsolateral to ventromedial gradient of increasing numbers of Fos-labeled neurons, regardless of treatment

Table 3
Numerical densities of dark Fos-labeled neurons[a].

Brain region	Vehicle	Armodafinil (mg/kg)	
		30	100
Nucleus accumbens, core	33.2±6.1	27.2±8.4 (−18)	38.7±10.7 (+17)
Nucleus accumbens, shell	7.4±0.7	3.7±1.3 (−50)	7.9±2.1 (+7)
Striatum	5.5±1.3	5.1±0.9 (−7)	33.6±3.7*** (+511)
Anterior cingulate cortex	12.2±2.2	18.9±6.1 (+55)	46.8±10.2* (+284)
Tuberomammillary nucleus (TMN)	59.3±5.3	109.1±24.6 (+84)	181.7±32.1** (+206)
Pedunculopontine tegmentum (PPTg)	11.9±1.4	13.8±3.0 (+16)	52.0±10.1*** (+337)
Laterodorsal tegmentum (LDTg)	26.0±6.3	33.6±5.7 (+29)	63.6±6.7** (+145)
Dorsal raphe nucleus (DR)	15.5±2.9	25.0±4.4 (+61)	65.4±18.8* (+322)
Locus coeruleus (LC)	8.5±0.9	13.3±2.3 (+56)	30.8±4.6*** (+262)

[a] Densities of neurons (number/mm^2, mean±SEM) and in parentheses, percent change from vehicle. Significant differences by post hoc tests for the 100-mg/kg armodafinil group from the vehicle group are denoted by asterisks. *$P<0.05$, **$P<0.01$, and ***$P<0.001$.

(Fig. 4G–I). The overall ANOVA was significant for dark Fos-labeled cells (F[2, 9]=49.698, $P<0.001$). In striatum, 100 mg/kg of armodafinil increased densities of dark Fos-labeled cells (Table 3).

The portion of anterior cingulate cortex quantified was the caudally located mid-cingulate cortex (areas 24b′ and 24a′ of Vogt et al. (2004)), where numbers of Fos-labeled neurons were highest in all 3 treatment groups (Fig. 4J–L). Additionally, there were more Fos-labeled neurons in the ventral area 24a′ than in the dorsal area 24b′ (Fig. 1C). Most of the Fos-labeled neurons were located in layers III, V, and VI. Dark Fos-labeled neurons were increased in density in the animals treated with 100 mg/kg armodafinil (F[2, 9]=6.931, P=0.015). Light Fos-labeled neurons were increased in both armodafinil groups (F[2, 9]=18.581, $P<0.001$; 30 mg/kg — $P<0.001$ and 100 mg/kg — $P<0.05$). Low-dose armodafinil produced light Fos labeling while the high dose produced increased light and dark labeling in anterior cingulate cortex.

Several subcortical arousal centers were activated by 100 mg/kg armodafinil. The TMN, a thin band of large histaminergic neurons along the ventrolateral surface of the posterior hypothalamus (Fig. 4M–O) (Panula et al., 1984), had more dark Fos-labeled cells in the 100-mg/kg armodafinil group than in the vehicle group (F[2, 7]= 8.883, P=0.012). PPTg (Fig. 5A–C) and LDTg (Fig. 5D–F) were activated by 100 mg/kg armodafinil (PPTg — F[2, 11]=16.753, $P<0.001$ and LDTg — F[2, 12]=10.152, P=0.003). Fos activation within the PPTg was similar to the level of activation in the surrounding midbrain and pontine tegmentum for each of the treatment groups (Fig. 5A–C). This suggested that 100 mg/kg of armodafinil produced a generalized activation of the PPTg and the surrounding reticular formation.

Both DR (Fig. 5G–I) and LC (Fig. 5J–L) were activated by 100 mg/kg armodafinil (DR — F[2, 9]=5.523, P=0.027 and LC — F[2, 12]=15.135, $P<0.001$). Fos-labeled neurons were sparse within the core of the DR and tended to predominate at the lateral edges of the nucleus in the 100-mg/kg armodafinil group. The distribution of Fos-labeled neurons in the DR was consistent with the distribution of wake-active dopaminergic neurons of the DR and ventral periaqueductal gray rather than of the serotonergic DR neurons (Lu et al., 2006). The ventral tegmental area and substantia nigra were qualitatively assessed. These nuclei contained few dark Fos-labeled neurons in all of the treatment groups and did not appear activated by armodafinil.

Three subnuclei of the lateral parabrachial complex were qualitatively evaluated and each responded differently. The central nucleus contained a low number of Fos-labeled neurons, while the dorsal nucleus contained numerous dark Fos-labeled neurons in all 3 groups of rats. The external nucleus was the only subdivision that was qualitatively modulated by armodafinil treatment at 100 mg/kg. In vehicle-treated and 30-mg/kg armodafinil-treated rats, there were few dark Fos-labeled neurons. Treatment with 100 mg/kg armodafinil induced a marked increase in numbers of dark Fos-labeled neurons in the external nucleus.

4. Discussion

4.1. Wake-promoting effect of armodafinil

Armodafinil was significantly wake promoting over a 2-hour period at 100 mg/kg and over a 30-minute period for 30 mg/kg in Sprague Dawley rats. Alterations in other measures consistent with wake promotion were also observed, including increases in wake, motor activity, and onset latencies for SWS and REMS at 30 and 100 mg/kg; and decreases in SWS and REMS times at 100 mg/kg. Similar dose-dependent, wake-promoting effects of armodafinil were reported in WKY rats (Wisor et al., 2006). Motor intensity in WKY rats was significantly increased by 30 mg/kg of armodafinil, but not at higher doses (Wisor et al., 2006). This finding was replicated in the present study in Sprague Dawley rats. Although body temperature was elevated by armodafinil at 30 mg/kg in WKY rats (Wisor et al., 2006), it was not changed by armodafinil treatment in Sprague Dawley rats.

The wake-promoting effects of armodafinil and modafinil at comparable doses had a similar time course in the Sprague Dawley rat (data not shown for modafinil). Additionally, plasma levels and pharmacokinetic parameters were similar at comparable doses of armodafinil and modafinil in the Sprague Dawley rat (data not shown for modafinil). The comparative profile of modafinil and armodafinil in rat contrasts with the profile in human where 200 mg armodafinil showed higher plasma concentrations, improved wakefulness, and longer sustained attention than 200 mg modafinil (Dinges et al., 2006).

4.2. Fos activation by armodafinil

Armodafinil had differential effects across brain regions in its ability to increase Fos labeling of neurons. Armodafinil at the wake-promoting dose of 100 mg/kg increased densities of Fos-labeled neurons in the striatum, anterior cingulate cortex, TMN, PPTg, LDTg, DR, and LC, but did not alter densities of Fos-labeled neurons in the nucleus accumbens core and shell. Armodafinil at 30 mg/kg only increased densities of light Fos-labeled neurons in the anterior cingulate cortex.

4.3. Comparison of Fos activation by modafinil and armodafinil

Four previous reports on Fos induction by modafinil are relevant to the data reported here but produced conflicting results (Engber et al., 1998; Lin et al., 1996; Scammell et al., 2000; Willie et al., 2005). Modafinil (5 mg/kg, p.o.), administered to cats at 1100 h, produced continuous wakefulness for almost 10 h suggesting that the cat has greater sensitivity than the rat to modafinil, and produced few quantitative regional increases in Fos labeling 90 and 150 min after dosing (Lin et al., 1996). Striatum (caudate and putamen), nucleus accumbens, and cerebral cortex had only slightly and nonsignificantly higher numbers of Fos-labeled neurons in modafinil-treated cats than in vehicle-treated cats. Negligible Fos activation was observed in both vehicle- and modafinil-treated cats in TMN, DR, LC, and mesopontine tegmentum.

Engber et al. (1998) administered modafinil (300 mg/kg, i.p. injection) to rats in the early afternoon. Both vehicle- and 300-mg/kg modafinil-treated rats were rated as negative (0% to 5% of cells labeled) for Fos labeling in the striatum and frontal cortex. Scammell et al. (2000) also used similar methods to those of Engber et al. (1998), administration of modafinil (300 mg/kg, i.p. injection) at noon, but observed Fos activation in the striatum.

Scammell et al. (2000) performed the most extensive evaluation, by administering modafinil (75 or 150 mg/kg, i.p. catheter) to rats at either 1200 or 2400 h. Their methods were most comparable to those used in the present study. Modafinil (150 mg/kg) increased the numbers of Fos-labeled neurons in the striatum and TMN at both times and in the cingulate cortex and LC at midnight. The TMN was activated by 75 mg/kg modafinil as well. Numbers of Fos-labeled neurons were not increased in the nucleus accumbens core and shell by modafinil (75 and 150 mg/kg, i.p. catheter) administered at 2400 h. All of these findings were comparable to the changes observed in the present study with armodafinil (100 mg/kg, i.p. catheter).

Scammell et al. (2000) failed to find any differences in the PPTg, LDTg, and DR in Fos-labeled neurons for modafinil (75 or 150 mg/kg, i.p. catheter) given at 2400 h. Armodafinil (100 mg/kg, i.p. catheter) in the present study produced increases in Fos-labeled neurons in these brain regions. PPTg, LDTg, and DR show increased c-*fos* and Fos during waking in rats (Pompeiano et al., 1994; Semba et al., 2001). The additional Fos activation produced by modafinil during the nocturnal phase compared with Fos activation due to normal wakefulness, may not have been sufficient to detect a difference in Fos labeling in these nuclei (Scammell et al., 2000). Armodafinil was given during the middle of the diurnal phase so that a greater difference in Fos labeling could be expected in PPTg, LDTg, and DR between sleeping rats, with minimal Fos activation, and armodafinil-induced waking rats.

Willie et al. (2005) described changes in Fos labeling in mice administered modafinil (100 mg/kg i.p.) at 1900 h (lights off), generally confirming the findings of Scammell et al. (2000). In the mouse, striatum, cingulate cortex, and TMN all qualitatively contained more Fos-labeled neurons after 100 mg/kg modafinil (Willie et al., 2005), consistent with the findings in rat for modafinil (Scammell et al., 2000) and armodafinil. LC was only rarely labeled after 100 mg/kg modafinil in the mouse, while there was a moderate increase in numbers of Fos-labeled neurons in the nucleus accumbens core and shell (Willie et al., 2005). These results differed from the findings in the rat with both modafinil (Scammell et al., 2000) and armodafinil. In the mouse, Willie et al. (2005) found moderately increased Fos labeling in the DR, consistent with armodafinil but not with modafinil in the rat (Scammell et al., 2000).

In two of the studies, the greatest Fos activation by modafinil was seen in the hypothalamus (Engber et al., 1998; Lin et al., 1996). In the cat, Fos activation was observed in the anterior preoptic area, anterior hypothalamic nucleus, suprachiasmatic nucleus, and posterior hypothalamus (not TMN) (Lin et al., 1996). In the rat, Fos activation was observed in the anterior hypothalamus, paraventricular nucleus, and suprachiasmatic nucleus (Engber et al., 1998). In the mouse, moderate increases in the hypothalamus were found in the parvocellular region of the paraventricular nucleus, lateral hypothalamus, and posterior hypothalamus (Willie et al., 2005). In the second study in rat (Scammell et al., 2000), no increase in Fos labeling was found in the anterior hypothalamus, suprachiasmatic nucleus, or paraventricular nucleus after treatment with modafinil at either 1200 or 2400 h. Qualitatively, anterior hypothalamus, suprachiasmatic nucleus, and paraventricular nucleus were also not altered by armodafinil treatment in the present study at either 30 or 100 mg/kg.

4.4. Regional Fos activation by armodafinil and wake promotion

Multiple neuronal systems with distinct neurotransmitters interact to produce and maintain arousal and cortical activation (for review, Jones, 2003; España and Scammell, 2004). Putative glutamatergic neurons of the ascending reticular formation are found in the mesencephalic and pontine oral reticular formation and form the main core of the arousal system (for review, Jones, 2003). The mesencephalic reticular formation surrounding the PPTg contained Fos-activated neurons in the armodafinil-treated rats at 100 mg/kg but not at 30 mg/kg. The remainder of the ascending reticular formation did not appear qualitatively activated by armodafinil relative to vehicle.

The role of the dopaminergic system in arousal was initially underestimated because dopaminergic neurons do not fire more frequently during waking but rather change their firing pattern to fire in bursts of spikes (for review, Jones, 2003; España and Scammell, 2004). The alteration in firing pattern results in an elevation of dopamine release during waking. The brain regions that receive dopaminergic input, the striatum and nucleus accumbens, were differentially affected by armodafinil treatment. The striatum showed increased levels of Fos activation after treatment with 100 mg/kg of armodafinil while the nucleus accumbens did not.

The brainstem nuclei PPTg, LDTg, LC, DR, and TMN are all more active during waking (for review, Jones, 2003; España and Scammell, 2004). Although each of these systems alone can produce arousal, España and Scammell (2004) have suggested that their coordinated activity may be necessary for complete arousal and cortical activation. Armodafinil induced Fos activation in TMN, PPTg, LDTg, DR, and LC. The regional Fos activation induced by armodafinil is consistent with its wake-promoting activity in the rat.

Acknowledgements

The authors thank Dr. D. Bozyczko-Coyne for critical discussions, Dawn Clas for technical assistance, and J. Herman for measurements of armodafinil levels in plasma. This work was supported by Cephalon, Inc. These experiments were conducted on a proprietary Cephalon compound while the authors were employed by Cephalon, Inc.

References

Abercrombie M. Estimation of nuclear population from microtome sections. Anat Rec 1946;94:239–47.
Bastuji H, Jouvet M. Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. Prog Neuropsychopharmacol Biol Psychiatry 1988;12:695–700.
Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, et al. Narcolepsy in *orexin* knockout mice: molecular genetics of sleep regulation. Cell 1999;98: 437–51.
Cirelli C, Tononi G. On the functional significance of c-*fos* induction during the sleep–waking cycle. Sleep 2000;23:453–69.
Czeisler CA, Walsh JK, Roth T, Hughes RJ, Wright KP, Kingsbury L, et al. Modafinil for excessive sleepiness associated with shift-work sleep disorder. N Engl J Med 2005;353:476–86.
Dinges DF, Arora S, Darwish M, Niebler GE. Pharmacodynamic effects on alertness of single doses of armodafinil in healthy subjects during a nocturnal period of acute sleep loss. Curr Med Res Opin 2006;22:159–67.
Edgar DM, Seidel WF. Modafinil induces wakefulness without intensifying motor activity or subsequent rebound hypersomnolence in the rat. J Pharmacol Exp Ther 1997;283:757–69.
Engber TM, Koury EJ, Dennis SA, Miller MS, Contreras PC, Bhat RV. Differential patterns of regional c-Fos induction in the rat brain by amphetamine and the novel wakefulness-promoting agent modafinil. Neurosci Lett 1998;241:95–8.
España RA, Scammell TE. Sleep neurobiology for the clinician. Sleep 2004;27:811–20.
Gibaldi M, Perrier D. Pharmacokinetics. Marcel Dekker: New York; 1982.
Harsh JR, Hayduk R, Rosenberg R, Wesnes KA, Walsh JK, Arora S, et al. The efficacy and safety of armodafinil as treatment for adults with excessive sleepiness associated with narcolepsy. Curr Med Res Opin 2006;22:761–74.
Hermant JF, Rambert FA, Duteil J. Awakening properties of modafinil: effect on nocturnal activity in monkeys (*Macaca mulatta*) after acute and repeated administration. Psychopharmacology 1991;103:28–32.
Hirshkowitz M, Black JE, Wesnes K, Niebler G, Arora S, Roth T. Adjunct armodafinil improves wakefulness and memory in obstructive sleep apnea/hypopnea syndrome. Respir Med 2007;101:616–27.
Jones BE. Arousal systems. Front Biosci 2003;8:s438-51.
Kopp C, Petit JM, Magistretti P, Borbély AA, Tobler I. Comparison of the effects of modafinil and sleep deprivation on sleep and cortical EEG spectra in mice. Neuropharmacology 2002;43:110–8.

Lagarde D, Milhaud C. Electroencephalographic effects of modafinil, an alpha-1-adrenergic psychostimulant, on the sleep of rhesus monkeys. Sleep 1990;13:441–8.

Lin JS, Roussel B, Akaoka H, Fort P, Debilly G, Jouvet M. Role of catecholamines in the modafinil and amphetamine induced wakefulness, a comparative pharmacological study in the cat. Brain Res 1992;591:319–26.

Lin JS, Hou Y, Jouvet M. Potential brain neuronal targets for amphetamine-, methylphenidate-, and modafinil-induced wakefulness, evidenced by c-*fos* immunohistochemistry in the cat. Proc Natl Acad Sci USA 1996;93:14128–33.

Lu J, Jhou TC, Saper CB. Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. J Neurosci 2006;26:193–202.

Opp MR, Krueger JM. Anti-interleukin-1 beta reduces sleep and sleep rebound after sleep deprivation in rats. Am J Physiol 1994;266(Pt 2):R688-695.

Pack AI, Black JE, Schwartz JR, Matheson JK. Modafinil as adjunct therapy for daytime sleepiness in obstructive sleep apnea. Am J Respir Crit Care Med 2001;164:1675–81.

Panula P, Yang HYT, Costa E. Histamine-containing neurons in the rat hypothalamus. Proc Natl Acad Sci USA 1984;81:2572–6.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. Sydney: Academic Press; 1986.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 5th ed. Amsterdam: Elsevier Academic Press; 2005.

Pompeiano M, Cirelli C, Tononi G. Immediate-early genes in spontaneous wakefulness and sleep: expression of c-*fos* and NGFI-A mRNA and protein. J Sleep Res 1994;3:80–96.

Roth T, Czeisler CA, Walsh JK, Wesnes KA, Williams M, Neibler GE, et al. Randomized, double-blind, placebo-controlled study of armodafinil for the treatment of excessive sleepiness associated with chronic shift work sleep disorder. Sleep 2005;30(Suppl 1):s140.

Roth T, White D, Schmidt-Nowara W, Wesnes KA, Niebler G, Arora S, et al. Effects of armodafinil in the treatment of residual excessive sleepiness associated with obstructive sleep apnea/hypopnea syndrome: a 12-week, multicenter, double-blind, randomized, placebo-controlled study in nCAP-adherent adults. Clin Ther 2006;28:689–706.

Sagar SM, Sharp FR, Curran T. Expression of c-*fos* protein in brain: metabolic mapping at the cellular level. Science 1988;240:1328–31.

Scammell TE, Estabrooke IV, McCarthy MT, Chemelli RM, Yanagisawa M, Miller MS, et al. Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 2000;20:8620–8.

Semba K, Pastorius J, Wilkinson M, Rusak B. Sleep deprivation-induced c-*fos* and *junB* expression in the rat brain: effects of duration and timing. Behav Brain Res 2001;120:75–86.

Shelton J, Nishino S, Vaught J, Dement WC, Mignot E. Comparative effects of modafinil and amphetamine on daytime sleepiness and cataplexy of narcoleptic dogs. Sleep 1995;18:817–26.

Touret M, Sallanon-Moulin M, Jouvet M. Awakening properties of modafinil without paradoxical sleep rebound: comparative study with amphetamine in the rat. Neurosci Lett 1995;189:43–6.

US Modafinil in Narcolepsy Multicenter Study Group. Randomized trial of modafinil for the treatment of pathological somnolence in narcolepsy. Ann Neurol 1998;43:88–97.

US Modafinil in Narcolepsy Multicenter Study Group. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy. Neurology 2000;54:1166–75.

Vogt BA, Vogt L, Farber NB. Cingulate cortex and disease models. In: Paxinos G, editor. The rat nervous system. 3rd ed. Amsterdam: Elsevier Academic Press; 2004. p. 705–27.

Willie JT, Renthal W, Chemelli RM, Miller MS, Scammell TE, Yanagisawa M, et al. Modafinil more effectively induces wakefulness in orexin-null mice than in wild-type littermates. Neuroscience 2005;130:983–95.

Wisor JP, Dement WC, Aimone L, Williams M, Bozyczko-Coyne D. Armodafinil, the R-enantiomer of modafinil: wake-promoting effects and pharmacokinetic profile in the rat. Pharmacol Biochem Behav 2006;85:492–9.