Psychopharmacology (1991) 103:28–32

Psychopharmacology

Awakening properties of modafinil: effect on nocturnal activity in monkeys *(Macaca mulatta)* after acute and repeated administration

Jean-François Hermant, Francis A. Rambert, and Jacques Duteil

Centre de Recherches du Laboratoire L. Lafon, 19, avenue du Professeur Cadiot, B.P. 22, F–94701 Maisons-Alfort Cedex, France

Received March 28, 1990 / Final version June 12, 1990

Abstract. Single oral administration of modafinil (16–64 mg/kg) in monkeys induced an increase in nocturnal activity and in behavioural arousal without stereotyped behaviour. Modafinil-induced increase in nocturnal activity was prevented by the centrally acting α_1 adrenoceptor antagonist prazosin. After repeated oral administration of modafinil, b.i.d. for 5 days, nocturnal activity was still increased, but was lower than after a single administration; the gradual decrease appeared on the second night. No rebound of sleep and no residual effect on night 5 (when modafinil was administered for 4 days and placebo on day 5) were seen after the withdrawal of the drug. Therefore, modafinil appeared to produce strong behavioural stimulation and awakening in a non-human primate species, after single, as well as after repeated administration, without other obvious side-effects (no sign of anxiety, no behavioural trouble). These awakening properties involved a modulation (stimulation) of a central α_1 adrenergic system.

Key words: Modafinil – Prazosin – Awakening – α_1-Adrenoceptor – Nocturnal activity – Rhesus monkey

Narcolepsy and idiopathic hypersomnia are disabling sleep disorders usually treated with amphetamine-like psychostimulants and tricyclic antidepressants. However, these therapeutics are unsatisfactory, so we searched for new drugs with high activity against these syndromes. Recently, adrafinil [(diphenylmethyl) sulfinyl-2 acetohydroxamic acid] demonstrated an original profile characterized by hyperlocomotion in mice (Rambert et al. 1986) and awakening effect in monkeys (Milhaud and Klein 1985). Since a partial metabolic transformation of adrafinil into modafinil [(diphenylmethyl) sulfinyl-2 acetamide] has been demonstrated (Moachon, personnal communication), the pharmacological activity of this last compound was studied and an increased locomotor activity was evidenced in mice, without stereotyped behaviour in mice and rats (Duteil et al. 1990). This increase in locomotor activity was prevented by centrally acting α_1-adrenoceptor antagonists. No obvious peripheral sympathetic effect was seen in mice and rats. Pharmacological activities of modafinil were then considered to be investigated in monkeys.

It is already known that the locomotor stimulant effect of drugs is related to the activity cycle (diurnal or nocturnal) of the species; the diurnal activity of monkeys was decreased or unchanged after administration of stimulant drugs such as amphetamine, caffeine or methylphenidate (Davis 1957; Alexander and Isaac 1965; Stinnette and Isaac 1975; Lowther and Isaac 1976; Isaac and Kallman 1979). In contrast, the nocturnal activity of monkeys was increased after the administration of amphetamine, caffeine (Lagarde and Milhaud 1989) or adrafinil (Milhaud and Klein 1985). Therefore, the nocturnal period seemed to be the most suitable to observe a drug-induced locomotor stimulant effect. Moreover, the night period is noiseless and without external stimulation, compared with daytime.

The present study was therefore undertaken to evaluate the pharmacological effects of modafinil on behaviour and nocturnal activity, after either single or repeated oral administration of the drug, in the rhesus monkey.

In addition, since the pharmacological effects of adrafinil (Duteil et al. 1979) and modafinil (Duteil et al. 1990) involve a central α_1-adrenergic link, the α_1-adrenoceptor antagonist prazosin, was used in order to attempt to antagonize the modafinil-induced stimulating effects.

Materials and methods

Animals

The experimental subjects were ten male (Charles River, Saint-Aubin-lès-Elbeuf, France), 3–4 years old, 3.7–5.6 kg, rhesus monkeys *(Macaca mulatta)*. Usually, the monkeys were either housed in a large living cage (2 × 2 × 2 m) for six to eight animals, allowing

Offprint requests to: J. Duteil

them more space for exercise, or paired in a stainless steel cage (0.5 × 0.6 × 0.7 m), located in an environmentally controlled room with regulated temperature (21 ± 1° C) under a constant 12 h light/12 h dark cycle (lights on from 06.00 hours to 18.00 hours). They were fed 3 times a day on a diet consisting of standard monkey pellets (Extra-Labo) supplemented with fresh fruits and vegetables, and were given free access to water.

All animals were accustomed to the experimental conditions and were free of drugs for a period of 4 weeks before the study.

Experimental design

Animals were assigned to treatment groups using appropriate randomization procedures according to a latin square design (Lellouch and Lazar 1985). Drugs were administered in a flavoured syrup by the oral route in a volume of 10 ml per animal. Control animals always received the same number of administrations of deionized water or tragacanth gum in a flavoured syrup at the same time intervals.

Experimental procedure

Nocturnal activity was investigated in four pairs of monkeys. Two animals of the same pair were moved from the housing unit and singly placed in cages in a sound attenuated room. The two cages were distant from 60 cm and separated by a transparent screen to avoid ultrasonic interference. Gross behaviour was assessed by intermittent videotape recording. Total nocturnal mobility time was individually recorded for 12 h (from 18.00 hours to 06.00 hours) and for each third of the night (from 18.00 hours to 22. 00 hours, 22.00 hours to 02.00 hours and 02. 00 hours to 06. 00 hours) by an ultrasound device (an ultrasound field was generated in each cage so that each movement interrupting this ultrasound field could be detected by a receiving transducer).

Experiment 1: Acute administration of modafinil. At 17.00 hours, the two animals of the same pair received modafinil (16, 32 or 64 mg/kg) or vehicle. Two drug administrations to the same animal were spaced by 1 week.

Experiment 2: Interaction with prazosin. At 16.00, hours the two animals of the same pair received prazosin (2 mg/kg) or vehicle, followed by modafinil (32 mg/ kg) or vehicle 1 h later.

Experiment 3: Repeated administration of modafinil. Nocturnal activity was investigated for 5 days. Four treatments were alternated in a randomized order in each pair of subjects at 1-month interval. Each treatment consisted of a b. i. d. (09. 00 hours and 17. 00 hours) oral dosage of modafinil (32 mg/kg) or vehicle:

Treatment 1: placebo (from day 1 to day 5),
Treatment 2: placebo (from day 1 to day 4) followed by modafinil (on day 5),
Treatment 3: modafinil (from day 1 to day 4) followed by placebo (on day 5),
Treatment 4: modafinil (from day 1 to day 5).

Drugs

Prazosin hydrochloride (Pfizer) was dissolved in deionized water, modafinil (Lafon) was suspended in a 0.5% tragacanth gum solution. All doses refer to the free base.

Statistics

The data are expressed as means ± SEM. Drug effects were assessed (after logarithmic transformation to reduce variation between groups) using a one-way ANOVA, followed by the two-tailed Dunnett's test for comparison of multiple experimental groups with a single control group (experiment 1), a two-way ANOVA followed by the Newman-Keuls test for multiple comparisons between groups (experiment 2) and a repeated measure design (ANOVAR) followed by either the two-tailed Dunnett's test or the Newman-Keuls test (experiment 3). A P value of 0.05 was accepted as the level of statistical significance (Schwartz 1981).

Results

Experiment 1: Acute administration of modafinil

The overall observation of animals receiving oral doses of modafinil ranging from 16 to 64 mg/kg demonstrated a quiet awakening behaviour without stereotypies.

A significant increase (Table 1) in mobility duration was observed after modafinil administration (16–64 mg/kg, Fig. 1). This effect was prominent during the first third of the night (18.00 hours to 22.00 hours).

Experiment 2: Interaction with prazosin

The overall observation of animals receiving prazosin (2 mg/kg) did not show behavioural change.

Table 1. Summary of analysis of variance: experiment 1

Period	Treatment		
	F	df	P
18.00–22.00 h	62.2	(3,18)	$P<0.001$
22.00–02.00 h	9.8	(3,18)	$P<0.001$
02.00–06.00 h	11.3	(3,18)	$P<0.001$
18.00–06.00 h	59.0	(3,18)	$P<0.001$

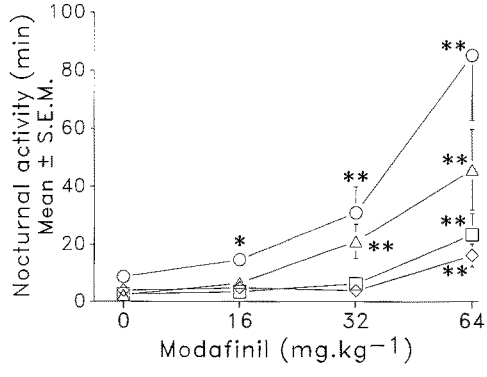

Fig. 1. Nocturnal activity after a single administration of modafinil in monkeys. Placebo (0 mg/kg) or modafinil (16–32 or 64 mg/kg) was given orally at 17.00 hours (8 animals/dose). Mobility time, recorded by an ultrasound device from 18.00 hours to 22.00 hours *(open triangle)*, from 22.00 hours to 02.00 hours *(open square)* and from 02.00 hours to 06.00 hours *(open diamond)* is shown. Total mobility time from 18.00 hours to 06.00 hours *(open circle)* is the sum of the three periods. Significant differences from placebo group (Dunnett's test), are represented as: * $0.01<P\leq0.05$; ** $0.001<P\leq0.01$

Table 2. Summary of analysis of variance: experiment 2

Period	Treatment A (Modafinil)			Treatment B (Prazosin)			Interaction A × B		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
18.00–22.00 h	88.6	(1,18)	$P<0.001$	2.0	(1,18)	$P>0.05$	0.4	(1,18)	$P>0.05$
22.00–02.00 h	5.4	(1,18)	$P<0.05$	2.7	(1,18)	$P>0.05$	0.2	(1,18)	$P>0.05$
02.00–06.00 h	4.9	(1,18)	$P<0.05$	0.7	(1,18)	$P>0.05$	1.6	(1,18)	$P>0.05$
18.00–06.00 h	51.4	(1,18)	$P<0.001$	3.0	(1,18)	$P>0.05$	1.1	(1,18)	$P>0.05$

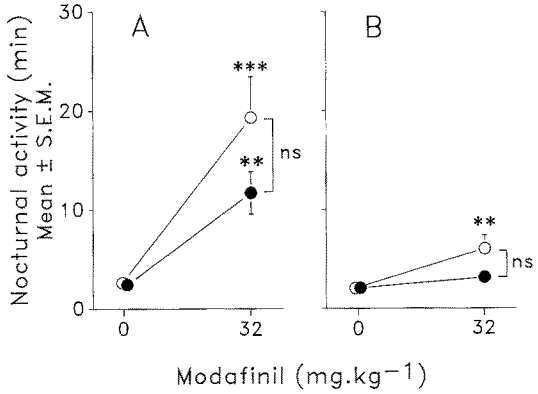

Fig. 2A, B. Effect of prazosin on modafinil-induced increase in nocturnal activity in monkeys. Prazosin (2 mg/kg) and modafinil (32 mg/kg) were given orally at 16.00 hours and 17.00 hours, respectively (8 animals/dose). Mobility time, recorded by an ultrasound device from 18.00 hours to 22.00 hours (panel **A**), and from 22.00 hours to 02.00 hours, (panel **B**) is shown. Prazosin 0 mg/kg: *open circle*, prazosin 2 mg/kg: *filled circle*. Significant differences between appropriate groups (Newman-Keuls test), are represented as: ns: not significant; ** $0.001<P\leq0.01$; *** $P\leq0.001$

The nocturnal mobility duration in placebo or prazosin (2 mg/kg) treated animals were similar.

Modafinil (32 mg/kg) induced a significant increase in mobility duration which was only obvious from 18.00 hours to 22.00 hours (Fig. 2A) and from 22.00 hours to 02.00 hours (Fig. 2B). Prazosin pretreatment reduced modafinil-induced increase in mobility duration (Fig. 2) although the two-way ANOVA did not show a significant effect (Table 2).

Experiment 3: Repeated administration of modafinil

In treatment group 1, the overall observation of animals displayed a quiet normal sleep every night.

In treatment group 2, a quiet normal sleep for nights 1–4 and a total arousal on night 5 were observed.

In treatment group 3, almost continuous arousal was observed on night 1 and 2, but the awakening activity of modafinil gradually decreased on night 3 and 4. The behavioural sleep was quite normal on night 5.

In treatment group 4, the same behaviour as in group 3 was seen, but the awakening activity of modafinil gradually decreased on nights 4 and 5.

Otherwise, in treatment groups 3 and 4, abnormal short lasting head-weaving early appeared (30–60 min after drug administration) in three of eight animals following day 1 and day 2 modafinil administration only.

The nocturnal activity recording showed homogeneity in the responses according to the treatment: from day 1 to day 4, no significant difference appeared between treatment 1 and treatment 2 (receiving placebo for 4 days) or between treatment 3 and treatment 4 (receiving modafinil for 4 days).

The ANOVAR revealed significant overall treatment effect, time effect and treatment by time interaction (Table 3). In the modafinil groups, the enhancement in nocturnal activity gradually decreased on the second night (from 02.00 hours to 06.00 hours, Fig. 3C) and the third night (from 22.00 hours to 02.00 hours, Fig. 3B). For the 18.00 hours to 22.00 hours period, the hyperactivity decreased only on day 4 and onwards (Fig. 3A).

After 4 days of placebo administration, the subsequent administration of modafinil on day 5, significantly increased nocturnal activity (Fig. 3D). This increase (which did not significantly differ from those observed on day 1 in treatment groups 3 and 4) was more obvious during the first part of the night (Fig. 3A).

After 4 days of modafinil administration, the subsequent administration of placebo on day 5 did not significantly modify nocturnal activity as compared with the group receiving placebo for 5 days.

Table 3. Summary of analysis of variance: experiment 3

Period	Treatment			Time			Treatment × Time		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
18.00–22.00 h	30.2	(3,28)	$P<0.001$	4.7	(4,112)	$P<0.001$	18.4	(12,112)	$P<0.001$
22.00–02.00 h	8.2	(3,28)	$P<0.001$	4.7	(4,112)	$P<0.01$	8.3	(12,112)	$P<0.001$
02.00–06.00 h	1.3	(3,28)	$P<0.001$	3.4	(4,112)	$P<0.01$	3.7	(12,112)	$P<0.001$
18.00–06.00 h	15.4	(3,28)	$P>0.05$	5.8	(4,112)	$P<0.05$	18.4	(12,112)	$P<0.001$

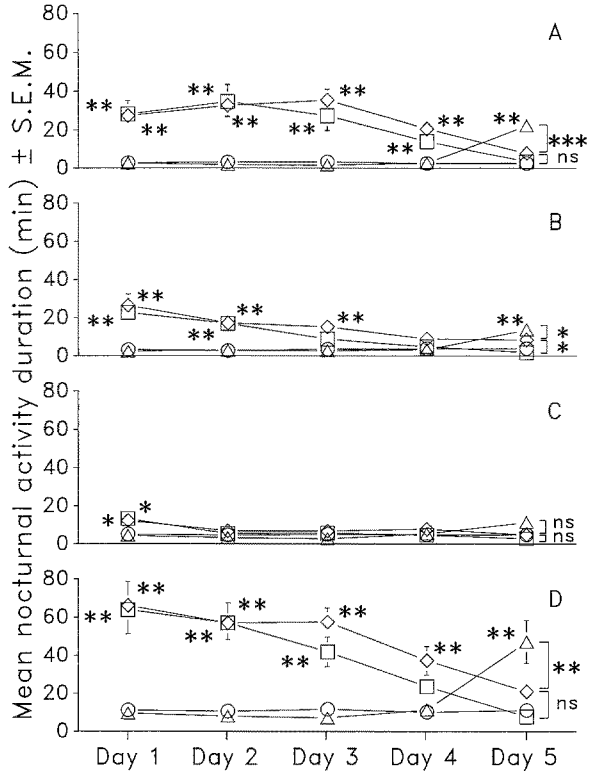

Fig. 3A–D. Nocturnal activity after repeated administrations of modafinil in monkeys. Modafinil (32 mg/kg) or placebo was given orally twice a day at 09.00 hours and 17.00 hours (8 animals/dose). Mobility time recorded each day by an ultrasound device from 18.00 hours to 22.00 hours (panel **A**), from 22.00 hours to 02.00 hours (panel **B**) and from 02.00 hours to 06.00 hours (panel **C**) is shown. Total mobility time from 18.00 hours to 06.00 hours (panel **D**) is the sum of the three periods. Placebo (control group) from day 1 to day 5: *open circle;* placebo from day 1 to day 4 followed by modafinil on day 5: *open triangle;* modafinil from day 1 to day 4 followed by placebo on day 5: *open square;* modafinil from day 1 to day 5: *open diamond.* Significant differences from control group (day 1 to day 5, Dunnett's test) and between appropriate groups (day 5, Newman-Keuls test), are represented as: ns: not significant; * $0.01 < P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$

Discussion

In the rhesus monkey, nocturnal behaviour is characterized by the presence of nycthemeral rhythms, sitting or crouching during sleep time and low nocturnal locomotor activity, but the percentage of nocturnal arousal periods were relatively high compared with other primate species (Yellin and Hauty 1971; Adams and Barratt 1974).

The two methods used in these studies complemented each other. The ultrasonic method did not appear to induce obvious behavioural changes; it allowed a continuous measure of activity, but did not fully take into account the drug-induced arousal duration in monkeys. On the other hand, the intermittently-programmed videotape recording did not allow continuous observation of the behaviour, but was able to clearly differentiate quiet arousal from sleep. The ultrasonic method demonstrated an increase in nocturnal activity duration after acute oral administration of modafinil compared with placebo. The highest dose of modafinil induced hyperactivity for only 12 % of the observation time; in fact, even after the lower doses of modafinil, intermittent video observation samples showed that, whatever the time, monkeys stayed awake throughout the night, often motionless in a sitting position that was not taken into account by the ultrasonic method.

EEG results after single or repeated administration of modafinil are in keeping with the nocturnal awakening effect of modafinil in monkeys (Milhaud, personal communication). Diurnal arousal and structural organisation of sleep (when it reappeared) were not altered.

Video recording also allowed observation of movements. In non-human primates, central nervous system stimulants such as amphetamine (Randrup and Munkvad 1967; Scraggs and Ridley 1978; Ridley and Baker 1982; Miczek and Gold 1983) elicited stereotyped behaviour (repetitive movements of head, neck, limbs and trunk) and repetitive behavioural sequences (self-grooming, staring at the hands).

On the other hand, in rhesus monkeys, apomorphine elicited hyperactivity and repetitive stereotyped movements, i.e. chewing, tongue movements, licking, biting and vocalizations (Porsolt et al. 1982), which were antagonized by neuroleptics. In contrast to amphetamine or apomorphine, modafinil did not produce stereotyped patterns of repetitive movements associated with hyperactivity.

In mice, modafinil-induced hyperactivity was prevented by the central α_1-adrenoceptor antagonists, prazosin, phenoxybenzamine, and yohimbine in high doses (Duteil et al. 1990). This antagonism appeared to be of central origin because phentolamine, which did not easily cross the blood-brain barrier (Anden and Strömbom 1974), did not antagonize this hyperactivity. The importance of a modulatory role for noradrenaline in locomotor activity is well documented. In rats, specific and selective α_2- and α_1-adrenoceptor antagonists idazoxan and prazosin, respectively, differentially influenced amphetamine-induced hyperactivity: prazosin (but not idazoxan) markedly reduced low dose *d*-amphetamine-induced hyper locomotion and enhanced high dose *d*-amphetamine-induced stereotyped gnawing (Dickinson et al. 1988).

Prazosin, a drug known to block the central α_1-adrenoceptors (Menkes et al. 1981), at a dose which did not alter blood pressure (data not shown) and did not modify nocturnal activity, antagonized modafinil-induced hyperactivity in rhesus monkeys. This argued for a central α_1-adrenergic participation in modafinil-induced increase in arousal in rhesus monkeys according to the hypothesis of an involvement of noradrenergic system in the regulation of the sleep-waking cycle (Fuxe et al. 1974; De Sarro et al. 1987).

Repeated administration of modafinil induced an increase in nocturnal activity duration after each administration, but this hyperactivity was shortened following the second day of administration; this decrease first appeared in the third part of the night and was probably related to an enzymatic induction involved by

repeated high doses, as reported in mice (Rambert et al. 1987).

After the last administration of modafinil, no withdrawal syndrome was observed. The lack of increase in mobility duration is in line with the lack of residual effect of the drug. Likewise, the lack of decrease in mobility duration is in keeping with the lack of rebound effect; however, a reduction in mobility duration seemed difficult to demonstrate, since at that time the activity level was already very low.

In conclusion, single modafinil administration induced a dose-related hyperactivity and awakening effect in monkeys, which seem to be dependent upon an α_1-adrenergic activity. These two effects decreased after repeated administration without any withdrawal effect.

These studies, demonstrating awakening efficacy of oral administration of modafinil in non-human primates, were corroborated by clinical studies which confirmed the awakening activity of modafinil in human and its therapeutic efficacy in narcolepsy and idiopathic hypersomnia (Billiard et al. 1987; Laffont et al. 1987; Bastuji and Jouvet 1988).

References

Adams PM, Barratt ES (1974) Nocturnal sleep in squirrel monkeys. Electroencephalogr Clin Neurophysiol 36:201–204

Alexander M, Isaac W (1965) Effect of illumination and *d*-amphetamine on the activity of the rhesus macaque. Psychol Rep 16:311–313

Anden N-E, Strömbom U (1974) Adrenergic blocking agents: effects on central noradrenaline and dopamine receptors and on motor activity. Psychopharmacologia 38:91–103

Bastuji H, Jouvet M (1988) Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. Prog Neuropsychopharmacol Biol Psychiatry 12:695–700

Billiard M, Picard E, Besset A, Maurel A (1987) Treatment of narcolepsy-cataplexy with modafinil, an alpha$_1$-adrenoceptor agonist. Communication at the 5th International Congress of Sleep Research, Copenhagen, book of abstracts, p 471

Davis GD (1957) Effects of central excitant and depressant drugs on locomotor activity in the monkey. Am J Physiol 188:619–623

De Sarro GB, Asciotto C, Froio F, Libri V, Nistico G (1987) Evidence that the locus coeruleus is the site where clonidine and drugs acting at α_1- and α_2-adrenoceptors affect sleep and arousal mechanisms. Br J Pharmacol 90:675–685

Dickinson SL, Gadie B, Tulloch IF (1988) α_1- and α_2-adrenoceptor antagonists differentially influence locomotor and stereotyped behaviour induced by *d*-amphetamine and apomorphine in the rat. Psychopharmacology 96:521–527

Duteil J, Rambert FA, Pessonnier J, Gombert R, Assous E (1979) A possible α-adrenergic mechanism for drug (CRL40028)-induced hyperactivity. Eur J Pharmacol 59:121–123

Duteil J, Rambert FA, Pessonnier J, Hermant JF, Gombert R, Assous E (1990) Central alpha$_1$-adrenergic stimulation in relation to the behaviour stimulating effect of modafinil; studies with experimental animals. Eur J Pharmacol 180:49–58

Fuxe K, Lidbrink P, Hökfelt T, Blome P, Goldstein M (1974) Effects of piperoxan on sleep and waking in the rat. Evidence for increased waking by blocking inhibitory adrenaline receptors on the locus coeruleus. Acta Physiol Scand 91:566–567

Isaac W, Kallman MD (1979) Locomotor effects of *d*-amphetamine and methylphenidate in young squirrel monkeys. Bull Psychon Soc 14:315–317

Laffont F, Cathala HP, Kohler F (1987) Effect of modafinil on narcolepsy and idiopathic hypersomnia. Communication at the 5th International Congress of Sleep Research, Copenhagen, book of abstracts, p 536

Lagarde D, Milhaud C (1989) Distinction between wakening and agitating effects of amphetamine in rhesus monkeys. Med Sci Res 17:961–963

Lellouch J, Lazar Ph (1985) Méthodes statistiques en expérimentation biologique. Flammarion Médecine Sciences, Paris

Lowther WR, Isaac W (1976) The effects of *d*-amphetamine and illumination on behaviors of the squirrel monkey. Psychopharmacology 50:231–235

Menkes DB, Baraban JM, Aghajanian GK (1981) Prazosin selectively antagonizes neuronal responses mediated by α_1-adrenoceptors in brain. Naunyn Schmiedeberg's Arch Pharmacol 317:273–275

Miczek KA, Gold LH (1983) *d*-Amphetamine in squirrel monkeys of different social status: effects on social and agonistic behaviour, locomotion and stereotypies. Psychopharmacology 81:183–190

Milhaud CL, Klein MJ (1985) Effet de l'adrafinil sur l'activité nocturne du macaque rhésus *(Macaca mulatta)*. J Pharmacol (Paris) 16:372–380

Porsolt RD, Roux S, Jalfre M (1982) Antiapomorphine and locomotor effects of neuroleptics in rhesus monkeys. Pharmacol Biochem Behav 17:1309–1312

Rambert FA, Pessonnier J, De Séréville JE, Pointeau AM, Duteil J (1986) Profil pharmacologique original de l'adrafinil chez la souris. J Pharmacol (Paris) 17:37–52

Rambert FA, Moachon G, Multon MF, Matinier D, Pessonnier J, Clenet M, Duteil J (1987) Correlation of modafinil plasma levels to pharmacological activity in mice. Communication at the 5th International Congress of Sleep Research, Copenhagen, book of abstracts, p 124

Randrup A, Munkvad I (1967) Stereotyped activities produced by amphetamine in several animal species and man. Psychopharmacologia 11:300–310

Ridley RM, Baker HF (1982) Stereotypy in monkeys and human. Psychol Med 12:61–72

Schwartz D (1981) Méthodes statistiques à l'usage des médecins et biologistes. Flammarion Médecine Sciences, Paris

Scraggs PR, Ridley RM (1978) Behavioural effects of amphetamine in a small primate: relative potencies of the *d*- and *l*-isomers. Psychopharmacology 59:243–245

Stinnette MJ Isaac W (1975) Behavioral effects of *d*-amphetamine and caffeine in the squirrel monkey. Eur J Pharmacol 30:268–271

Yellin AM, Hauty GT (1971) Activity cycles of the rhesus monkey *(Macaca mulatta)* under several experimental conditions, both in isolation and in a group situation. J Interdisciplinary Cycle Res 2:475–490