

# Comparison of pramipexole and modafinil on arousal, autonomic, and endocrine functions in healthy volunteers

*Journal of Psychopharmacology*  
00(00) (2005) 000–000  
© 2005 British Association  
for Psychopharmacology  
ISSN 0269-8811  
SAGE Publications Ltd,  
London, Thousand Oaks,  
CA and New Delhi  
10.1177/0269881105060770

E. R. Samuels *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

R. H. Hou *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

R. W. Langley *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

E. Szabadi *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

C. M. Bradshaw *Psychopharmacology Section, Division of Psychiatry, University of Nottingham, Nottingham, UK.*

## Abstract

The noradrenergic locus coeruleus is a major wakefulness-promoting nucleus of the brain, which is also involved in the regulation of autonomic and endocrine functions. The activity of the locus coeruleus is believed to be tonically enhanced by a mesocoerulear dopaminergic pathway arising from the ventral tegmental area of the midbrain. Both modafinil, a wakefulness-promoting drug, and pramipexole, a  $D_2/D_3$  receptor agonist with sedative properties, may act on this pathway, with modafinil increasing and pramipexole decreasing locus coeruleus activity. The aim of this study was to compare the two drugs on alertness, autonomic and endocrine functions in healthy volunteers. Pramipexole (0.5 mg), modafinil (200 mg), and their combination were administered to 16 healthy males in a double-blind, placebo-controlled design. Methods included tests of alertness (pupillographic sleepiness test, critical flicker fusion frequency, visual analogue scales), autonomic functions (resting pupil diameter, light and darkness reflex responses,

heart rate, blood pressure, salivation, core temperature), and endocrine functions (blood concentrations of prolactin, growth hormone, and thyroid stimulating hormone). Data were analysed by ANOVA.

Pramipexole reduced alertness, caused pupil dilatation, increased heart rate, reduced prolactin and thyroid stimulating hormone, and increased growth hormone level. Modafinil caused small increases in blood pressure and core temperature, and reduced prolactin levels. The sedative effect of pramipexole and the autonomic effects of modafinil are consistent with altered activity in the mesocoerulear pathway; the pupil dilatation following pramipexole suggests reduced dopaminergic excitation of the Edinger-Westphal nucleus.

## Keywords

pramipexole, modafinil, locus coeruleus, ventral tegmental area, Edinger-Westphal nucleus, arousal

## Introduction

Pramipexole is a non-ergot dopamine  $D_2/D_3$  receptor agonist used in the treatment of Parkinson's disease (PD) (Parkinson Study Group, 1997, 2000; Piercey, 1998; Reichmann *et al.*, 2003). PD is a gradual-onset, neurodegenerative disorder associated with a loss of dopaminergic neurones in the substantia nigra, resulting in motor deficits including muscular rigidity, tremor, postural instability, and hypokinesia. Recently, *excessive* daytime sleepiness (EDS) has also been associated with PD (O'Suilleabhain and Dewey, 2002; Adler *et al.*, 2003). Paradoxically, the dopamine

receptor agonists used to treat PD and stimulate the dopamine receptors in the striatum are often sedative, including pramipexole (pramipexole: Hauser *et al.*, 2000a; ropinirole: Ferreira *et al.*, 2002; pergolide: Ulivelli *et al.*, 2002; bromocriptine and cabergoline: Paus *et al.*, 2003; piribedil: Tan, 2003). Thus a situation occurs where both dopamine neurone loss (causing dopamine deficiency) and dopamine receptor agonist administration result in sedation.

It has been suggested that the sedative effect of dopamine receptor agonists may be mediated via dopaminergic neurones located in the ventral tegmental area (VTA) of the midbrain and

projecting to the noradrenergic locus coeruleus (LC), a major wakefulness-promoting nucleus (Ornstein *et al.*, 1987; Maeda *et al.*, 1994). This mesocoerulear pathway is assumed to activate the LC via the stimulation of postsynaptic D<sub>2</sub> receptors. D<sub>2</sub> receptors have also been identified on the dopaminergic neurones themselves and these autoreceptors are inhibitory in function (Bagetta *et al.*, 1988). Thus, the sedative effect of dopamine receptor agonists may be explained by the activation of these inhibitory autoreceptors, leading to the 'switching off' of the mesocoerulear dopaminergic neurone and the withdrawal of the tonic dopaminergic stimulation of the LC. A biphasic dose-response curve for dopaminomimetics has been suggested, where low doses promote sedation and sleep through pre-synaptic autoreceptors and high doses enhance wakefulness through post-synaptic receptor activation (Rye and Jankovic, 2002; Keating and Rye, 2003).

In contrast to pramipexole, modafinil is a novel wakefulness-promoting drug and is used in the treatment of EDS in narcolepsy (US Modafinil in Narcolepsy Multicentre Study Group, 1998, 2000). It has also been shown to alleviate the EDS associated with a number of other conditions (Parkinson's disease: Nieves and Lang, 2002; idiopathic hypersomnia: Ivanenko *et al.*, 2003; night shift sleep disorder: Walsh *et al.*, 2004; obstructive sleep apnoea: Pack *et al.*, 2001; multiple sclerosis: Rammohan *et al.*, 2002; myotonic dystrophy: MacDonald *et al.*, 2002; depression: DeBattista *et al.*, 2003; schizophrenia: Rosenthal and Bryant, 2004; sleep deprivation: Pigeau *et al.*, 1995; and drug-induced sedation: Webster *et al.*, 2003). Interestingly, in a case study of a PD patient, modafinil was found to reverse the sedative effect of pramipexole (Hauser *et al.*, 2000b). The mode of action of modafinil is unclear, but it appears to involve dopaminergic and/or noradrenergic arousal pathways (Saper and Scammell, 2004) and has been found to increase extracellular levels of dopamine (Wisor *et al.*, 2001). It may, therefore, be promoting alertness by enhancing the tonic excitatory activation of the LC by the dopaminergic VTA.

The sedative and alerting effects of these treatments are measurable using various tests: visual analogue scales (VAS), critical flicker fusion frequency (CFFF) test, and the pupillographic sleepiness test (PST). The PST derives two measures from the changing diameter of the pupil over time: total power of pupil fluctuations and the pupillary unrest index (PUI), which sums the mean movement of the diameter of the pupil per minute over the 11 minutes of the test (Lüdtke *et al.*, 1998). Therefore, an increase in PUI relates to an increase in pupil fluctuation and an increase in sedation (Wilhelm *et al.*, 2001). It has been shown that the PST has been used successfully to detect the sedative effect of drugs (Phillips *et al.*, 2000a, 2000b; Hou *et al.*, 2005). It is hypothesized that pramipexole will reduce the subjective rating of alertness in the VAS and the frequency detectable in the CFFF, while increasing the PST total power and PUI measures. Conversely, modafinil is expected to increase the subjective rating of alertness in the VAS and the frequency detectable in the CFFF, while decreasing the PST total power and PUI measures.

The noradrenergic LC, apart from promoting arousal, is also involved in autonomic regulation. In general, and together with other noradrenergic nuclei, it contributes to central sympathetic

outflow and exerts an inhibitory influence on parasympathetic activity (Szabadi and Bradshaw, 1996). LC activation therefore results in alerting, sympathomimetic, and parasympatholytic effects. The autonomic consequences of LC activation include pupil dilatation, alterations in the kinetics of the light reflex response (increase in latency, reduction in amplitude, shortening of recovery time) and the darkness reflex response (increases in initial velocity and amplitude, decrease in latency), increases in blood pressure and heart rate, a reduction in salivation, and an increase in core body temperature (Szabadi and Bradshaw, 1996; Hou *et al.*, 2005). In a previous experiment in our laboratory we obtained evidence consistent with the activation of the LC by modafinil (Hou *et al.*, 2005). Therefore, it was predicted that pramipexole is likely to evoke effects opposite to those of modafinil.

As central noradrenergic and dopaminergic neurones are involved in neuroendocrine function, the levels of prolactin, growth hormone (GH), and thyroid stimulating hormone (TSH) are expected to be influenced by drugs which interact with central catecholaminergic neurotransmission. Indeed, it has been reported that pramipexole reduces prolactin and TSH levels and increases GH levels (Schilling *et al.*, 1992). No effects on GH levels have been found following modafinil (Brun *et al.*, 1998), but to our knowledge the effects of modafinil on TSH and prolactin have not been reported.

The aim of this paper was therefore to examine the effects of single doses of pramipexole, modafinil, and the combination of pramipexole and modafinil, on measures of arousal, autonomic function (pupillary activity, cardiovascular functions, core temperature, and salivary output), and endocrine function (blood concentrations of prolactin, GH, and TSH) in healthy human volunteers. The results will be important to further understand the role of dopamine in normal and pathological sleep-wake regulation, and for elucidating the mechanisms of action of pramipexole and modafinil.

## Methods and materials

### Subjects

Sixteen healthy male volunteers aged 18–36 years (mean  $\pm$  SEM: 20.94 years  $\pm$  1.08 years), 166–190 cm (mean  $\pm$  SEM: 180.69  $\pm$  1.48 cm) in height and weighing 58.6–93.5 kg (mean  $\pm$  SEM: 75.17 kg  $\pm$  8.65 kg) participated in the study. Subjects were all medication free for at least 3 months prior to the start of the study and completed a brief medical history and physical examination before inclusion in the study. Volunteers were required to smoke less than five cigarettes a day and were requested to avoid drinking alcohol, coffee and other caffeine-containing beverages for at least 24 h before each experimental session and to avoid taking any medication for the duration of the study. Women were excluded from the study due to the slower renal clearance of pramipexole in this population (Wright *et al.*, 1997). The study protocol was approved by the University of Nottingham Medical School Ethics Committee, and all volunteers

gave their written consent after reading a detailed information sheet.

### Drugs

Pramipexole 0.5 mg, modafinil 200 mg, pramipexole 0.5 mg + modafinil 200 mg, and placebo were administered orally in matching capsules for double-blind administration. The doses were chosen on the basis of the current literature (pramipexole: Wright *et al.*, 1997; modafinil: Robertson and Hellriegel, 2003; Hou *et al.*, 2005). In each session the subjects ingested two capsules 2 hours prior to post-treatment testing: one contained pramipexole 0.5 mg or placebo and one contained modafinil 200 mg or placebo.

### Design

Subjects participated in four sessions at weekly intervals, returning to the laboratory at the same time each week. Subjects were allocated to drug conditions according to a double-blind, balanced, cross-over design. The time course of the sessions was designed with regard to the pharmacokinetic profile of the two active drugs:  $t_{\text{MAX}}$  is approximately 2 h following the ingestion of a single dose of pramipexole (Wright *et al.*, 1997) or modafinil (Robertson and Hellriegel, 2003).

### Tests and apparatus

**Measures of alertness** Level of alertness was assessed using the pupillographic sleepiness test (PST), critical flicker fusion frequency (CFFF), and a battery of visual analogue scales (VAS).

The PST was used to record spontaneous pupillary fluctuations in darkness over 11 min using a dedicated monocular television pupillometer (setup version 1.20: AMTech, Weinheim, Germany) (see for details Hou *et al.*, 2005). The PST quantitatively analyses pupil fluctuations, which are regarded a physiological index of level of alertness (Lowenstein *et al.*, 1963; Yoss *et al.*, 1970). The method yields two measures of pupillary fluctuations, the pupillary unrest index (PUI: the distance travelled by the margin of the pupil over 1 min) and the total power of the pupil diameter fluctuations (obtained from a Fast Fourier Transformation).

The CFFF test, defined as the frequency at which a flickering light appears to be continuous (Smith and Misiak, 1976), was conducted conventionally. The Leeds Psychomotor Tester (Psychopharma Ltd, Surrey, UK) was used to collect eight measurements of the threshold, four with increasing frequencies and four with decreasing frequencies. The mean of the eight measurements was taken as the value of CFFF for each testing session (Abduljawad *et al.*, 1997).

Self-ratings of alertness, contentedness, and anxiety were made using a computerized version of VAS (Norris, 1971; Bond and Lader, 1974; see for details Hou *et al.*, 2005).

**Autonomic functions** Static pupillometry was used to obtain resting pupil diameter measures using a binocular infra-red video pupillometer with a calibrated internal light source (Procyon Ltd, London, UK) in darkness and at three luminance levels (6, 91, and 360  $\text{cd m}^{-2}$ ) (see for details Hou *et al.*, 2005).

Dynamic pupillometry was used to record light reflex and darkness reflex responses using a binocular infra-red television pupillometer (TVC 1015B Applied Science Laboratories, Waltham, MA, USA) with a sampling rate of 50 Hz and detection accuracy in excess of 0.05 mm. The pupillary light reflex response was evoked using light stimuli (green, 565 nm peak wavelength) of 200 ms duration at four incremental luminance levels (5.2, 41, 320, and 2050  $\text{cd m}^{-2}$ ) presented at 35 s intervals. The stimuli were presented via a light-emitting diode positioned 1 cm from the cornea of the subject's right eye, whilst the subject fixed his gaze upon a dim red spot of light approximately 5 m in front of him. The parameters studied were: latency (time from the onset of the light stimulus to the onset of the pupil response, s), amplitude (change in pupil diameter from maximum to minimum diameter as a response to presentation of the light stimulus, mm), and 75% recovery time (the time from the minimum pupil diameter to 75% of the initial pupil diameter in darkness, s) (see for details Hou *et al.*, 2005).

The pupillary darkness response was evoked by presenting an illuminated screen (light intensity: 1370  $\text{cd m}^{-2}$ ) approximately 90 cm in front of the subject for 10 s after a period of dark adaptation. As the screen was switched to darkness for 20 s the darkness reflex was recorded. This cycle was repeated and the means of the parameters of the two responses were calculated. The parameters studied were: amplitude (change in pupil diameter from minimum to maximum diameter as a response to the removal of the background luminance, mm), latency (time from the offset of the light to the onset of the pupil response, s), and initial velocity (time required to obtain 25% of the maximum response following the onset of the response,  $\text{mm s}^{-1}$ ).

Blood pressure and heart rate were measured conventionally using an electroneroid sphygmomanometer, in both the standing and supine positions. Temperature was measured using a Braun Pro4000 tympanic thermometer (Welch Allyn UK Ltd, Buckinghamshire, UK) placed in the ear canal. A measure of salivation was derived by placing three cotton wool dental rolls in the mouth (two buccally and one sublingually) and recording the increase in their weight over a 1 minute period (Peck, 1959; Arya *et al.*, 1997; Szabadi and Tavernor, 1999). The test was repeated twice with a 5 minute interval, and the mean of the two measurements was taken as an index of salivary output.

**Endocrine functions** A 10 ml blood sample was taken and analysed for concentrations of the hormones prolactin and thyroid stimulating hormone by enzyme immunoassay and for growth hormone by chemiluminescence immunoassay in the Clinical Chemistry Laboratory (Queen's Medical Centre, Nottingham).

### Procedure

After a 15 minute acclimatization period, subjects completed 30 minutes of pre-treatment testing, including standing and supine heart rate and blood pressure, temperature, salivation, CFFF, VAS, and pupil diameter. Two hours after ingestion of the capsules the post-treatment tests were conducted over 45 minutes, including standing and supine heart rate and blood pressure, tem-

perature, salivation, CFFF, VAS, pupil diameter, PST, light reflex responses, darkness reflex responses, and blood sampling.

### Data analysis

**Measures of alertness** Pre-treatment/post-treatment differences for the CFFF were calculated for further analysis. Self-rated values of ‘alertness’, ‘anxiety’ and ‘contentedness’ were derived from the VAS scores after weighting on these factors (Bond and Lader, 1974). Pre-treatment/post-treatment differences for these ratings were calculated for further analysis. Post-treatment measures of the PST parameters power and PUI were used for statistical analysis.

**Autonomic functions** Autonomic function parameters recorded included pupillary and non-pupillary measures. Resting pupil diameter measures in darkness and at different levels of luminance were averaged across the right and left eyes and analysed using pre-treatment and post-treatment values separately. Reflex measures (latency, amplitude, and 75% recovery time of the light reflex response; latency, amplitude, and initial velocity of the darkness reflex response) taken from the left eye were analysed using post-treatment values. Pre-treatment/post-treatment differences were not calculated for these pupillary functions since measurements were taken at different luminance levels, and calculating the difference would have eliminated the effect of luminance on the measures studied. Non-pupillary measures included cardiovascular functions (standing and supine heart rate, standing and supine diastolic and systolic blood pressure), salivation, and temperature, and were analysed using pre-treatment/post-treatment differences. Prolactin, GH, and TSH effects were analysed using post-treatment blood concentration values.

### Statistics

All pre-treatment values were analysed using one- or two-way ANOVA (treatment, or luminance  $\times$  treatment) to find any session effects within the results. When no significant pre-treatment session effects were found, pre-treatment/post-treatment differences were taken as the dependent variable where appropriate. All data were analysed using repeated measures ANOVA. The data were initially checked for homogeneity of variance and skew, and subjected to a  $\log_{10}$  or reciprocal transformation where indicated (transformations were conducted for severe skew only, as ANOVA is robust against moderate violations of the assumption of normality; Howell, 2002). One-way ANOVA with drug condition (four levels) as the within subjects factor was used to compare the effects of drug condition on PST, CFFF, VAS, darkness reflexes, blood pressure, heart rate, salivation, temperature, prolactin levels, GH levels, and TSH levels. Two-way ANOVA with drug condition (four levels) and light intensity (four levels) as the within subjects factors was used to compare the effects of drug and light intensity on pupil diameter and light reflex responses. All significant main effects were further analysed using Dunnett’s corrected t-test ( $df=45$ ,  $k=4$ ): active treatment conditions were compared with placebo (criterion of significance  $p < 0.05$ ).

For some measures of autonomic function the whole dataset derived from the group of 16 subjects could not be included in the analysis due to the corruption of some data points for technical reasons. Therefore subjects whose datasets were not complete were excluded from the analysis (light reflex response amplitude: three subjects [5.2  $\text{cdm}^{-2}$ : three subjects, 41  $\text{cdm}^{-2}$ : one subject, 320  $\text{cdm}^{-2}$ : one subject, 2050  $\text{cdm}^{-2}$ : one subject], light reflex response latency: three subjects [5.2  $\text{cdm}^{-2}$ : three subjects, 41  $\text{cdm}^{-2}$ : one subject, 320  $\text{cdm}^{-2}$ : one subject, 2050  $\text{cdm}^{-2}$ : one subject], light reflex response 75% recovery time: 11 subjects [5.2  $\text{cdm}^{-2}$ : four subjects, 41  $\text{cdm}^{-2}$ : four subjects, 320  $\text{cdm}^{-2}$ : four subjects, 2050  $\text{cdm}^{-2}$ : six subjects], supine heart rate: four subjects, standing heart rate: one subject, supine systolic blood pressure: one subject, standing systolic blood pressure: two subjects, supine diastolic blood pressure: one subject, standing diastolic blood pressure: two subjects, temperature: eight subjects).

## Results

All pre-treatment values were analysed initially using ANOVA to find any session effects within the results. No significant differences were found between drug conditions pre-treatment ( $p > 0.05$ ) and so pre-treatment/post-treatment differences were used as dependent measures of condition effect, where appropriate.

### Alertness

The effects of drug condition on the CFFF, the PST, and the three factors of the VAS are shown in Fig. 1.

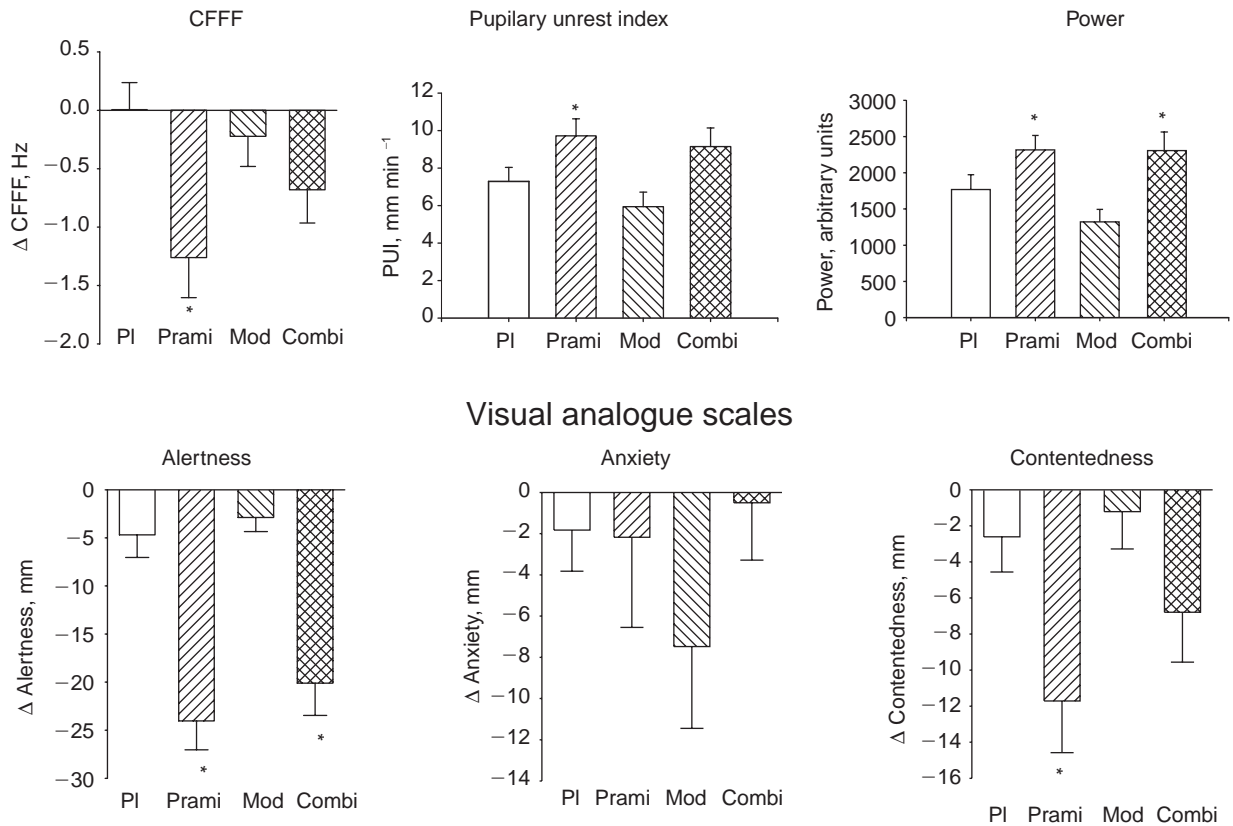
**CFFF** There was a significant drug condition effect ( $F_{3,45} = 3.63$ ,  $p < 0.05$ ), with pramipexole significantly reducing this measure.

**PST** The pupillary unrest index (PUI;  $\log_{10}$  transformation) showed a significant effect of drug condition ( $F_{3,45} = 7.65$ ,  $p < 0.001$ ), where pramipexole increased this measure. A significant effect of drug condition was also found in total power of pupil diameter fluctuations ( $\log_{10}$  transformation;  $F_{3,45} = 8.62$ ,  $p < 0.001$ ), where both pramipexole and the combination treatment increased the measure.

**VAS** The alertness factor of the VAS showed a significant effect of drug condition ( $F_{3,45} = 17.99$ ,  $p < 0.001$ ), with reductions in alertness following pramipexole and the combination treatment. Similarly, the contentedness factor of the VAS showed a significant effect of drug condition ( $F_{3,45} = 5.26$ ,  $p < 0.01$ ), with pramipexole reducing contentedness. Drug condition had no effect on anxiety ( $F_{3,45} = 0.90$ , NS).

### Pupillary functions

**Pupil diameter** The effects of treatments on pupil diameter at different light intensities are shown in Fig. 2. There was a significant effect of drug condition ( $F_{3,45} = 4.66$ ,  $p < 0.01$ ) and light intensity ( $F_{3,45} = 303.45$ ,  $p < 0.001$ ), and a trend for a significant interaction ( $F_{9,135} = 1.72$ ,  $p = 0.091$ ). Analysis of pupil diameter at



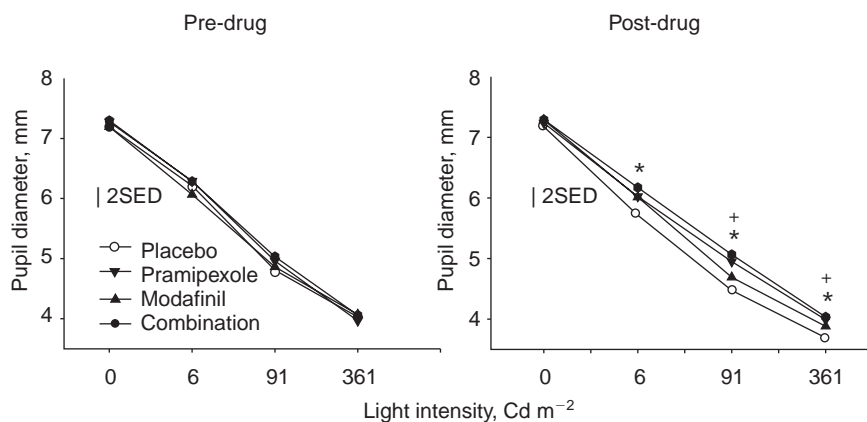
**Figure 1** Measures of arousal.

Pre-treatment/post-treatment differences in critical flicker fusion frequency (CFFF [Hz]; top left), pupillary unrest index (PUI [ $\text{mm min}^{-1}$ ]; top middle) and total power (arbitrary units; top right) of the PST, and visual analogue scales (VAS [mm]; bottom). Columns correspond to mean changes in the parameters for the group ( $n = 16$ ). Treatment condition is indicated at the bottom of the graphs: pl = placebo, prami = pramipexole, mod = modafinil, combi = combination of pramipexole and modafinil. The ordinate axis represents the change in the parameter from pre-treatment values. Vertical bars represent standard errors of the mean (SEM). \* $p < 0.05$  (Dunnett's test: comparison with placebo condition).

each light intensity condition separately, using one-way ANOVA, demonstrated a significant difference between active treatments and placebo at  $6 \text{ cd m}^{-2}$  ( $F_{3,45} = 2.87$ ,  $p < 0.05$ ), at  $91 \text{ cd m}^{-2}$  ( $F_{3,45} = 5.33$ ,  $p < 0.01$ ), and at  $360 \text{ cd m}^{-2}$  ( $F_{3,45} = 3.02$ ,  $p < 0.05$ ); there were no significant drug condition differences in darkness ( $F_{3,45} = 0.50$ , NS). Pramipexole ( $91 \text{ cd m}^{-2}$  and  $360 \text{ cd m}^{-2}$ ) and the combination of pramipexole and modafinil ( $6 \text{ cd m}^{-2}$ ,  $91 \text{ cd m}^{-2}$ , and  $360 \text{ cd m}^{-2}$ ) caused pupil dilatation.

**Light reflex** The effects of treatments on the amplitude and latency of the light reflex response are shown in Fig. 3. For *amplitude*, there was a significant effect of drug condition ( $F_{3,36} = 3.71$ ,  $p < 0.05$ ) and light intensity ( $F_{3,36} = 172.99$ ,  $p < 0.001$ ), but no interaction ( $F_{9,108} = 1.13$ , NS). Analysis of light reflex amplitude at each light intensity revealed a significant effect of drug condition at  $41 \text{ cd m}^{-2}$  ( $F_{3,42} = 4.07$ ,  $p < 0.05$ ), and a trend for significance at  $320 \text{ cd m}^{-2}$  ( $F_{3,42} = 2.42$ ,  $p = 0.08$ ), but not at  $5.2 \text{ cd m}^{-2}$  ( $F_{3,36} = 2.08$ , NS) or  $2050 \text{ cd m}^{-2}$  ( $F_{3,42} = 1.99$ , NS). Pramipexole reduced light

reflex amplitude at  $41 \text{ cd m}^{-2}$  and  $320 \text{ cd m}^{-2}$ . For *latency* there was a trend for an effect of drug condition ( $F_{3,36} = 2.24$ ,  $p = 0.1$ ) and a significant effect of light intensity ( $F_{3,36} = 30.72$ ,  $p < 0.001$ ) (Fig. 3), but again there was no interaction ( $F_{9,108} = 0.98$ , NS). Analysis of light reflex latency at each light intensity showed a trend for a difference at  $320 \text{ cd m}^{-2}$  ( $F_{3,42} = 2.69$ ,  $p = 0.058$ ), but no significant effects at  $5.2 \text{ cd m}^{-2}$  ( $F_{3,36} = 0.63$ , NS),  $41 \text{ cd m}^{-2}$  ( $F_{3,42} = 1.33$ , NS) or  $2050 \text{ cd m}^{-2}$  ( $F_{3,42} = 2.0$ , NS). The combination of pramipexole and modafinil caused an increase in latency ( $320 \text{ cd m}^{-2}$ ). For *75% recovery time* ( $\log_{10}$  transformation) there was a trend for an effect of drug condition ( $F_{3,12} = 2.69$ ,  $p = 0.093$ ), and a significant effect of light intensity ( $F_{3,12} = 8.4$ ,  $p < 0.01$ ), but no interaction ( $F_{9,36} = 0.84$ , NS). Analysis of 75% recovery time at each light intensity showed a trend for significance at  $5.2 \text{ cd m}^{-2}$  ( $F_{3,30} = 2.66$ ,  $p = 0.066$ ) and  $41 \text{ cd m}^{-2}$  ( $F_{3,30} = 2.66$ ,  $p = 0.066$ ), but no significant effects at  $320 \text{ cd m}^{-2}$  ( $F_{3,33} = 0.74$ , NS) or  $2050 \text{ cd m}^{-2}$  ( $F_{3,24} = 0.51$ , NS).



**Figure 2** Resting pupil diameter: relationship between level of luminance and pupil diameter pre- and post-treatment.

The four treatment conditions are indicated by different symbols (see inset). Ordinate: absolute pupil diameter (mm); abscissa: level of luminance ( $\text{cd m}^{-2}$ ). Each point corresponds to the mean obtained for the group ( $n = 16$ ). Vertical bars represent two standard errors of the difference (2SED) obtained from the interaction term of the analysis of variance. Pramipexole alone and in combination with modafinil increased pupil diameter. Pramipexole:  $+p < 0.05$ , pramipexole and modafinil:  $*p < 0.05$  (Dunnett's test: comparison with placebo condition).

**Table 1** Non-pupillary autonomic functions (mean  $\pm$  SEM)

	Pre-drug				Post-drug			
	placebo	pramipexole	modafinil	combination	placebo	pramipexole	modafinil	combination
<b>Heart rate (bpm)</b>								
supine	63.5 $\pm$ 2.71	58.25 $\pm$ 1.78	63 $\pm$ 2.24	59.29 $\pm$ 1.77	56.27 $\pm$ 2.02	57.06 $\pm$ 2.81	59.25 $\pm$ 2.71	57.2 $\pm$ 1.75
standing	76.06 $\pm$ 2.70	72.13 $\pm$ 2.63	75.06 $\pm$ 2.58	74.19 $\pm$ 2.70	63.63 $\pm$ 3.08	69.87 $\pm$ 3.21	65.87 $\pm$ 2.61	72.06 $\pm$ 2.84
orthostatic change	14 $\pm$ 2.61	13.86 $\pm$ 2.12	13.36 $\pm$ 2.29	15.71 $\pm$ 2.84	9.36 $\pm$ 2.98	13.82 $\pm$ 3.36	6.64 $\pm$ 1.76	16.27 $\pm$ 3.97
<b>Systolic BP (Hg mm)</b>								
supine	129.88 $\pm$ 2.65	126.13 $\pm$ 2.45	129 $\pm$ 2.83	128.13 $\pm$ 3.44	124.88 $\pm$ 3.36	126.56 $\pm$ 2.73	132.69 $\pm$ 3.05	129.81 $\pm$ 3.86
standing	127.25 $\pm$ 2.93	129.31 $\pm$ 3.15	128.38 $\pm$ 2.90	124.31 $\pm$ 3.47	127.38 $\pm$ 3.09	125.25 $\pm$ 3.32	136.87 $\pm$ 4.21	126.56 $\pm$ 4.18
orthostatic change	-2.13 $\pm$ 2.27	3.8 $\pm$ 3.21	-0.53 $\pm$ 3.43	-4.8 $\pm$ 2.69	2.53 $\pm$ 2.29	-0.6 $\pm$ 2.05	4.27 $\pm$ 3.37	-3.6 $\pm$ 1.78
<b>Diastolic BP (Hg mm)</b>								
supine	72.25 $\pm$ 1.86	69.69 $\pm$ 1.75	74.13 $\pm$ 2.82	71.4 $\pm$ 2.01	71.13 $\pm$ 2.64	71.63 $\pm$ 2.59	75.56 $\pm$ 1.80	73.5 $\pm$ 1.97
standing	76.25 $\pm$ 1.79	75.94 $\pm$ 1.83	75.69 $\pm$ 1.47	71 $\pm$ 2.79	76.75 $\pm$ 2.33	75.06 $\pm$ 2.20	82.6 $\pm$ 2.25	76.81 $\pm$ 3.06
orthostatic change	4.8 $\pm$ 1.97	6.6 $\pm$ 1.74	1.87 $\pm$ 2.80	-0.2 $\pm$ 2.64	5.93 $\pm$ 1.72	3.87 $\pm$ 1.83	7.2 $\pm$ 1.83	2.93 $\pm$ 1.97
<b>Salivation (g)</b>								
	0.98 $\pm$ 0.17	0.80 $\pm$ 0.19	0.86 $\pm$ 0.21	0.97 $\pm$ 0.25	0.95 $\pm$ 0.22	1.04 $\pm$ 0.23	0.94 $\pm$ 0.19	1.02 $\pm$ 0.21
<b>Temperature (<math>^{\circ}\text{C}</math>)</b>								
	36.37 $\pm$ 0.16	36.04 $\pm$ 0.19	36.04 $\pm$ 0.19	35.93 $\pm$ 0.16	36.3 $\pm$ 0.11	35.94 $\pm$ 0.10	36.27 $\pm$ 0.12	36.98 $\pm$ 0.13

**Darkness reflex** The effects of the treatments on the parameters of the darkness reflex response are shown in Fig. 3. There were no significant effects of drug condition on any of the darkness reflex parameters ( $p > 0.05$ ).

### Cardiovascular functions

The values obtained (mean  $\pm$  SEM) for heart rate and blood pressure both before and after treatment are shown in Table 1.

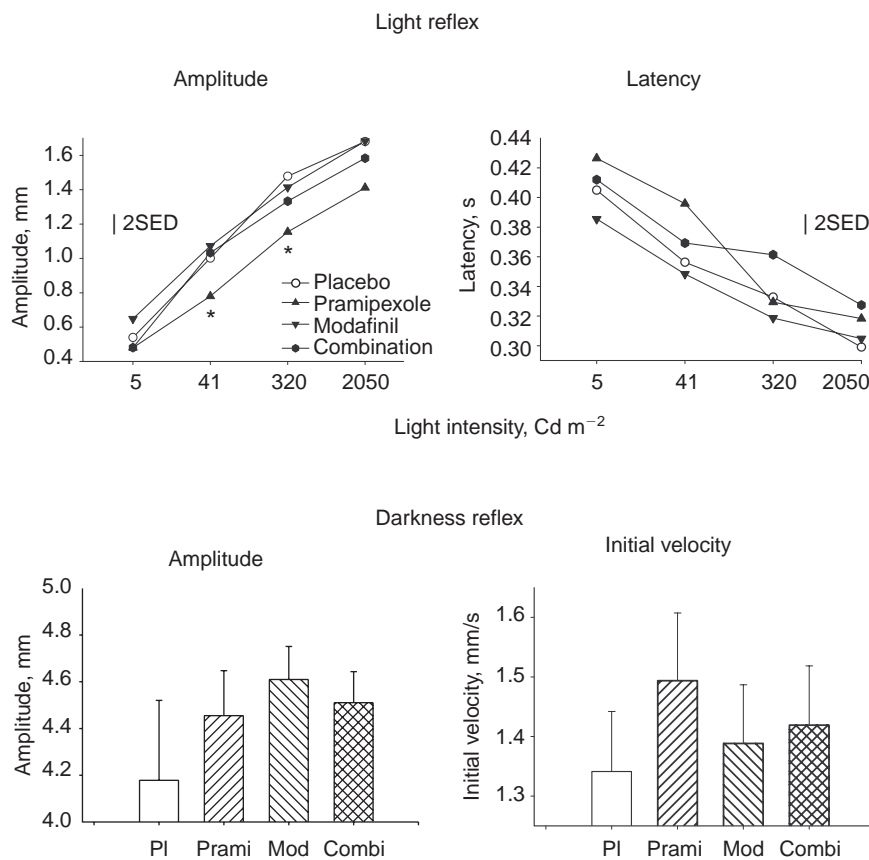
**Heart rate** Pre-treatment/post-treatment differences were used to analyse heart rate changes following drug administration in both supine and standing positions (Fig. 4). Standing values showed a significant effect of drug condition ( $F_{3,42} = 4.01$ ,  $p < 0.05$ ), where pramipexole and the combination of pramipexole

and modafinil both increased heart rate. No significant effect of drug condition was found in the supine position ( $F_{3,33} = 1.62$ ,  $p = 0.203$ ). There was no effect of treatment on the orthostatic change in heart rate from lying to standing position ( $F_{3,30} = 1.40$ , NS).

**Blood pressure** Pre-treatment/post-treatment differences were used to analyse systolic and diastolic blood pressure changes in both supine and standing positions (Fig. 4). Standing values showed a significant effect of drug condition on diastolic blood pressure ( $F_{3,39} = 3.89$ ,  $p < 0.05$ ), with modafinil increasing this measure, and a trend for an effect of drug condition on systolic blood pressure ( $F_{3,39} = 2.60$ ,  $p = 0.066$ ), with a trend for modafinil to increase this measure ( $p < 0.1$ ). Supine values showed a trend for an effect of drug condition on systolic blood pressure ( $F_{3,42} = 2.31$ ,  $p = 0.09$ ), where modafinil increased this measure.

**Figure 3** Pupillary reflex responses: light reflex (top) and darkness reflex (bottom).

**Light reflex:** relationship between light stimulus intensity and amplitude (top left) and latency (top right) of the response post-treatment. The four treatment conditions are indicated by different symbols (see inset). Each point corresponds to the mean obtained for the group ( $n = 16$ ). Ordinate (amplitude): maximal change in pupil diameter in response to the light stimulus (mm); ordinate (latency): time from the onset of the light stimulus to the onset of the pupil response (s); abscissa: light stimulus intensity ( $\text{cd m}^{-2}$ ). Pramipexole alone reduced light reflex amplitude and in combination with modafinil increased light reflex latency. Vertical bars represent two standard errors of the difference (2 SED) obtained from the interaction term of the analysis of variance. \* $p < 0.05$  (Dunnett's test: comparison with placebo condition). **Darkness reflex:** amplitude (bottom left) and initial velocity (bottom right) of the response post-treatment. Treatments are indicated at the bottom of the graphs (see Fig. 1). Columns correspond to mean changes in the parameters for the group ( $n = 16$ ). Ordinate (amplitude): maximal change in pupil diameter in response to removal of the light stimulus (mm); ordinate (initial velocity): time required to obtain 25% of the maximum response following the onset of the response ( $\text{mm s}^{-1}$ ). Vertical bars represent standard errors of the mean (SEM).



There was no significant effect of drug condition on supine diastolic blood pressure ( $F_{3,42} = 0.49$ , NS). There was no effect of treatment on the orthostatic change from lying to standing position in systolic ( $F_{3,39} = 1.03$ , NS) or diastolic ( $F_{3,39} = 2.23$ , NS) blood pressure.

### Salivation

The values obtained (mean  $\pm$  SEM) for salivation both before and after treatment are shown in Table 1.

Pre-treatment/post-treatment differences were used to analyse changes in salivation following the four drug conditions. No significant differences were found ( $F_{3,45} = 1.22$ , NS).

### Temperature

The values obtained (mean  $\pm$  SEM) for temperature both before and after treatment are shown in Table 1.

Pre-treatment/post-treatment differences (reciprocal transformation) were used to analyse changes in temperature following

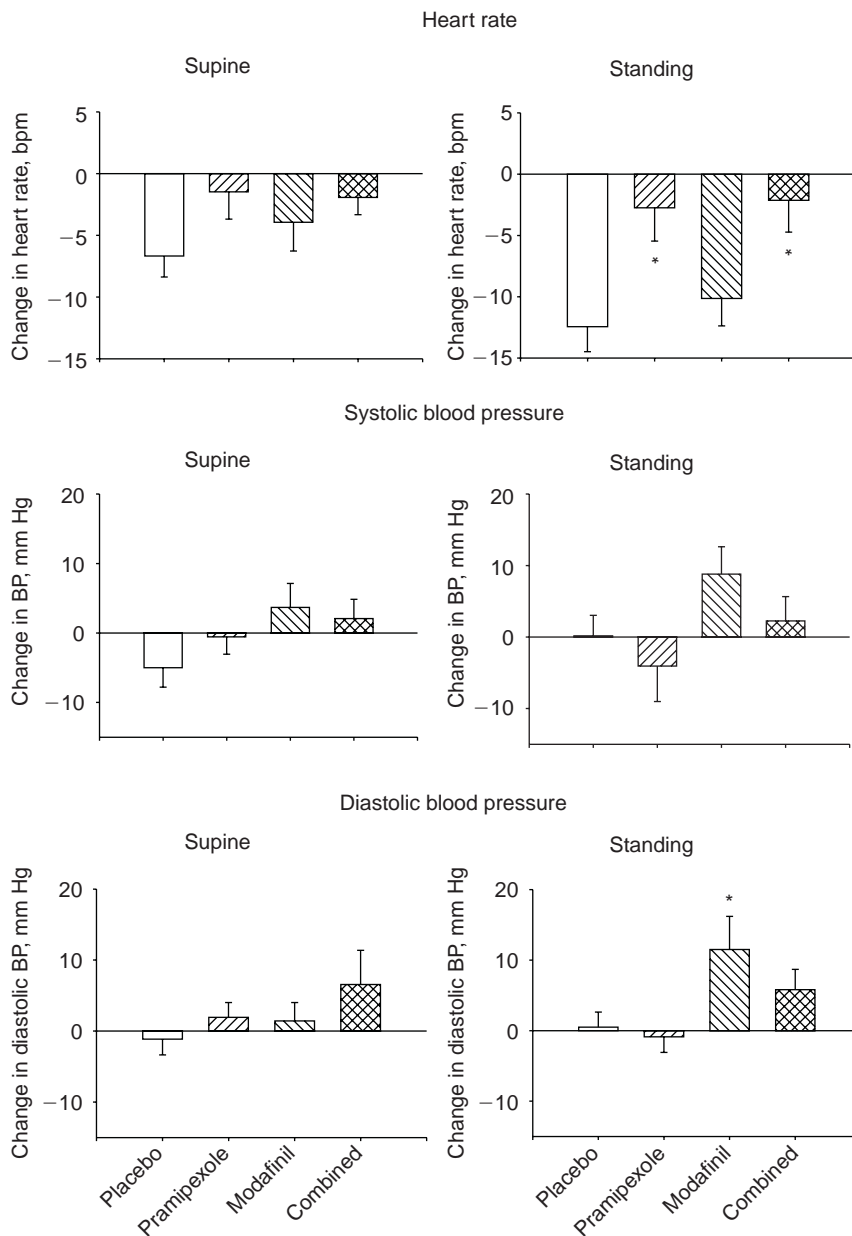
the four drug conditions. The ANOVA revealed a significant effect of drug condition on temperature ( $F_{3,21} = 4.02$ ,  $p < 0.05$ ), where modafinil increased temperature compared to placebo.

### Endocrine functions

The effect of drug treatments on endocrine functions is shown in Fig. 5.

**Prolactin** Post-treatment blood concentrations ( $\log_{10}$  transformation) of prolactin showed a significant effect of drug condition ( $F_{3,45} = 73.15$ ,  $p < 0.001$ ), where pramipexole, modafinil, and the combination of pramipexole and modafinil all reduced prolactin levels.

**Growth hormone** Post-treatment blood concentrations ( $\log_{10}$  transformation) of GH showed a significant effect of drug condition ( $F_{3,45} = 42.00$ ,  $p < 0.001$ ), where pramipexole and the combination of pramipexole and modafinil increased GH levels. There was a significant negative correlation between GH level in the



**Figure 4** Cardiovascular activity: pre-treatment/post-treatment differences in supine (left) and standing (right) heart rate (bpm; top), systolic blood pressure (mm Hg; middle) and diastolic blood pressure (mm Hg; bottom).

Columns correspond to mean changes in the parameters for the group ( $n = 16$ ). Treatment condition is indicated at the bottom of the graphs; the ordinate axis represents the change in the parameter from pre-treatment values. Vertical bars represent standard errors of the mean (SEM). \* $p < 0.05$  (Dunnett's test: comparison with placebo condition).

placebo condition (baseline) and the change from baseline following modafinil (Pearson's product moment correlation coefficient  $r = -0.77$ ).

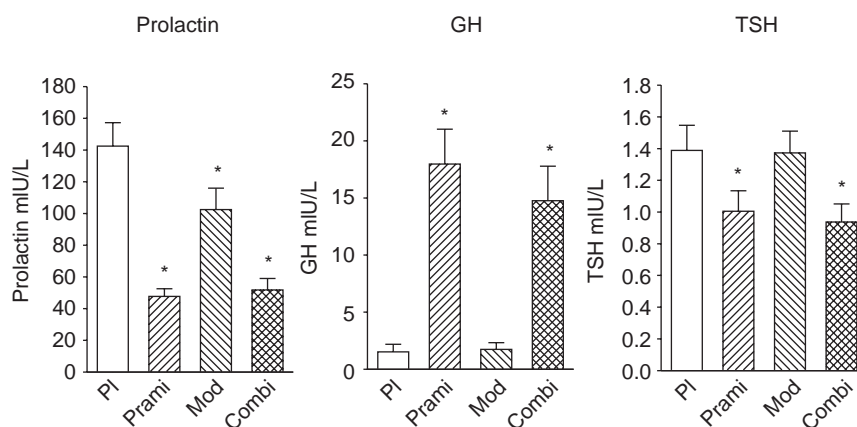
**Thyroid stimulating hormone** Post-treatment blood concentrations ( $\log_{10}$  transformation) of TSH showed a significant effect of drug condition ( $F_{3,45} = 10.15$ ,  $p < 0.001$ ), where pramipexole and the combination of pramipexole and modafinil reduced TSH levels.

### General behavioural effects of treatments

Most subjects reported feeling highly sedated following pramipexole, with frequent periods of sleeping occurring between pre-treatment testing and post-treatment testing. A number of these subjects reported sedation in only one of the four sessions (although this effect was not reflected in the laboratory measures of alertness, where both the VAS alertness factor and the total power of pupil diameter fluctuations in the PST show sedation in two sessions). Modafinil was generally well tolerated, however

**Figure 5** Endocrine functions: post-treatment blood concentrations of prolactin (left), GH (middle), and TSH (right).

Columns correspond to mean blood concentrations for the group ( $n = 16$ ). Treatment condition is indicated at the bottom of the graphs: pl = placebo, prami = pramipexole, mod = modafinil, combi = combination of pramipexole and modafinil. The ordinate axis represents the blood hormone concentration (mIU/L). Vertical bars represent standard errors of the mean (SEM). \* $p < 0.05$  (Dunnett's test: comparison with placebo condition).



pramipexole often resulted in nausea and dizziness and on five occasions in vomiting.

## Discussion

In this study we used single doses of the wakefulness-promoting drug modafinil and the anti-Parkinsonian drug pramipexole. The dosages used were selected on the basis of previous reports. Many investigators have used a single dose of 200 mg modafinil (Turner *et al.*, 2003; Randall *et al.*, 2003, 2004; Hou *et al.*, 2005), and the single dose of 0.5 mg pramipexole was within the range used in pharmacokinetic and endocrine reports with this drug in healthy volunteers (Schilling *et al.*, 1992; Wright *et al.*, 1997).

Single doses of 0.5 mg pramipexole showed robust sedative effects as evidenced by increases in PST measures and reductions in CFF and VAS alertness measures. This result is consistent with clinical observations of the sedative effects of pramipexole when used in the treatment of PD (Parkinson Study Group, 1997, 2000; Hauser *et al.*, 2000a; O'Suilleabhain and Dewey, 2002). The sedation induced by pramipexole has been attributed to reduction in the activity of dopaminergic neurones in the VTA brought about by the stimulation of inhibitory  $D_2$  autoreceptors on these neurones (Rye and Jankovic, 2002). The neural substrate of the sedative effect of pramipexole is likely to be the excitatory meso-coerulear pathway: the action of pramipexole at inhibitory  $D_2$  autoreceptors results in the withdrawal of the tonic dopaminergic stimulation of the LC, a major wakefulness-promoting nucleus (Keating and Rye, 2003; see also Fig. 6).

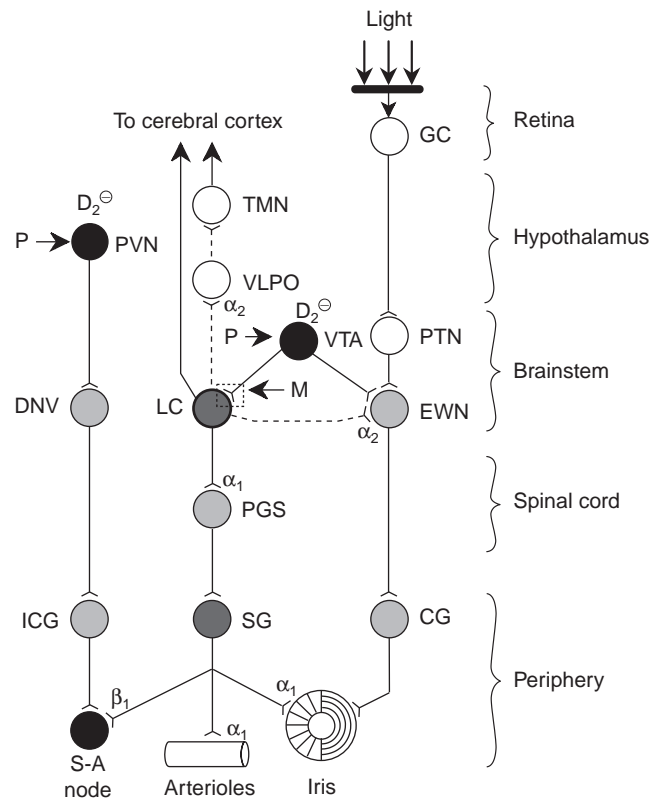
Single doses of 200 mg modafinil had little effect on measures of alertness. Modafinil has been found to have only minor effects on alertness in non-sleep-deprived individuals (Ellis *et al.*, 1999; Randall *et al.*, 2003, 2004; Hou *et al.*, 2005), and our results are consistent with this phenomenon. Although modafinil alone had no effect on alertness, it was expected that it would antagonize pramipexole-induced sedation since the alerting effect of modafinil is manifest when the level of alertness is reduced (Pigeau *et al.*, 1995; Stivalet *et al.*, 1998; Ellis *et al.*, 1999; Caldwell *et al.*, 2000; Szabadi *et al.*, 2002; Wesensten *et al.*, 2002).

Furthermore, in a clinical case study, modafinil was found to antagonize the sedative effect of pramipexole when used for the treatment of PD (Hauser *et al.*, 2000b). However, in the present experiment modafinil failed to antagonize the effects of pramipexole on measures of alertness in healthy volunteers. It is possible that the dose of modafinil used in this study was too low to supersede the robust sedative effect of pramipexole.

As pramipexole exerted prominent sedative effects, it was expected to cause miosis since there is a close association between the sedative and miotic effects of drugs (see pp. 786–788 in Loewenfeld, 1993). The miotic effect of sedative drugs is likely to be mediated by the locus coeruleus: a decrease in the level of arousal is associated with a decrease in LC activity, which would result in a reduction in the sympathetic outflow to the iris, and an increase in the parasympathetic output, due to the withdrawal of the inhibitory influence of the LC on the Edinger-Westphal nucleus (EWN) (Szabadi and Bradshaw, 1996; Phillips *et al.*, 2000a, 2000b; see also Fig. 6). Therefore, the observation of an increase in pupil diameter following pramipexole administration was an unexpected finding.

Analysis of the kinetics of the light reflex response (prolongation of latency and a reduction in amplitude) indicates that the pramipexole-induced mydriasis was due to a parasympatholytic (see pp. 726–727 in Loewenfeld, 1993), rather than a sympathomimetic effect on the pupil. As pramipexole has little affinity for muscarinic cholinergic receptors (Mierau, 1995; Millan *et al.*, 2002), it is unlikely that the parasympatholytic effect of the drug on the pupil was the result of the blockade of muscarinic cholinergic receptors in the iris. Indeed, in the present experiment pramipexole failed to modify salivary output, which is a very sensitive index of muscarinic cholinergic blockade (Szabadi and Tavernor, 1999). Therefore, the parasympatholytic effect of pramipexole on the pupil is likely to be due to a central mechanism.

The central parasympatholytic mechanism mediating the mydriatic effect of pramipexole is likely to involve the EWN, which is the preganglionic parasympathetic nucleus controlling pupil constriction. Dopamine receptors are also likely to be involved, since pramipexole's only pharmacological action is the stimulation of  $D_2/D_3$  dopamine receptors. Thus the question arises



**Figure 6** Catecholaminergic control of arousal and autonomic functions. The noradrenergic neurones in the locus coeruleus (LC) play a pivotal role in the regulation of both arousal and autonomic functions. The activity of LC neurones is modulated by dopaminergic neurones in the ventral tegmental area (VTA). Arousal mechanisms, apart from the LC and VTA, include the ventrolateral preoptic area (VLPO) and the tuberomammillary nucleus (TMN) of the hypothalamus. The LC and the TMN send major wakefulness-promoting excitatory outputs to the cerebral cortex. Sympathetic control includes the LC itself, preganglionic sympathetic neurones (PGS) of the spinal cord, and peripheral sympathetic ganglia (SG). Parasympathetic control includes preganglionic neurones in the dorsal nucleus of the vagus (DNV) and in the Edinger-Westphal nucleus (EWN), and postganglionic neurones in the intercardiac ganglia (ICG) and the ciliary ganglion (CG). The DNV is under a dopaminergic excitatory influence from the paraventricular nucleus (PVN) of the hypothalamus, and the EWN is an important relay station in the light reflex pathway. The light evoked response is relayed from the retinal ganglion cells (GC) via the pretectal nucleus (PTN) to the EWN. The peripheral targets of the autonomic outflow include the sino-atrial node (S-A node) of the heart, the arterioles of the vascular tree, and the dilator and constrictor muscles of the iris. The neurones making up the network are identified by their neurotransmitters: dopamine (black), noradrenaline (hatched), acetylcholine (stippled), and other transmitters (white). The other transmitters involved include GABA (VLPO), histamine (TMN), and presumably glutamate (GC and PTN). Excitatory connections = solid lines, inhibitory connections = broken lines. The adrenoceptors involved are  $\alpha_1$  and  $\beta_1$  (excitatory) and  $\alpha_2$  (inhibitory). The relevant dopamine receptors are D2: there are inhibitory D2 autoreceptors on neurones in the PVN and VTA, and excitatory postsynaptic receptors on the DNV, LC, and EWN neurones. Only the autoreceptors are indicated in the figure. *Pramipexole* (P) exerts its major effect by stimulating inhibitory D2 autoreceptors and thus 'switches off' the activity of dopaminergic PVN and VTA neurones. *Modafinil's* (M) presumed main action is the blockade of dopamine uptake at the mesocoerulear dopaminergic synapse, thereby activating the LC. See text for details. Modified from Szabadi and Bradshaw (1996) and Hou *et al.* (2005).

as to where these dopamine receptors may be located in relation to the EWN. Apart from a well documented dopaminergic output from the VTA to the LC (Swanson, 1982; Ornstein *et al.*, 1987), the VTA also sends projections to a number of other brainstem nuclei including neurones in the region of the peri-aqueductal grey matter of the midbrain (Swanson, 1982). Furthermore, cells expressing D<sub>2</sub> receptor mRNA have been identified in the peri-

aqueductal grey, the highest numbers being found in the ventral division (Mansour and Watson, 1995) where the oculomotor nuclear complex, incorporating the EWN, is situated. Therefore, the EWN may receive a dopaminergic input from the VTA, and we postulate that this input may exert a tonic excitatory influence on the EWN via postsynaptic D<sub>2</sub> receptors (see Fig. 6). Thus, the 'switching off' of the VTA neurones by pramipexole may lead to

the withdrawal of the tonic dopaminergic activation of the EWN, thereby leading to a central parasympatholytic effect on the pupil. It should be noted that this effect is expected to be attenuated by a reduction in LC activity, resulting from the 'switching off' of the tonic dopaminergic excitation of the LC by pramipexole which in turn would lead to a central parasympathomimetic effect on the pupil (Szabadi and Bradshaw, 1996). However, the direct effect of the 'switching off' of the VTA on the EWN is likely to supersede the indirect effect mediated via the LC since the observed change is an increase, rather than a decrease, in pupil diameter.

Any effect on pupil diameter mediated via the EWN is expected to be 'light-dependent', i.e. it should increase with increasing levels of ambient luminance as the contribution of the parasympathetic activity to pupil diameter increases (see Fig. 6). This effect of light has been demonstrated on the miotic effect of clonidine, a drug which indirectly increases EWN activity (Szabadi and Bradshaw, 1996). The present results show that not only a miotic effect due to increased activity of the EWN shows light dependence, but also a mydriatic effect due to a reduction in EWN activity: the pupil dilatation following pramipexole administration could be observed only at the two higher luminance levels, when the effect of the removal of the tonic excitation of the EWN was presumably the greatest.

Modafinil had no effect on pupil function in the present experiment. This is in contrast with a previous report from this laboratory which described marked effects of the same single dosage of modafinil on pupillary function, consistent with an increase in the sympathetic outflow to the iris (Hou *et al.*, 2005). There is no immediate explanation for the discrepancy between the results of the present study and those reported by Hou *et al.* (2005). As in both studies a relatively small number of subjects were used, it is possible that inter-individual variations in pharmacokinetics leading to different drug concentrations at relevant sites in the brain may have contributed to the different results.

Pramipexole increased heart rate in the standing position, consistent with previous reports (Schilling *et al.*, 1992; Wright *et al.*, 1997). This effect may be due to either an increase in sympathetically mediated cardio-acceleration or to a decrease in cardio-deceleration mediated by the parasympathetic vagus nerve. As the LC may contribute to sympathetically mediated cardio-acceleration via descending coeruleo-spinal neurones (Hancock and Fougereousse, 1976; Guyenet, 1980), the withdrawal of dopaminergic LC activation by pramipexole is expected to lead to a decrease rather than an increase in heart rate. However, the effect of pramipexole may have been mediated primarily via an action on dopaminergic neurones projecting from the paraventricular nucleus of the hypothalamus to the dorsal nucleus of the vagus (Swanson *et al.*, 1981), which may exert an activating influence on the vagus (See Fig. 6). As these hypothalamic dopaminergic neurones have been shown to be controlled by inhibitory D<sub>2</sub> autoreceptors (Moore and Lookingland, 1995), pramipexole, by stimulating these receptors, may have removed the dopaminergic facilitation of vagus activity leading to an increase in heart rate. In contrast to pramipexole, modafinil had no effect on heart rate, consistent with previous reports using the same single dose of

modafinil as in the present experiment (Rush *et al.*, 2002; Turner *et al.*, 2003; Makris *et al.*, 2004; Hou *et al.*, 2005).

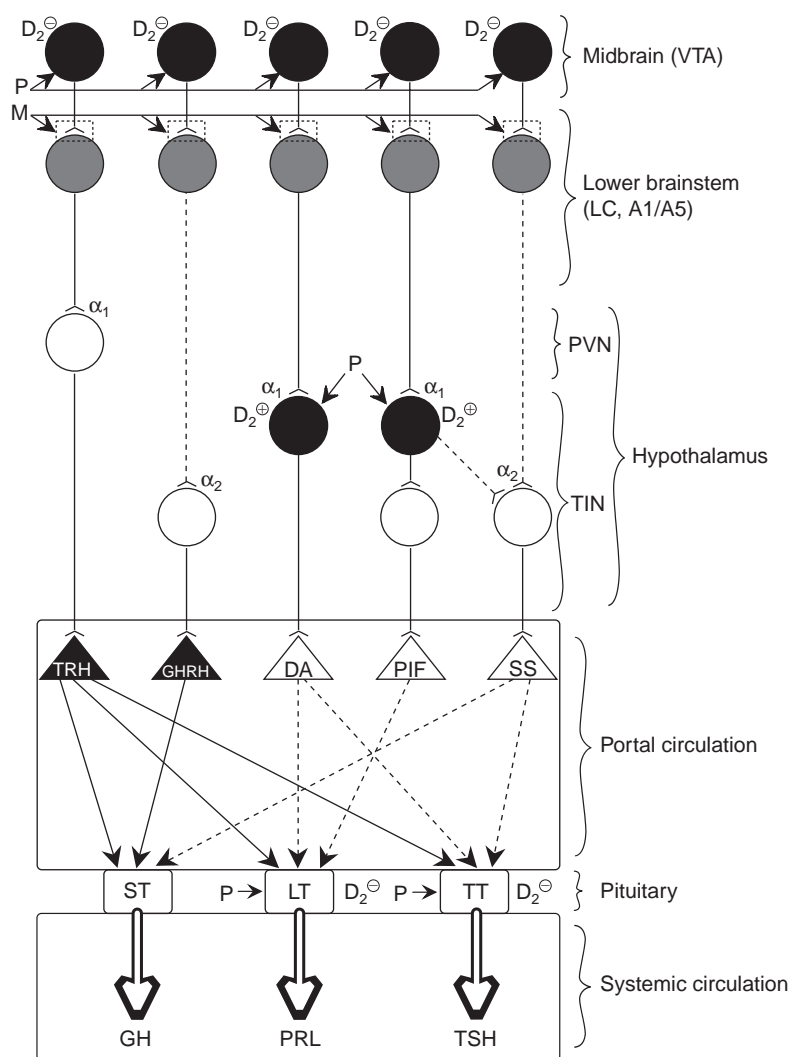
Pramipexole had no effect on either systolic or diastolic blood pressure in this study. This observation is in agreement with previous reports of the lack of effect of a range of doses of pramipexole, including the dose used in the present study, on blood pressure in healthy volunteers (Schilling *et al.*, 1992; Wright *et al.*, 1997). On the other hand, modafinil increased diastolic blood pressure in the standing position and there was a similar trend for systolic blood pressure. Previous reports on the effect of modafinil on blood pressure, using the same single dose as was used in the present experiment, vary between no effect (systolic blood pressure: Rush *et al.*, 2002; Makris *et al.*, 2004; Hou *et al.*, 2005; diastolic blood pressure: Turner *et al.*, 2003; Hou *et al.*, 2005; mean arterial pressure: Heitman *et al.*, 1999; Rush *et al.*, 2002) and small increases (systolic blood pressure: Turner *et al.*, 2003; diastolic blood pressure: Makris *et al.*, 2004). Nevertheless, higher single doses (400 mg and above) of modafinil have been reported to evoke consistent increases in both systolic and diastolic blood pressure (Caldwell *et al.*, 2000; Rush *et al.*, 2002; Makris *et al.*, 2004; Taneja *et al.*, 2005). The pressor effect of modafinil has been attributed to central sympathetic activation (Taneja *et al.*, 2005).

The central sympathetic activating effect of modafinil is also highlighted by an increase in body core temperature evoked by the drug in the present experiment. This observation is in agreement with a previous report (Brun *et al.*, 1998), and is opposite to the temperature lowering effect of the centrally acting sympatholytic drug clonidine (Arya *et al.*, 1997). In contrast to modafinil, pramipexole had no effect on core temperature.

Neither pramipexole nor modafinil affected salivary output. The lack of effect of modafinil on salivation is in agreement with an earlier report (Hou *et al.*, 2005).

We also examined the effects of pramipexole and modafinil on the blood concentration of three pituitary hormones whose secretion is either directly or indirectly, via peptidergic neurones (Reichlin, 1998), controlled by the dopaminergic and noradrenergic neuronal systems of the brain. The relevant dopaminergic neurones are situated in the infundibular nuclei (arcuate and periventricular nuclei) of the median eminence, whereas the noradrenergic neurones project to the peptidergic neurones of the median eminence from noradrenergic nuclei of the lower brainstem (mainly A1/A5 but also the LC) (see Fig. 7).

The secretion of prolactin by the lactotropes of the pituitary gland is largely controlled by dopaminergic neurones which secrete dopamine either directly into the portal circulation or synapse with peptidergic neurones which secrete prolactin release-inhibiting factor (PIF). Both dopamine and PIF reduce the secretion of prolactin. In the present experiment, pramipexole, like other D<sub>2</sub> dopamine receptor agonists (Svet-Moldovsky *et al.*, 1981; Ben-Jonathan and Hnasko, 2001), decreased the concentration of prolactin in the blood in agreement with a previous report (Schilling *et al.*, 1992). Modafinil also caused a reduction in the blood level of prolactin, albeit this was less than that evoked by pramipexole. This effect of modafinil, according to our knowledge, has not been reported previously. It is an intriguing



**Figure 7** Simplified diagram to indicate the principle pathways of the catecholaminergic control of the secretion of the pituitary hormones growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone (TSH). The four compartments involved are: brain (VTA = ventral tegmental area; LC = locus coeruleus; PVN = paraventricular nucleus; TIN = tuberoinfundibular nuclei, including arcuate nucleus and periventricular nucleus), portal circulation (TRH = thyrotropin releasing hormone; GHRH = growth hormone releasing hormone; DA = dopamine; PIF = prolactin inhibiting factor; SS = somatostatin), pituitary gland (ST = somatotropes; LT = lactotropes; TT = thyrotropes), and systemic circulation. The neurones involved are dopaminergic neurones (black circles), noradrenergic neurones (hatched circles), and peptidergic neurones (white circles). Excitatory connections are indicated by continuous lines and inhibitory connections by broken lines. Catecholamine receptors involved are:  $D_2$  dopamine receptor (excitatory or inhibitory, as indicated),  $\alpha_1$ -adrenoceptor (excitatory) and  $\alpha_2$ -adrenoceptor (inhibitory). The hypothalamus controls the secretion of pituitary hormones via hypophysiotropic hormones, which include dopamine and a number of peptides. These hormones are secreted into the portal circulation (stimulatory hormones = black triangles; inhibitory hormones = white triangles). Each hypophysiotropic hormone may regulate the secretion of more than one pituitary hormone by acting at somatotropes, lactotropes, and thyrotropes in the anterior pituitary. There are inhibitory  $D_2$  dopamine receptors on the lactotropes and thyrotropes. *Pramipexole* (P) acts at inhibitory  $D_2$  autoreceptors on dopaminergic neurones in the VTA, on stimulatory  $D_2$  receptors on dopaminergic neurones in the hypothalamus, and on inhibitory  $D_2$  receptors on lactotropes and thyrotropes in the pituitary. *Modafinil* (M) acts at the dopaminergic synapse on noradrenergic neurones by blocking the re-uptake of dopamine and thereby enhancing the dopaminergic excitation of these neurones. Based on Reichlin (1998).

possibility that this effect of modafinil may be due to the activation of central noradrenergic neurones which project to the arcuate nucleus (O'Donohue *et al.*, 1979) where they activate excitatory  $\alpha$ 1-adrenoceptors (Kang *et al.*, 2000) (see Fig. 7). The co-administration of modafinil with pramipexole evoked a reduction in prolactin level which was comparable to that produced by pramipexole alone, suggesting that the reduction evoked by pramipexole was maximal and could not be enhanced any further.

Growth hormone (GH) secretion is under the dual control of growth hormone releasing hormone (GHRH), which stimulates GH release, and somatostatin (SS), which inhibits GH release (Reichlin, 1998). Both dopaminergic and noradrenergic neurones modulate GH secretion by interacting with the GHRH and SS neurones (see Fig. 7). We have found that pramipexole, like other  $D_2$  dopamine receptor agonists (Lal *et al.*, 1975; Müller *et al.*, 1991; for review see Müller *et al.*, 1999), increased GH secretion in agreement with a previous report (pramipexole: Schilling *et al.*, 1992). It is believed that the GH release-promoting effect of the dopaminomimetic drugs is due primarily to a reduction in the release of SS (Vance *et al.*, 1987; Giustina and Veldhuis, 1998). In the present experiment, modafinil had no effect on GH level, as assessed by the group mean, in agreement with a previous report by Brun *et al.* (1998). It should be noted, however, that the GH levels in the placebo condition were extremely low ( $<0.5$  mIU/L), in fact near the limit of detection of the assay, in nine subjects, and therefore a possible reduction in GH level induced by modafinil could be detected only in the remainder of the group. When the relationship between GH level and the response to modafinil was examined in individual subjects, a significant negative correlation was found between baseline GH level and the response to modafinil, suggesting a possible inhibitory action of modafinil on GH output. The inhibitory effect of modafinil on GH secretion is further illustrated by the apparent attenuation of the pramipexole-evoked increase in GH secretion by modafinil (see Fig. 5). The inhibition of GH secretion by modafinil is likely to have been mediated via GHRH neurones: modafinil, by stimulating central noradrenergic neurones, may have increased the noradrenergic inhibition of GHRH neurones (see Fig. 7).

The secretion of thyroid-stimulating hormone (TSH; thyrotropin) is largely controlled by thyrotropin-releasing hormone (TRH) secreted by peptidergic neurones (Mason *et al.*, 1995). In addition to the stimulating effect of TRH, TSH secretion is inhibited by SS and dopamine released by neurones in the tuberoinfundibular area (Reichlin, 1998). Pramipexole reduced the level of TSH in agreement with a previous report (Schilling *et al.*, 1992). Pramipexole could have enhanced the release of dopamine into the portal circulation and stimulated inhibitory  $D_2$  dopamine receptors on the thyrotropes themselves (See Fig. 7). Modafinil had no effect on TSH secretion.

In conclusion, the effects of pramipexole on arousal, autonomic, and endocrine functions are consistent with the stimulatory effect of this drug on  $D_2$  autoreceptors in the midbrain and hypothalamus. Modafinil failed to modify the level of arousal in the present study but evoked cardiovascular, hyperthermic, and neuroendocrine effects consistent with the activation of the central noradrenergic system. The pupillary effect of pramipexole can

also be related to  $D_2$  dopamine autoreceptor activation on VTA neurones, leading to the postulate of a dopaminergic neural circuit involved in the regulation of pupil function. Furthermore, the pupillary effect of pramipexole provides an example of the dissociation between sedation and miosis.

## Acknowledgements

E. R. Samuels is a Scholar of the Institute of Neuroscience, University of Nottingham.

## References

- Abduljawad K A J, Langley R W, Bradshaw C M, Szabadi E (1997) Effects of clonidine and diazepam on the acoustic startle response and on its inhibition by 'prepulses' in man. *J Psychopharmacol* 11: 29–34
- Adler C H, Caviness J N, Hentz J G, Lind M, Tiede J (2003) Randomized trial of modafinil for treating subjective daytime sleepiness in patients with Parkinson's disease. *Mov Disord* 18: 287–293
- Arya D K, Langley R W, Szabadi E, Bradshaw C M (1997) Comparison of the effects of high ambient temperature and clonidine on autonomic functions in man. *Naunyn-Schmiedeberg's Arch Pharmacol* 355: 376–383
- Bagetta G, De Sarro G, Priolo E, Nistico G (1988) Ventral tegmental area: site through which dopamine  $D_2$ -receptor agonists evoke behavioural and electrocortical sleep in rats. *Br J Pharmacol* 95: 860–866
- Ben-Jonathan N, Hnasko R (2001) Dopamine as a prolactin (PRL) inhibitor. *Endocr Rev* 22: 724–763
- Bond A J, Lader M H (1974) The use of analogue scales in rating subjective feelings. *Br J Med Psychol* 47: 211–218
- Brun J, Chamba G, Khalfallah Y, Girard P, Boissy I, Bastuji H, Sassolas G, Claustrat B (1998) Effect of modafinil on plasma melatonin, cortisol and growth hormone rhythms, rectal temperature and performance in healthy subjects during a 36 h sleep deprivation. *J Sleep Res* 7: 105–114
- Caldwell J A Jr, Caldwell J L, Smythe N K, Hall K K (2000) A double-blind, placebo-controlled investigation of the efficacy of modafinil for sustaining the alertness and performance of aviators: a helicopter simulator study. *Psychopharmacology (Berl.)* 150: 272–282
- DeBattista C, Doghramji K, Menza M A, Rosenthal M H, Fieve R R, Modafinil in Depression Study Group (2003) Adjunct modafinil for the short-term treatment of fatigue and sleepiness in patients with major depressive disorder: a preliminary double-blind, placebo-controlled study. *J Clin Psychiatry* 64: 1057–1064
- Ellis C M, Monk C, Simmons A, Lemmens G, Williams S C R, Brammer M, Bullmore E, Parkes J D (1999) Functional magnetic resonance imaging neuroactivation studies in normal subjects and subjects with the narcoleptic syndrome. *Actions of modafinil. J Sleep Res* 8: 85–93
- Ferreira J J, Galitzky M, Thalamus C, Tiberge M, Montastruc J L, Sampaio C, Rascol O (2002) Effect of ropinirole on sleep onset: a randomised, placebo-controlled study in healthy volunteers. *Neurology* 58: 460–462
- Guistina A, Veldhuis J D (1998) Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocr Rev* 19: 717–797
- Guyenet P G (1980) The coeruleospinal noradrenergic neurons: anatomical and electrophysiological studies in the rat. *Brain Res* 189: 121–133
- Hancock M B, Fougereousse C L (1976) Spinal projections from the nucleus locus coeruleus and nucleus subcoeruleus in the cat and monkey as demonstrated by the retrograde transport of horseradish peroxidase. *Brain Res Bull* 1: 229–234
- Hauser R A, Gauger L, McDowell Anderson W, Zesiewicz T A (2000a) Pramipexole-induced somnolence and episodes of daytime sleep. *Mov Disord* 15: 658–663

- Hauser R A, Wahba M N, Zesiewicz T A, McDowell Anderson W (2000b) Modafinil treatment of pramipexole associated somnolence. *Mov Disord* 15: 1269–1271
- Heitmann J, Cassel W, Grote L, Bickel U, Hartlaub U, Penzel T, Peter J H (1999) Does short-term treatment with modafinil affect blood pressure in patients with obstructive sleep apnea? *Clin Pharmacol Ther* 65: 328–335
- Hou R H, Freeman C, Langley R W, Szabadi E, Bradshaw C M (2005) Does modafinil activate the locus coeruleus in man? Comparison of modafinil and clonidine on arousal and autonomic functions in human volunteers. *Psychopharmacology (Berl)*, 181: 537–549
- Howell D C (2002) *Statistical Methods for Psychology*, 5th edn. Duxbury, Pacific Grove, CA, p. 340
- Ivanenko A, Tauman R, Gozal D (2003) Modafinil in the treatment of excessive daytime sleepiness in children. *Sleep Med* 4: 579–582
- Kang Y-M, Ouyang W, Chen J-Y, Qiao J-T, Dafny N (2000) Norepinephrine modulates single hypothalamic arcuate neurons via  $\alpha_1$  and  $\beta$  adrenergic receptors. *Brain Res* 869: 146–157
- Keating G L, Rye D B (2003) Where you least expect it: dopamine in the pons and modulation of sleep and REM-sleep. *Sleep* 26: 788–789
- Lal S, Nair N P, Iskandar H L, Etienne P, Wood P L, Schwartz G, Guyda H (1975) Effect of domperidone on apomorphine-induced growth hormone secretion in normal men. *J Neural Transm* 54: 75–84
- Loewenfeld I E (1993) *The Pupil: Anatomy, Physiology, and Clinical Applications*. Wayne State University Press, Detroit, MI
- Lowenstein O, Feinbeig R, Lowenfeld I E (1963) Pupillary movements during acute and chronic fatigue. *Invest Ophthalmol* 2: 138–157
- Lüdtke H, Wilhelm B, Adler M, Schaeffel F, Wilhelm H (1998) Mathematical procedures in data recording and processing of pupillary fatigue waves. *Vision Res* 38: 2889–2896
- MacDonald J R, Hill J D, Tarnopolsky M A (2002) Modafinil reduces excessive somnolence and enhances mood in patients with myotonic dystrophy. *Neurology* 59: 1876–1880
- Maeda T, Kitahama K, Geffard M (1994) Dopaminergic innervation of rat locus coeruleus: a light and electron microscope immunohistochemical study. *Microsc Res Tech* 29: 211–218
- Makris A P, Rush C R, Frederich R C, Kelly T H (2004) Wake-promoting agents with different mechanisms of action: comparison of effects of modafinil and amphetamine on food intake and cardiovascular activity. *Appetite* 42: 185–195
- Mansour A, Watson S J Jr (1995) Dopamine receptor expression in the central nervous system. In Bloom F E, Kupfer D J (eds), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp. 207–219
- Mason G A, Garbutt J C, Prange A J Jr (1995) Thyrotropin-releasing hormone: focus on basic neurobiology. In Bloom F E, Kupfer D J (eds), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp. 493–504
- Mierau J (1995) Pramipexole: a dopamine-receptor agonist for treatment of Parkinson's disease. *Clin Neuropharmacol* 18 (Suppl. 1): S195–S206
- Millan M J, Maioufiss L, Cussac D, Audinot V, Boutin J-A, Newman-Tancredi A (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. *J Pharmacol Exp Ther* 303: 791–804
- Moore K E, Lookingland K J (1995) Dopaminergic neuronal systems in the hypothalamus. In Bloom F E, Kupfer D J (eds), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp. 245–256
- Müller E E, Locatelli V, Cocchi D (1999) Neuroendocrine control of growth hormone secretion. *Physiol Rev* 79: 511–607
- Müller E E, Locatelli V, Ghigo E, Cella S G, Loche S, Pintor C, Camanni F (1991) Involvement of brain catecholamines and acetylcholine in growth hormone deficiency states: pathophysiological, diagnostic and therapeutic implications. *Drugs* 41: 161–177
- Nieves A V, Lang A E (2002) Treatment of excessive daytime sleepiness in patients with Parkinson's disease with modafinil. *Clinical Neuropharmacology* 25: 111–114
- Norris H (1971) The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology* 10: 181–189
- O'Donohue T L, Crowley W R, Jacobowitz D M (1979) Biochemical mapping of the noradrenergic ventral bundle projection sites: evidence for a noradrenergic-dopaminergic interaction. *Brain Res* 172: 87–100
- O'Suilleabhain P E, Dewey R B (2002) Contributions of dopaminergic drugs and disease severity to daytime sleepiness in Parkinson's disease. *Arch Neurol* 59: 986–989
- Ornstein K, Milon H, McRae-Degueurce A, Alvarez C, Berger B, Würzner H P (1987) Biochemical and radioautographic evidence for dopaminergic afferents of the locus coeruleus originating in the ventral tegmental area. *J Neural Transm* 70: 183–191
- Pack A I, Black J E, Schwartz J R L, Matheson J K (2001) Modafinil as adjunct therapy for daytime sleepiness in obstructive sleep apnea. *Am J Respir Crit Care Med* 164: 1675–1681
- Parkinson Study Group (1997) Safety and efficacy of pramipexole in early Parkinson disease: a randomized dose-ranging study. *JAMA* 278: 125–130
- Parkinson Study Group (2000) Pramipexole vs. levodopa as initial treatment for Parkinson disease: a randomized controlled trial. *JAMA* 284: 1931–1938
- Paus S, Brecht H M, Koster J, Seeger G, Klockgether T, Wullner U (2003) Sleep attacks, daytime sleepiness, and dopamine agonists in Parkinson's disease. *Mov Disord* 18: 659–667
- Peck R E (1959) The SHP test – an aid in the detection and measurement of depression. *Arch Gen Psychiatry* 1: 35–40
- Phillips M A, Szabadi E, Bradshaw C M (2000a) Comparison of the effects of clonidine and yohimbine on spontaneous pupillary fluctuations in healthy human volunteers. *Psychopharmacology* 150: 85–89
- Phillips M A, Bitsios P, Szabadi E, Bradshaw C M (2000b) Comparison of the antidepressants reboxetine, fluvoxamine and amitriptyline upon spontaneous pupillary fluctuations in healthy human volunteers. *Psychopharmacology* 149: 72–76
- Piercey M F (1998) Pharmacology of pramipexole, a dopamine D3-preferring agonist useful in treating Parkinson's disease. *Clin Neuropharmacol* 21: 141–151
- Pigeau R, Naitoh P, Buguet A, McCann C, Baranski J, Taylor M, Thompson M, Mack I I (1995) Modafinil, d-amphetamine and placebo during 64 hours of sustained mental work. I. Effects on mood, fatigue, cognitive performance and body temperature. *J Sleep Res* 4: 212–228
- Rammohan K W, Rosenberg J H, Lynn D J, Blumenfeld A M, Pollak C P, Nagaraja H N (2002) Efficacy and safety of modafinil (Provigil) for the treatment of fatigue in multiple sclerosis: a two centre phase 2 study. *J Neurol Neurosurg Psychiatry* 72: 179–183
- Randall D C, Fleck N L, Shneerson J M, File S E (2004) The cognitive-enhancing properties of modafinil are limited in non-sleep-deprived middle-aged volunteers. *Pharmacol Biochem Behav* 77: 547–555
- Randall D C, Shneerson J M, Plaha K K, File S E (2003) Modafinil affects mood, but not cognitive function, in healthy young volunteers. *Hum Psychopharmacol* 18: 163–173
- Reichlin S (1998) Neuroendocrinology. In Wilson J D, Foster D W, Kronenberg H M, Larsen P R (eds), *Williams Textbook of Endocrinology*. WB Saunders Company, Philadelphia, pp. 165–248
- Reichmann H, Brecht M H, Köster J, Kraus P H, Lemke M R (2003)

- Pramipexole in routine clinical practice: a prospective observational trial in Parkinson's disease. *CNS Drugs* 17: 965–973
- Robertson P, Hellriegel E T (2003) Clinical pharmacokinetic profile of modafinil. *Clin Pharmacokinet* 42: 123–137
- Rosenthal M H, Bryant S L (2004) Benefits of adjunct modafinil in an open-label, pilot study in patients with schizophrenia. *Clin Neuropharmacol* 27: 38–43
- Rush C R, Kelly T H, Hays L R, Baker R W, Wooten A F (2002) Acute behavioural and physiological effects of modafinil in drug abusers. *Behav Pharmacol* 13: 105–115
- Rye D B, Jankovic J (2002) Emerging views of dopamine in modulating sleep/wake state from an unlikely source: PD. *Neurology* 58: 341–346
- Saper C B, Scammell T E (2004) Modafinil: a drug in search of a mechanism. *Sleep* 27: 11–12
- Schilling J C, Adamus W S, Palluk R (1992) Neuroendocrine and side effect profile of pramipexole, a new dopamine receptor agonist, in humans. *Clin Pharmacol Ther* 51: 541–548
- Smith J M, Misiak H (1976) Critical flicker frequency (CFF) and psychotropic drugs in normal human subjects – a review. *Psychopharmacology* 47: 175–182
- Stivalet P, Esquivie D, Barraud P A, Leiffren D, Raphel C (1998) Effects of modafinil on attentional processes during 60 hours of sleep deprivation. *Hum Psychopharmacol* 13: 501–507
- Svet-Moldovsky I, Svet-Moldovsky G, Zinzar S, Holland J, Vergara C, Arlin Z, Koziner B, Clarkson B (1981) The extracerebral dopamine antagonist domperidone block the suppressive effect of bromocriptine on prolactin and TSH secretion in man. *Biomedicine* 35: 142–144
- Swanson L W (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull* 9: 321–353
- Swanson L W, Sawchenko P E, Béroed A, Hartman B K, Helle K B, Vanorden D E (1981) An immunohistochemical study of the organisation of catecholaminergic cells and terminal fields in the paraventricular and supra optic nuclei of the hypothalamus. *J Comp Neurol* 196: 271–285
- Szabadi E, Bradshaw C M (1996) Autonomic pharmacology of  $\alpha$ 2-adrenoceptors. *Journal of Psychopharmacology* 10 (Suppl. 3): 6–18
- Szabadi E, Tavernor S (1999) Hypo- and hypersalivation induced by psychoactive drugs. *CNS Drugs* 11: 449–466
- Szabadi E, Langley R W, Bradshaw C M (2002) Comparison of single doses of noradrenergic drugs on pupillary fatigue waves in a patient with excessive daytime sleepiness. *J Sleep Res* 11 (Suppl. 1): 220
- Tan E K (2003) Piribedil-induced sleep attacks in Parkinson's disease. *Fundam Clin Pharmacol* 17: 117–119
- Taneja I, Diedrich A, Black B K, Byrne D W, Paranjape S Y, Robertson D (2005) Modafinil elicits sympathomedullary activation. *Hypertension* 45: 612–618
- Turner D C, Robbins T W, Clark L, Aron A R, Dowson J, Sahakian B J (2003) Cognitive enhancing effects of modafinil in healthy volunteers. *Psychopharmacol* 165: 260–269
- Ulivelli M, Rossi S, Lombardi C, Bartalini S, Rocchi R, Giannini F, Passero S, Battistini N, Lugaesi E (2002) Polysomnographic characterization of pergolide-induced sleep attacks in idiopathic PD. *Neurology* 58: 462–465
- US Modafinil in Narcolepsy Multicentre Study Group (1998) Randomized trial of modafinil for the treatment of pathological somnolence in narcolepsy. *Ann Neurol* 43: 88–97
- US Modafinil in Narcolepsy Multicentre Study Group (2000) Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy. *Neurology* 54: 1166–1175
- Vance M L, Kaiser D L, Frohman L A, Rivier J, Vale W W, Thorne M O (1987) Role of dopamine in the regulation of growth hormone secretion: dopamine and bromocriptine augment growth hormone (GH)-releasing hormone – stimulated GH secretion in normal man. *J Clin Endocrinol Metab* 64: 1136–1141
- Walsh J K, Randazzo A C, Stone K L, Schweitzer P K (2004) Modafinil improves alertness, vigilance, and executive function during simulated night shifts. *Sleep* 27: 434–439
- Webster L, Andrews M, Stoddard G (2003) Modafinil treatment of opioid-induced sedation. *Pain Med* 4: 135–140
- Wesensten N J, Belenky G, Kautz M A, Thorne D R, Reichardt R M, Balkin T J (2002) Maintaining alertness and performance during sleep deprivation: modafinil versus caffeine. *Psychopharmacol* 159: 238–247
- Wilhelm B, Giedke H, Lütke H, Bittner E, Hofmann A, Wilhelm H (2001) Daytime variations in central nervous system activation measured by a pupillographic sleepiness test. *J Sleep Res* 10: 1–7
- Wisor J P, Nishino S, Sora I, Uhl G H, Mignot E, Edgar D M (2001) Dopaminergic role in stimulant-induced wakefulness. *Journal of Neuroscience* 21: 1787–1794
- Wright C E, Lasher Sisson T, Ichhpurani A K, Peters G R (1997) Steady-state pharmacokinetic properties of pramipexole in healthy volunteers. *J Clin Pharmacol* 37: 520–525
- Yoss R E, Moyer N J, Hollenurst R W (1970) Pupil size and spontaneous pupillary waves associated with alertness, drowsiness, and sleep. *Neurology* 20: 545–554