Psychopharmacology (2008) 196:167–175
DOI 10.1007/s00213-007-0949-y

ORIGINAL INVESTIGATION

The 5-min pupillary alertness test is sensitive to modafinil: a placebo controlled study in patients with sleep apnea

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Received: 24 May 2007 / Accepted: 10 September 2007 / Published online: 25 September 2007

Abstract
Rationale The extent of pupillary miosis during 5 min in darkness is a simple, recently introduced alertness test which may become useful in the clinical assessment of normal and pathological sleepiness.
Objectives In this study, we further validated this test by testing its sensitivity to the effects of modafinil, a non-stimulant, alertness-promoting drug.
Methods Twelve unmedicated patients recently diagnosed with obstructive sleep apnea (OSA) after polysomnography, received placebo or modafinil (200 mg), according to a double-blind, cross-over design. The patients' resting pupil diameter (RPD) was sampled over 5 min in darkness before (10:00 A.M.) and after treatment (2:00 P.M.), and their light reflexes were elicited and recorded in darkness with an infrared video pupillometer.
Results We found a circadian miosis at 2:00 P.M. in the placebo treatment condition, which was reversed by modafinil. This effect correlated with modafinil-induced increase in subjective alertness, and it was greater in the most severely affected patients in terms of lowest oxygen saturation, independently of body mass index, age, or apneic episodes during sleep. Modafinil reduced the light reflex amplitude, suggesting an increase in the inhibitory input at the pupilloconstrictor Edinger-Westphal nucleus.
Conclusions These effects of modafinil are best explained via an activation of the hypoxia-sensitive nucleus locus coeruleus. The 5-min pupillary alertness test has promising predictive validity, and it holds promise as a fast and sensitive method for the objective assessment of excessive daytime sleepiness, monitoring of disease progression, and response to treatment.

Keywords Pupil · Arousal · Modafinil · Locus coeruleus · Excessive daytime sleepiness · Obstructive sleep apnea

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Introduction

It has long been known that the pupils of healthy, well-rested subjects eventually begin to decrease in size when the subjects remain unstimulated in a dark and quiet environment, and this phenomenon is thought to reflect the gradual reduction in the central arousal state of the brain (Lowenstein et al. 1963; Lowenstein and Loewenfeld 1964). The functions of the pupil are intimately linked to the regulation of arousal via activity in the nucleus locus coeruleus (LC), a structure implicated in the regulation of attention, arousal (Berridge and Waterhouse 2003), maintenance of wakefulness (Nelson et al. 2002, 2003), and pupillary control (Koss et al. 1984; Koss 1986; Szabadi and Bradshaw 1996). Recent theoretical models emphasize the importance of arousal in the expression of sleepiness (Johns 1998) and predict lower arousal levels in patients with excessive daytime sleepiness (EDS).

We have recently launched the 5-min Pupillary Alertness Test (5-min PAT), which is based on pupillary physiology, to test this prediction in patients with obstructive sleep apnea (OSA) who suffer from EDS. The test yields the resting pupil diameter (RPD) averaged over 5 min of recording in

darkness, as an immediate quantitative measure of arousal. We found that the patients had lower tonic RPD levels compared to controls and that RPD correlated inversely with objective polysomnographic (PSG) indices of OSA severity and subjective sleepiness within the patient group (Bitsios et al. 2006). In light of the failure of the Multiple Sleep Latency Test (MSLT) to correlate with subjective measures of sleepiness (Benbadis et al. 1999; Johns 2000) or with the frequency of respiratory disturbances during sleep (Chervin et al. 1995), its high cost and intrusiveness, and low repeatability, the 5-min PAT emerges as a promising, fast, and highly repeatable alternative. OSA has a high prevalence and cost to society (Hillman et al. 2006), and if the 5-min PAT is to become a useful clinical tool for the objective assessment of EDS, then patients with OSA will be the most frequent candidates for undergoing the test. An important issue is, whether this test is sensitive to the effects of treatments currently used to restore alertness in patients with excessive daytime sleepiness (EDS).

Modafinil is a new well-tolerated, non-stimulant, alertness-provoking drug licensed for the treatment of narcolepsy (Moldofsky et al. 2000), but it may have a place in the treatment of EDS regardless of its etiology, as chronic modafinil treatment has also been shown to improve EDS and fatigue associated with several CNS and sleep disorders including residual EDS in treatment-resistant OSA patients (Pack et al. 2001). A single dose of modafinil has been previously reported to increase alertness (Ellis et al. 1999) and reduce pupillary oscillations in patients with EDS (Szabadi et al. 2002) and healthy alert individuals (Hou et al. 2005, 2007). This study was designed to test the predictive validity of the 5-min PAT, by exploring whether it would be sensitive to the alerting effects of a single modafinil dose. The predictions were that (a) the alertness-provoking drug modafinil would increase the patients' RPD and (b) the latter effect would be greater in the most severely affected patients.

Modafinil cannot affect directly the peripheral alpha-1 adrenergic and acetylcholine receptors located on the iris dilator and constrictor muscles, respectively, because it does not bind to cholinergic or adrenergic receptors (Mignot et al. 1994). Therefore, a putative modafinil-induced mydriasis could only be of central origin, as a result of increased sympathetic or reduced parasympathetic influences on the iris or both. The pupillary light reflex may help to elucidate the effects of a drug on the sympathetic and parasympathetic inputs to the iris, as the time-course of the light reflex response is determined by the successive activation of the parasympathetic and sympathetic inputs; the amplitude (the depth of pupillary constriction in response to a light stimulus) reflects activation of the midbrain parasympathetic Edinger-Westphal nucleus (Gamlin et al. 1997; Barbur 2004) and the recovery time (the redilation of the pupil to its original size in response to light stimulus offset) reflects mainly sympathetic activation (Smith and Smith 1999; Loewenfeld 1999; Bitsios et al. 1998). In healthy alert individuals, modafinil has little (Hou et al. 2007) or no mydriatic effect in darkness (Samuels et al. 2006, 2007), but it is possible that a ceiling effect in the pupils of alert subjects militates against the detection of such an effect in the dark. Indeed, a mydriatic effect of modafinil is more apparent in the light (Hou et al. 2005, 2007; Samuels et al. 2007). This effect may be mediated by sympathetic activation by the drug, as modafinil failed to inhibit the amplitude of the light reflex (Hou et al. 2005, 2007), while it increased the velocity of the darkness reflex, a sympathetically mediated response (Hou et al. 2005). A subsidiary aim of the study, therefore, was to dissect the relative central influences of modafinil on the sympathetic vs the parasympathetic branches of the autonomic nervous system, using the pupillary light reflex as a test system.

Materials and methods

Patients

The study was approved by the Ethics Committee of the University of Crete, and all participants gave their written informed consent before screening. We restricted our sample to unmedicated patients (age range: 30–50 years) with a recent (<10 days) diagnosis of OSA, with a full range (mild to severe) of disease severity and with normal laboratory findings who were not yet under any type of treatment. Exclusion criteria were the presence of common comorbid conditions such as hypertension, cardiopulmonary disease, diabetes mellitus, and a body mass index (BMI) ≥33. Additional exclusion criteria were: unrelated active clinically significant disease, ocular conditions or operations, a history of drug abuse, or a positive urine drug-screening test, excessive caffeine consumption (greater than five cups per day) or any prescribed or over-the-counter medication, which affects the autonomic nervous system. Twelve patients (one female) were recruited from the Sleep Disorders Unit, Department of Thoracic Medicine, Medical School, University of Crete on the basis of the above criteria. Daytime sleepiness was assessed subjectively using the Epworth Sleepiness Scale (ESS) (Johns 1991). The presence and severity of OSA were diagnosed on the basis of clinical and overnight PSG assessments, which have been described in detail previously (Bitsios et al. 2006). The patients' characteristics are shown in Table 1.

Drugs, design, and procedures

Modafinil 200 mg and placebo were administered orally in two weekly sessions, according to a double-blind, balanced,

Table 1 Characteristics of 12 patients diagnosed with OSA

Patient number	Age (years)	BMI (kg/m^2)	ESS score	AHI	AI	Severity
1	41	27.58	3	26	20	Mild
2	35	28.00	3	19	12	Mild
3	42	31.06	2	15	7	Mild
4	40	26.50	14	27	25	Moderate
5	39	29.01	5	38	22	Moderate
6	48	26.81	16	60	42	Severe
7	47	31.00	20	63	60	Severe
8	49	28.00	18	81	63	Severe
9	37	28.04	6	45	42	Severe
10	34	32.15	6	74	36	Severe
11	31	32.08	19	28	23	Severe
12	41	33.05	7	79	65	Severe
Mean	40.3	29.4	9.9	46.2	34.7	
SD	5.6	2.3	6.9	24.2	19.9	

BMI Body mass index, *ESS* Epworth Sleepiness Scale, *AHI* apnea–hypopnea index, *AI* arousal index

cross-over design. The procedures were identical in the two experimental sessions. Onset of sessions was always at 10:00 in the morning for all patients with a 30-min predrug testing immediately followed by ingestion of the capsule (placebo or modafinil 200 mg). Because peak plasma levels of modafinil are reached between 2 and 4 h after ingestion (Robertson and Hellriegel 2003), an identical postdrug testing took place 3.5 h after capsule ingestion, i.e., always at 2:00 in the afternoon. This timing was chosen because at 2:00 in the afternoon the pupil size reaches a circadian nadir (Bitsios et al. 2006; Merritt et al. 1998), thus, maximizing the chances of detecting an alerting effect of modafinil on pupillary outcome measures. Following the postdrug testing, the patients' light reflex was elicited and recorded (see below) and after that, the patients were assessed on tests of sensorimotor gating and cognition, the results of which will be presented in subsequent reports.

Tests and apparatuses

The 5-min PAT

A commercial binocular infrared video pupillometer (PROCYON, P2000D, sampling rate: 25 Hz, spatial resolution: >0.05 mm, accuracy >±3%) was used to monitor RPD in darkness, once at 10:00 A.M. (RPD_{am}) and once at 2:00 P.M. (RPD_{pm}). The technique has been described in detail previously (Bitsios et al. 2006). The recordings took place in a dark, sound-attenuated room, and subjects were allowed to sit comfortably in a forward leaning position. They were encouraged to stay still but resist sleep and blink as little as possible, while they were looking through the eye-tubes of the pupillometer for 5 min. Pupil diameter was sampled for 15 consecutive 20-s periods with a total monitoring time of 300 s. The outcome measures were the average RPDs for each 1 of the 15 20-s periods and the collapsed RPD for the entire 300-s recording. Recording periods were excluded if artifacts were disrupting more than two of three of a 20-s recording period (i.e., excessive blinking or eye closures amounting to a time >13 s) in both eyes. Only six recording periods (3.3% of total) were discarded based on these criteria.

The light reflex

The light reflex was elicited and recorded in darkness at 2:00 P.M., in previously dark-adapted eyes (15 min). The stimuli were light flashes of 200 ms duration delivered via a light emitting diode, presented to the subject's right and left eye in an alternating fashion, as a white disk of 8° diameter, providing "full retinal field" light stimulation (at four levels of stimulus luminance: 0.35, 5, 50, and 140 cd m^{-2}), while the non-stimulated eye was fixating a target dot projected at

Table 2 Changes in physiologic and psychological measures under the two treatment conditions (mean±SE mean)

Physiologic and psychological measures	Placebo	Modafinil	*t*	*p*
ΔVAS alertness (cm)	0.01±0.17	0.80±0.37	−2.4	0.035*
Δ VAS anxiety (cm)	0.01±0.47	−0.55±0.44	0.76	>0.4
Δ VAS discontentment (cm)	−0.39±0.29	−0.58±0.38	0.38	>0.7
Δ Heart rate (beats/min)	−6.00±1.59	−1.33±2.72	−1.35	>0.2
Δ Systolic BP (mmHg)—sitting	0.83±3.42	−5.83±3.19	1.32	>0.2
Δ Diastolic BP (mmHg)—sitting	−1.67±2.41	−2.50±5.13	0.12	>0.9
Δ Systolic BP (mmHg)—standing	−0.83±3.36	−5.00±3.64	0.81	>0.4
Δ Diastolic BP (mmHg)—standing	0.00±2.54	−3.33±3.45	0.66	>0.5

a distance of approximately 10 m. Each one of the four levels of stimulus luminance was presented in a block of four stimuli, the average of which was the response for that luminance level. The interstimulus interval within blocks was fixed at 5 s. Therefore, the total time for the elicitation and recording of the light reflex was 80 s. Stimulus presentation was computer controlled, and pupillary measures were digitized and stored for off-line analysis. The parameters studied were: light reflex response amplitude [i.e., the difference between the baseline (defined as the mean pupil diameter recorded over 500 ms before the onset of the light stimulus) and the diameter reached at the trough of the pupillary response to the light stimulus] and 75% recovery time (i.e., the time taken for the pupil to reach the 75% of its original size from the moment it reached its maximum constriction to a light flash).

Psychological and Physiological measures

Patients rated their mood and feelings on 16-item 100-mm visual analog scales (VAS) in each session, before and after treatment. For each patient, the raw values (cm) for each item were weighted by multiplication with their respective factor loading, and the weighted values for each item were then allocated to "alertness", "anxiety", and "discontentment" factors, based upon a principal component analysis (Bond and Lader 1974). The average of the weighted values for each factor was entered in the statistical analysis. Heart rate and blood pressure (sitting and standing positions) were also recorded before and after treatment.

Data reduction and analysis

The collapsed RPD data for the entire 300-s recording entered the statistical analysis. A three-way repeated measures analysis of variance (ANOVA) with treatment (placebo and modafinil) and time-of-day (pretreatment 10:00 A.M. and posttreatment 2:00 P.M.) as the within-subject factors, and the order of treatment as the between-subject factor was used to analyze the effects of treatment on RPD. The order was considered as a salient factor because participation in an experiment may have an arousing effect especially in the first session. Exploratory Pearson's correlations and multiple linear regression analyses were used as appropriate, to examine the relationship between the effect of modafinil on RPD and subjective (ESS) and objective (PSG) indices of disease severity. The light reflex amplitude and recovery time were analyzed with separate repeated measures ANOVAs with treatment (placebo, modafinil) and light intensity (four levels) as the within-subject factors. Pre–posttreatment changes in the VAS and cardiovascular data were calculated and compared with paired samples *t* tests.

Results

Psychological and cardiovascular measures

The changes in psychological and cardiovascular measures under the two treatment conditions are shown in Table 2. Modafinil significantly increased VAS-rated "alertness" but it did not affect anxiety, discontentment, or the cardiovascular measures.

5-min PAT

Figure 1 (top left) shows the pretreatment (10:00 A.M.) and posttreatment (2:00 P.M.) RPD values for each one of the 15 20-s periods for the placebo and the modafinil treatment conditions. In the placebo treatment condition, the RPD was becoming progressively smaller from the 1st to the 15th period, and it was always smaller at 2:00 P.M. compared to 10:00 A.M. In contrast, in the modafinil treatment condition, this effect was reversed, and RPD at 2:00 P.M. was elevated at greater than 10:00 A.M. levels, and it was not reduced over the course of recording from the 1st to the 15th period. Figure 1 (top right) shows the pre- and posttreatment RPDs averaged across the 15 20-s periods. Analysis of the collapsed data with a 2×2×2 (order×treatment×time-of-day) repeated measures ANOVA showed a significant main effect of treatment [$F(1,10)=6.1$, $p<0.033$], significant treatment×time-of-day interaction [$F(1,10)=28.06$, $p<0.001$] and nonsignificant main effects of time-of-day and order or any other interactions (ps>0.1).

The pre-drug (10:00 A.M. baseline) RPDs in the placebo and modafinil treatment conditions did not differ (paired samples *t* test: $t=0.65$, $p>0.1$) and were highly correlated (Pearson's $r=0.964$, $p<0.001$). Therefore, the pre–post differences (ΔRPDs) were calculated for the placebo and modafinil treatment conditions. Figure 1 (bottom) shows the ΔRPDs for the placebo and modafinil treatment conditions for each one of the 15 20-s periods (bottom left) and the collapsed ΔRPDs (bottom right). RPD was clearly reduced at 2:00 P.M. with placebo treatment, and this effect was reversed by modafinil. Paired samples *t* test comparison of the collapsed RPDs showed that this difference was significant ($t=-5.4$, $p<0.001$).

The difference between $\Delta RPD_{modafinil}-\Delta RPD_{placebo}$ was calculated as the "pure" modafinil effect on RPD (shown in Fig. 1 bottom right), and this was significantly correlated ($r=0.856$; $p<0.001$) with the "pure" modafinil effect on VAS-rated alertness ($\Delta alertness_{modafinil}-\Delta alertness_{placebo}$). The "pure" modafinil effect on RPD was also used as the dependent variable in a regression analysis with the key PSG variables apnea–hypopnea index and lowest oxygen saturation and also including ESS, age, and BMI as the independent variables. Entering all these independent varia-

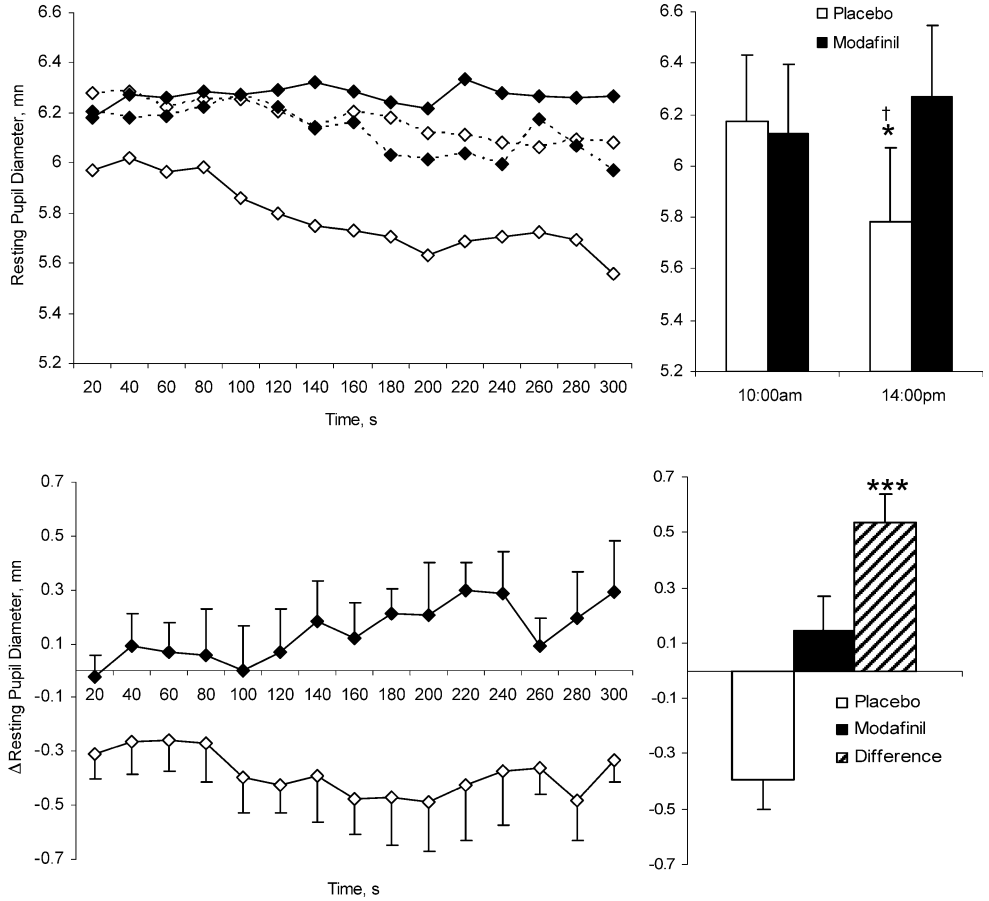

Fig. 1 *Top left panel*: (*ordinate*) resting pupil diameter (mm); (*abscissa*) time in seconds. The 15 data points are means (n=12) of the average resting pupil diameter sampled over periods of 20 s, for 15 consecutive periods. *Open symbols:* placebo; *closed symbols:* modafinil 200 mg; *dotted line:* morning (10:00 A.M.) measurements; *solid line:* afternoon (2:00 P.M.) measurements. *Top Right panel*: (*ordinate*) as above. The *bars* represent data collapsed across the 15 20-s periods for the two treatment conditions (mean SEM, n=12). *Open bars:* placebo; *closed bars:* modafinil 200 mg. *Bottom left panel*: Group means (n=12) of the pre–post (Δ) resting pupil diameter (*ordinate*) plotted against time (s) (*abscissa*) in the placebo (*open symbols*) and the modafinil (*close symbols*) treatment conditions. *Bars* represent standard errors of the mean. *Bottom right panel*: The *bars* represent the Δ resting pupil diameter collapsed across the 15 20-s periods for the two treatment conditions (mean SEM, n=12). *Open bar:* placebo; *closed bar:* modafinil 200 mg; *shaded bar:* the difference between modafinil–placebo. *asterisk*: RPDpm < RPDam; *dagger*: RPDpm placebo < RPDpm modafinil; ***p<0.001

bles revealed a nonsignificant model [$F(5,6)=3.11$, $p>0.1$], but a backward regression analysis revealed that 64.6% (adjusted $R^2=0.567$) of the variance of the pure modafinil effect on RPD was significantly [$F(2,9)=8.2$, $p<0.009$] predicted by apnea-hypopnea index and lowest oxygen saturation, but only the latter was a significant predictor ($t=-1.88$, $p=0.093$; partial correlation $r=-0.53$ and $t=-3.83$ $p=0.004$; partial correlation $r=-0.79$, respectively). Table 3 shows the correlations matrix between the "pure" modafinil effect on RPD and the above variables. Partialling out the

Table 3 Correlation matrix between the pure modafinil effect on RPD ($\Delta RPD_{modafinil} - \Delta RPD_{placebo}$) and indices of disease severity, age, and BMI in 12 patients

Parameter	ESS	AHI	$LSatO_2$	AGE	BMI
ΔRPD	0.70[a]	0.26	−0.71[a]	−0.44	−0.16
Controlled for BMI, neck, waist & hip circumference					
ΔRPD	0.70[b]	0.37	−0.89[a]	−0.26	

Values represent Pearson's correlation coefficients

RPD Refers to resting pupil diameter, *ESS* Epworth Sleepiness Scale, *AHI* apnea–hypopnea index, *LSatO2* lowest Oxygen saturation, *BMI* body mass index

[a] $p<0.01$

[b] $p=0.051$

effects of BMI and other body fat indices did not alter these relationships.

Light reflex

Figure 2 shows the group means for light reflex amplitude (top) and 75% recovery time (bottom) over the four light intensities. Light reflex amplitude was increased with increasing light intensity as expected, but it was reduced by modafinil at all light intensities. ANOVA of the amplitude data showed significant main effects of treatment and light intensity [$F(1,11)=28.58$; $p<0.001$ and $F(3,33)=146.23$; $p<0.001$, respectively], but not a significant interaction ($F<1$). In contrast to the amplitude data, 75% recovery time was not affected by either light intensity or modafinil treatment (Fs<1).

Discussion

The main finding of the present study is that the 5-min PAT is sensitive to the alerting effects of a single 200 mg dose of modafinil in OSA patients. Pupil size decreased significantly in the afternoon compared with in the morning, only in the placebo treatment condition, as evidenced by the significant treatment×time-of-day interaction (Fig. 1 top left). This is consistent with a mid-afternoon circadian nadir in the levels of alertness, which has previously been demonstrated in both patient and control groups with the MSLT (Clodore et al. 1990) and pupillometry (Bitsios et al. 2006; Merritt et al. 1998). Modafinil reversed this circadian effect on pupil size as evidenced by the significant treatment effect. Indeed, tonic levels of pupil diameter were higher already from the first 20-s period in the modafinil treatment condition and remained so at all times (Fig. 1-top left), suggesting higher tonic arousal levels. Modafinil significantly increased subjective alertness, consistent with previous acute studies in patients suffering from EDS (Ellis et al. 1999; Szabadi et al. 2002), but not in healthy alert subjects (Hou et al. 2005, 2007; Samuels et al. 2006, 2007). It is possible that patients with EDS are more sensitive to the alerting effects of the drug. Indeed, modafinil induces alertness more readily in healthy sleep-deprived (Pigeau et al. 1995) compared to healthy alert subjects (Randall et al. 2004) or in orexin-null mice Willie et al. 2005) who are less aroused compared to wild-type littermates (Estabrooke et al. 2001). Interestingly, the alerting effect of modafinil, as measured by the VAS ratings correlated with its effect on the 5-min PAT, suggesting that a common mechanism may underlie both these effects.

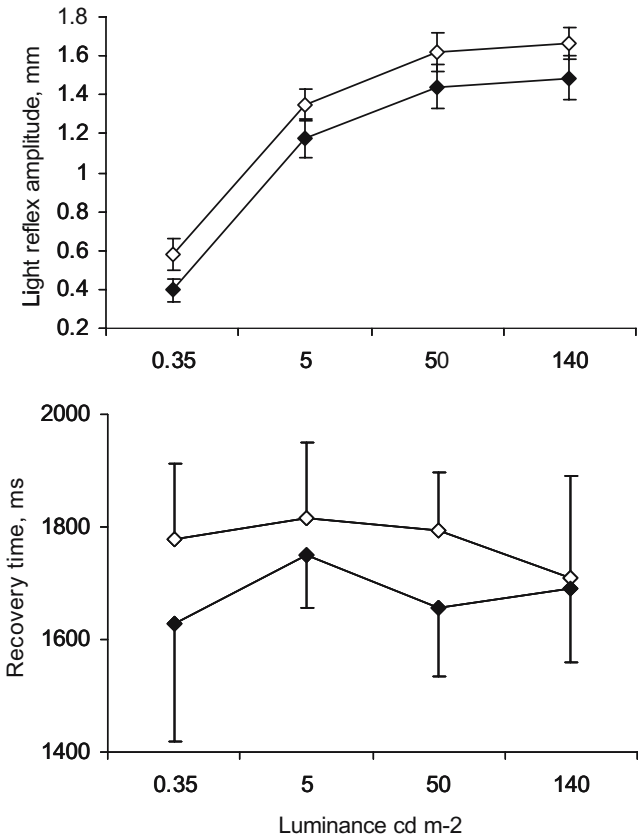

Fig. 2 Light reflex amplitude (*top*) and 75% recovery time (*bottom*) obtained at four graded luminance levels in the two treatment conditions. *Ordinate*: light reflex amplitude (mm) and 75% recovery time (ms), respectively. *abscissa*: light intensity (cd m^{-2}). Data points correspond to the mean obtained in the group ($n=12$). *Open symbols*: placebo; *closed symbols*: modafinil 200 mg

Importantly, the modafinil-induced increase in RPD was greater in the most severely affected OSA patients, as suggested by significant positive correlations with ESS scores and negative correlations with oxygen saturation. It is also noteworthy that these relationships were independent of body fat tissue (see Table 3). It is not possible to ensure whether this is a specific effect of modafinil in the brains of the sleepier and more severe OSA patients, but in favor of this possibility is the fact that the effect of modafinil on RPD correlated with its effect on VAS-rated alertness. With regard to PSG indexes of disease severity, the oxygen saturation during sleep was the only significant predictor of the "pure" modafinil effect on RPD (64.6% of the variance), as evidenced by the regression analysis. This suggests that the extent of the modafinil-induced reversal of circadian miosis in OSA patients (a) depends mainly on the presence and severity of the associated hypoxia and (b) may be in itself, an index of OSA severity. We found a reversal of the circadian pupillary miosis by modafinil, which correlated with an increase in subjective alertness and was greater in the more severely affected patients in terms of OSA-associated hypoxia. These findings indicate promising predictive validity of the 5-min PAT.

The light reflex findings suggest an increase in the inhibitory input to the Edinger-Westphal nucleus by modafinil, as the light reflex amplitude was equally reduced

at all levels of stimulus luminance, in the modafinil treatment condition. In contrast, the recovery time was not affected, in agreement with a previous report in healthy individuals (Hou et al. 2005). The cardiovascular measures were also not affected by modafinil, in agreement with previously reported minimal or no effect on sympathetic function with therapeutic single doses of modafinil in patient groups (Moldofsky et al. 2000) or healthy volunteers (Hou et al. 2005). These results taken together suggest that the reversal of circadian miosis by modafinil was primarily, the result of an increase in central inhibition of the parasympathetic Edinger-Westphal nucleus, rather than central sympathetic activation by the drug. However, the contribution of sympathetic activation by modafinil cannot be entirely excluded. In healthy subjects, modafinil has been found to increase the velocity of the sympathetically mediated darkness reflex (Hou et al. 2005), and there is independent evidence demonstrating the sympathetic activating effect of modafinil (Taneja et al. 2005). A better way to dissect the relative contributions of the sympathetic and parasympathetic systems in modafinil-induced mydriasis in OSA might be peripheral monocular sympatholysis with dapiprazole eyedrops, as in the paradigm developed by Giakoumaki et al. (2005).

Our findings are not entirely consistent with studies in healthy alert subjects. In the present study, we observed a robust (>0.5 mm) increase in RPD, while studies in healthy alert subjects show no effect (Samuels et al. 2006, 2007) or a very small mydriasis in darkness (Hou et al. 2007). It is possible that the large pupils of alert subjects in darkness, militate against detecting the mydriatic effect of modafinil, and indeed, a mydriatic effect of the drug is apparent in the light, when the pupils are driven to a lower baseline (Hou et al. 2005, 2007). This "ceiling effect" is less likely to operate in patients with OSA because they have EDS associated with smaller pupils in darkness compared to age-matched controls (Bitsios et al. 2006). The modafinil-induced reduction in light reflex amplitude observed here suggests that modafinil increases the central inhibition of the EW nucleus, but no such effect has been found in healthy alert subjects (Hou et al. 2005, 2007). One important procedural difference, which may account for these discrepancies, is that we studied the modafinil effects when autonomic arousal and the pupil size reach a circadian nadir (Merritt et al. 1998; Bitsios et al. 2006). This may have considerably increased the sensitivity of our tests to the alerting effects of modafinil on pupillary variables. However, the lack of a control group in the present study limits the interpretation of our findings and does not allow for a direct demonstration of their specificity or sensitivity for OSA patients.

Our findings are consistent with (a) evidence of circadian regulation of autonomic arousal through a functional circuit from the suprachiasmatic nucleus (SCN) to the LC (Aston-Jones et al. 2001), and (b) evidence that the LC and the associated reticular noradrenergic nuclei ("subcoeruleus") provide a direct (Gamlin and Reiner 1991; Koss et al. 1984; Koss 1986; Szabadi and Bradshaw 1996) inhibitory input to the EW nucleus via postsynaptic inhibitory alpha-2 adrenoceptors. An indirect input from the SCN through the olivary pretectal nucleus to the EW nucleus is also possible and cannot be excluded (Smeraski et al. 2004). Reduction of activity in the SCN-LC circuit will result in reduced alertness and loss of the inhibitory Edinger-Westphal control, which leads to circadian miosis. Although modafinil clearly reversed this circadian effect on pupil size, it is not possible to ascertain its central site of action. However, it seems reasonable to assume that it activated directly or indirectly the functional SCN-LC circuit, thus, leading to an increase in the LC inhibitory input to the Edinger-Westphal nucleus, and therefore, reversal of the circadian miosis and inhibition of the light reflex amplitude. Our results are also interesting in the light of evidence showing that chronic hypoxia causes adaptive hypometabolism (Hochachka et al. 1994) and a reduction in norepinephrine turnover in the brainstem (Soulier et al. 1992) due to reduced excitability of the noradrenergic LC neurons. The latter is a result of chronic hypoxia-induced up-regulation of α_2-somatodendritic inhibitory autoreceptors of the LC neurons (Chang et al. 2006). Because this nucleus is heavily implicated in the regulation of arousal and inhibitory control of the pupil, reduction of its excitability by hypoxia in OSA could greatly contribute towards reductions both in arousal and RPD in this condition. Taking all of the above together, it is tempting to assume that the modafinil effect on RPD was due to restoration of inhibitory control of the pupil via stimulation of the hypoxia-sensitive nucleus LC (see Hou et al. 2005 and Szabadi 2006 for arguing the point that modafinil may stimulate the LC). Although speculative, this interpretation fits with evidence that the LC is a critical "hub" in an identified circuit regulating circadian autonomic responses and in the arousal/sleep–wake network of the brain. It is also in agreement with recent theoretical and neurobiological models that emphasize the importance of arousal (Johns 1998) and the locus coeruleus (Nelson et al. 2002, 2003) respectively, in the expression of sleepiness.

These findings should not be taken to infer that the 5-min PAT can be used to assess the alerting effects of drugs. Although in the case of modafinil, there seems to be a good correlation between changes in alertness and pupil diameter induced by the drug, such a relationship may not exist in the case of other drugs, which may alter pupil diameter independently of their actions on arousal mechanisms. For instance, single doses of reboxetine (Phillips et al. 2000) and pentagastrin (Tavernor et al. 2000) increase pupil

diameter without affecting alertness. Therefore, while pupil diameter changes may reflect physiological changes in the level of alertness, this relationship may be disrupted when drugs are used to alter the level of alertness.

In summary, we found that the circadian reduction in pupil size in OSA patients was abolished by the alertness-promoting drug modafinil, which increased the inhibitory input to the parasympathetic pupilloconstrictor Edinger-Westphal nucleus in the midbrain. This effect was greater for the most severely affected OSA patients in terms of hypoxia, and it is best explained by activation of the hypoxia-sensitive nucleus locus coeruleus by modafinil. The 5-min PAT may offer insights in the pathophysiology of sleepiness associated with OSA, has promising predictive validity, and holds promise as a simple, fast, and feasible method for the objective assessment of excessive daytime sleepiness and its severity, monitoring of disease progression, and response to treatment.

Acknowledgements This work is part of the PENED 03ED375 research project funded by E.U-European Social Fund (75%) and the Greek Ministry of Development-General Secretariat of Research and Technology (25%). The experiments described in the study comply with the current laws of Greece, where they were performed.

Conflict of interest statement The authors have no conflict of interest or any involvement, financial or otherwise, that might have biased the present work.

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