

Modulation of the Acoustic Startle Response by the Level of Arousal: Comparison of Clonidine and Modafinil in Healthy Volunteers

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A sudden loud sound evokes an electromyographic (EMG) response from the orbicularis oculi muscle in humans together with an auditory evoked potential (AEP) and an increase in skin conductance (SC). Startle responses are inhibited by weak prepulses (prepulse inhibition, (PPI)) and may also be modified by the level of alertness. We compared the sedative drug clonidine and the alerting drug modafinil on sound-evoked EMG, AEP, and SC responses, on the PPI of these responses and on level of arousal and autonomic functions. Sixteen healthy male volunteers participated in four weekly sessions (clonidine 0.2 mg, modafinil 400 mg, their combination, placebo) in a double-blind, cross-over, balanced design. Responses were evoked by sound pulses of 115 and 85 dB (PPI) for 40 ms and recorded conventionally. Level of alertness, autonomic functions (pupil diameter, blood pressure, heart rate, salivation, temperature) and the plasma levels of the hormones prolactin, thyroid-stimulating hormone and growth hormone were also measured. Data were analyzed with analysis of variance with multiple comparisons. Both prepulses and clonidine attenuated all three startle responses and modafinil antagonized clonidine's effects on the EMG and AEP responses. None of the drugs affected PPI. Clonidine showed sedative and sympatholytic effects, and modafinil showed alerting and sympathomimetic effects. In conclusion, startle responses were susceptible not only to PPI but also to the level of arousal.

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INTRODUCTION

The startle response involves rapid involuntary contractions of facial and skeletal musculature in response to a sudden intense stimulus. In the case of the acoustic startle response, this reaction occurs in response to a sudden loud sound and is measurable in humans as an electromyographic (EMG) response from the orbicularis oculi muscle (eyeblink startle response). This response is believed to be mediated via a pathway below the level of the diencephalon consisting of between three and five central synapses, originating from the sensory receptors and terminating on the motor neurons, where the principal relay is the caudal pontine reticular nucleus (Davis *et al*, 1982; for review, see Yeomans and Frankland, 1996; Koch, 1999). In addition to this muscular response, changes in electroencephalographic (EEG) activity can be observed in response to the auditory

stimulus, and these changes are termed auditory evoked potentials (AEP). In particular, the N1/P2 component of the AEP is measurable following the presentation of the acoustic startle stimulus (for examples, see Abduljawad *et al*, 1999, 2001; Phillips *et al*, 2000a, b; Graham *et al*, 2001, 2004; Scaife *et al*, 2005, 2006). Both the muscular and the AEP responses are fast responses; longer-latency responding to the startle stimulus can be observed in autonomic activity, in particular in sympathetically mediated skin conductance. The skin conductance response (SCR) is an increase in conductance maximal approximately 6 s following the presentation of the stimulus (Turpin *et al*, 1999; Graham *et al*, 2005; Scaife *et al*, 2005).

The presentation of a brief, low-intensity acoustic stimulus 30–500 ms before the presentation of the loud, startle-eliciting stimulus attenuates the amplitude of the muscular startle response, a phenomenon termed prepulse inhibition (PPI; Graham, 1975; Davis, 1980; Swerdlow *et al*, 1992; Koch, 1999). The AEP response is also affected by PPI (Abduljawad *et al*, 1999; Phillips *et al*, 2000a, b; Graham *et al*, 2004). PPI of the AEP response may have a neural mechanism that is different from the mechanism underlying PPI of the muscular response because the two responses show different pharmacological sensitivities (eg bromocrip-

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tine (Abduljawad *et al*, 1999), amitriptyline (Phillips *et al*, 2000a), acute tryptophan depletion (Phillips *et al*, 2000b), and clozapine (Graham *et al*, 2001)). PPI is thought to represent a form of 'gating', in which the influence of irrelevant or intrusive stimuli on ongoing behavior is prevented (Swerdlow *et al*, 1992; Koch, 1999). There are currently no reports on the effect of a prepulse on the SCR.

Apart from PPI, the acoustic startle response may also be modulated by the level of arousal. Indeed, it has been reported that the EMG startle reflex amplitude can be reduced by sedative drugs of different classes, for example, benzodiazepines (diazepam: Scaife *et al*, 2005), 5HT₂ receptor antagonists (ketanserin: Graham *et al*, 2002), α_2 -adrenoceptor agonists (clonidine: Kumari *et al*, 1996; Abduljawad *et al*, 1997, 2001), tricyclic antidepressants (amitriptyline: Phillips *et al*, 2000a), and atypical antipsychotics (clozapine and quetiapine: Graham *et al*, 2001, 2004). In contrast to the EMG startle response, the AEP is relatively resistant to sedative drugs, with only the benzodiazepines reducing N1/P2 amplitude consistently (Lader, 1977; Bond *et al*, 1983; Pooviboonsuk *et al*, 1996; Curran *et al*, 1998; Abduljawad *et al*, 2001; Scaife *et al*, 2005). However, no information is available on the effects of sedative drugs on SCR.

Because sedative drugs consistently suppress the EMG startle response, it could be expected that alerting drugs could have an opposite effect, that is, they would enhance startle response amplitude. There is some indication that this may be the case, as it has been reported that the psychostimulant amphetamine enhances the acoustic startle response (Davis *et al*, 1975; Kokkinidis and MacNeill, 1982; Swerdlow *et al*, 1990). However, it is not known whether other alerting drugs share this effect. Modafinil is a novel wakefulness-promoting drug used in the treatment of narcolepsy (US Modafinil in Narcolepsy Multicentre Study Group, 1998, 2000). Although the mode of action of modafinil is currently unknown, it has been suggested that it interacts with the central noradrenergic arousal pathway involving the locus coeruleus (LC) (Saper and Scammell, 2004; Saper *et al*, 2005) and also with dopaminergic mechanisms involved in the regulation of alertness (Mignot *et al*, 1994; Nishino *et al*, 1998; Wisor *et al*, 2001; Wisor and Eriksson, 2005). Clonidine is an α_2 -adrenoceptor agonist with potent sedative effects that may be related to its inhibitory action on the LC (De Sarro *et al*, 1987). It has been shown previously that modafinil and clonidine influence the level of alertness and sympathetic functions in opposite directions (Hou *et al*, 2005), consistent with their interactions with central noradrenergic mechanisms.

In the present study we compared the effects of single doses of modafinil and clonidine on all three startle responses (EMG, AEP, SCR), as well as PPI of those responses, in a group of healthy male volunteers. We also examined the possible functional antagonistic interaction between the sedative and alerting drug on all these measures. The effects of the treatments were monitored on quantitative measures of arousal together with autonomic and endocrine functions, which are influenced by central noradrenergic mechanisms (Hou *et al*, 2005; Samuels *et al*, 2006).

MATERIALS AND METHODS

Subjects

Sixteen healthy male volunteers aged 18–45 years (mean \pm standard error of mean (SEM): 22.7 \pm 1.6 years), 171–190 cm (mean \pm SEM: 181.4 \pm 1.45 cm) in height and weighing 56.4–92.9 kg (mean \pm SEM: 74.7 \pm 2.8 kg), with a hearing threshold of 5–19 dB (mean \pm SEM: 6.9 \pm 0.9 dB), participated in the study. Subjects were all medication free for at least 3 months before the start of the study and completed a brief medical history and physical examination before inclusion in the study. Volunteers were all non-smokers and were asked 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. 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

Modafinil (400 mg), clonidine (0.2 mg), modafinil (400 mg) + clonidine (0.2 mg), and placebo were administered orally in matching capsules for double-blind administration. The doses were chosen on the basis of the current literature (modafinil: Taneja *et al*, 2005; clonidine: Morley *et al*, 1991; Bitsios *et al*, 1996; Phillips *et al*, 2000c, d). The time required to attain peak plasma concentrations (t_{MAX}) of clonidine and modafinil after the ingestion of single doses is approximately 2 h (modafinil: Wong *et al*, 1998; clonidine: Lowenthal *et al*, 1988), and the time course of the sessions was designed to ensure peak plasma concentrations of the drugs at the start of the post-treatment testing.

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 (see above).

Tests and Apparatus

Acoustic startle response. The method used was similar to that of Abduljawad *et al* (2001). All recordings were made while the subject was seated in an armchair.

A Kamplex AC30 Clinical Audiometer (P.C. Werth Ltd, London, UK) was used to generate binaural auditory stimuli; a background tone of 70 dB [A] at 1 kHz was present throughout, and sound pulses of 115 [A] or 85 dB [A] at 1 kHz were presented for 40 ms at varying intervals. The period of recording started with a 60-s adaptation period in which only the background sound was presented. This was followed by a single 115-dB [A] pulse, the responses to which were discarded. The remainder of the recording period consisted of 24 trials separated by varying inter-trial intervals of between 15 and 35 s, with a mean inter-trial interval of 25 s. The two stimulus combinations

presented in the trials were (i) a single 40-ms 115-dB [A] pulse (12 pulse-alone trials) and (ii) a 40-ms 85-dB [A] pulse followed by a 40-ms 115-dB [A] pulse with an inter-stimulus interval of 120 ms (12 prepulse/pulse trials). The order of presentation of the two types of trial was randomized, with the constraint that the same type of trial did not occur more than three times in succession.

A CED 1401 + computer with a 1902 interface (Cambridge Electronic Design Ltd, Cambridge, UK) was used to record the EMG response of the right orbicularis oculi muscle to the presentation of the acoustic stimuli. The response was measured using two silver/silver chloride disc electrodes of 0.5-cm diameter placed on the lower eyelid with a ground electrode placed over the right mastoid. The EMG data were rectified using a 1-Hz high-pass filter with a notch filter set at 50 Hz to minimize mains electrical interference.

Auditory evoked potential. A second CED 1902 interface was used to obtain single-channel recordings of the EEG response, the AEP, to the presentation of the acoustic stimuli simultaneously with the EMG recordings. The response was measured using a 0.5-cm silver/silver chloride disc electrode placed on the scalp at the Cz (vertex) position and an electrode pad placed on the left mastoid. The ground electrode was placed on the bridge of the nose. The vertex potentials were displayed in a 'positive up' configuration.

Skin conductance response. A CED 2502 interface connected to the same 1401 + computer used to record EMG and EEG responses was used to record the SCR to the presentation of the acoustic stimuli. The response was measured using electrode pads on the terminal phalanges of the first and third digits of the right hand.

Measures of alertness. The Leeds Psychomotor Tester (Psychopharma Ltd, Surrey, UK) was used to collect critical flicker fusion frequency (CFFF) measurements, defined as the frequency at which a flickering light appears to be continuous (Smith and Misiak, 1976). The CFFF test was conducted conventionally, with eight threshold measurements collected per session: four with increasing frequencies and four with decreasing frequencies. The mean of the eight measurements was taken as the value of the CFFF (see Samuels *et al*, 2006).

A computerized battery of 16 visual analogue scales (VASs) was used to collect self-ratings of alertness, calmness, and contentedness. Sixteen contrasting statements were rated along a continuous 10-cm line to represent the participant's subjective emotional and physiological state, for example, 'happy' vs 'sad' and 'alert' versus 'drowsy' (Norris, 1971; Bond and Lader, 1974; see Samuels *et al*, 2006). The position of the poles (left or right) and the order of presentation of the scales were randomized between subjects and sessions.

A dedicated monocular television pupillometer (setup version 1.20: AMtech, Weinheim, Germany) was used to collect the pupillographic sleepiness test (PST) measurements, defined as the spontaneous pupillary fluctuations in darkness over an 11-min period. These pupil fluctuations are regarded as a physiological index of alertness level

(Lowenstein *et al*, 1963; Yoss *et al*, 1970; Lüdtke *et al*, 1998). The PST quantitatively analyzes these pupil fluctuations and yields two measurements: pupillary unrest index (PUI: the distance traveled by the margin of the pupil over a 1-min period) and the total power of the pupil diameter fluctuations (obtained from a fast Fourier transform). Pupil diameter was measured continuously at a frequency of 25 Hz (see Samuels *et al*, 2006).

Autonomic functions. A binocular infra-red video pupillometer (Procyon Ltd, London, UK) with a calibrated internal light source was used to obtain resting pupil diameter measurements (static pupillometry). Pupillometry was conducted in a darkened room in darkness and at three luminance levels (6, 91, and 360 cd m^{-2}), with each measurement recorded over 2 s at 4 Hz (see Samuels *et al*, 2006).

An electroneroid sphygmomanometer was used to record blood pressure and heart rate in both standing and supine positions. A Braun Pro4000 tympanic thermometer (Welch Allyn UK Ltd, Buckinghamshire, UK) was used to measure temperature from the ear canal. A measure of salivation was derived by placing three cotton wool dental rolls in the subject's mouth (two buccally and one sublingually) and recording the increase in their weight over a 1-min period (Peck, 1959; Arya *et al*, 1997; Szabadi and Tavernor, 1999). The test was repeated twice with a 5-min 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 from an antecubital vein and analyzed for concentrations of the hormones prolactin and thyroid-stimulating hormone (TSH) by enzyme immunoassay and for growth hormone (GH) by chemiluminescence immunoassay in the Clinical Chemistry Laboratory (Queen's Medical Centre, Nottingham, UK).

Procedure

After a 15-min acclimatization period, subjects completed 30 min of pretreatment testing, including standing and supine heart rate and blood pressure, temperature, salivation, CFFF, VAS, PST, and resting pupil diameter. Two hours after ingestion of the capsule the post-treatment tests were conducted over 45 min, including standing and supine heart rate and blood pressure, temperature, salivation, CFFF, VAS, PST, resting pupil diameter, acoustic startle paradigm, and blood sampling.

Data Analysis and Statistics

The EMG responses recorded in each type of trial were averaged across the 12 trials using Spike 2 software (Cambridge Electronic Design Ltd, Cambridge, UK). The latencies (time taken from the onset of the stimulus to the onset of the response, in seconds), rise times (time taken from the onset of the response to the peak, in seconds) and amplitudes (maximal change in EMG potential, in millivolts) of the responses to the pulse alone and the prepulse/pulse were obtained from the averaged responses and used for further analysis. Percentage PPI was calculated for

further analysis using the formula

$$100 \times (A_{\text{pulse alone}} - A_{\text{prepulse/pulse}}) / A_{\text{pulse alone}}$$

where $A_{\text{pulse alone}}$ and $A_{\text{prepulse/pulse}}$ are the amplitudes of the mean responses to the 115-dB pulses in the pulse-alone and prepulse/pulse trials, respectively (Abduljawad *et al*, 2001). An example EMG trace is shown in Figure 1 (top).

The EEG AEPs recorded in each type of trial were averaged across the 12 trials (as EMG, above). The time to the N1 and P2 components of the response (in seconds) and the amplitude of the N1/P2 complex (in millivolts; taken as the amplitude difference from the peak of the N1 wave to the peak of the P2 wave, Mauguière *et al*, 1995) were obtained from the averaged responses to the pulse alone

and the prepulse/pulse and were used for further analysis. The range of latencies to the peak of the wave following stimulus onset was taken as 81–140 ms for N1 and 141–240 ms for P2. Percentage PPI of the N1/P2 complex was calculated using the formula stated above (Abduljawad *et al*, 2001). A sample EEG trace is shown in Figure 1 (center).

The SCRs recorded in each type of trial, measured from the onset of the response to the maximum conductance achieved within 10 s of the stimulus delivery (see Scaife *et al*, 2005), were averaged across the 12 trials (as EMG, above). The latencies (time taken from the onset of the stimulus to the onset of the response, in seconds), rise times (time taken from the onset of the response to the peak, in

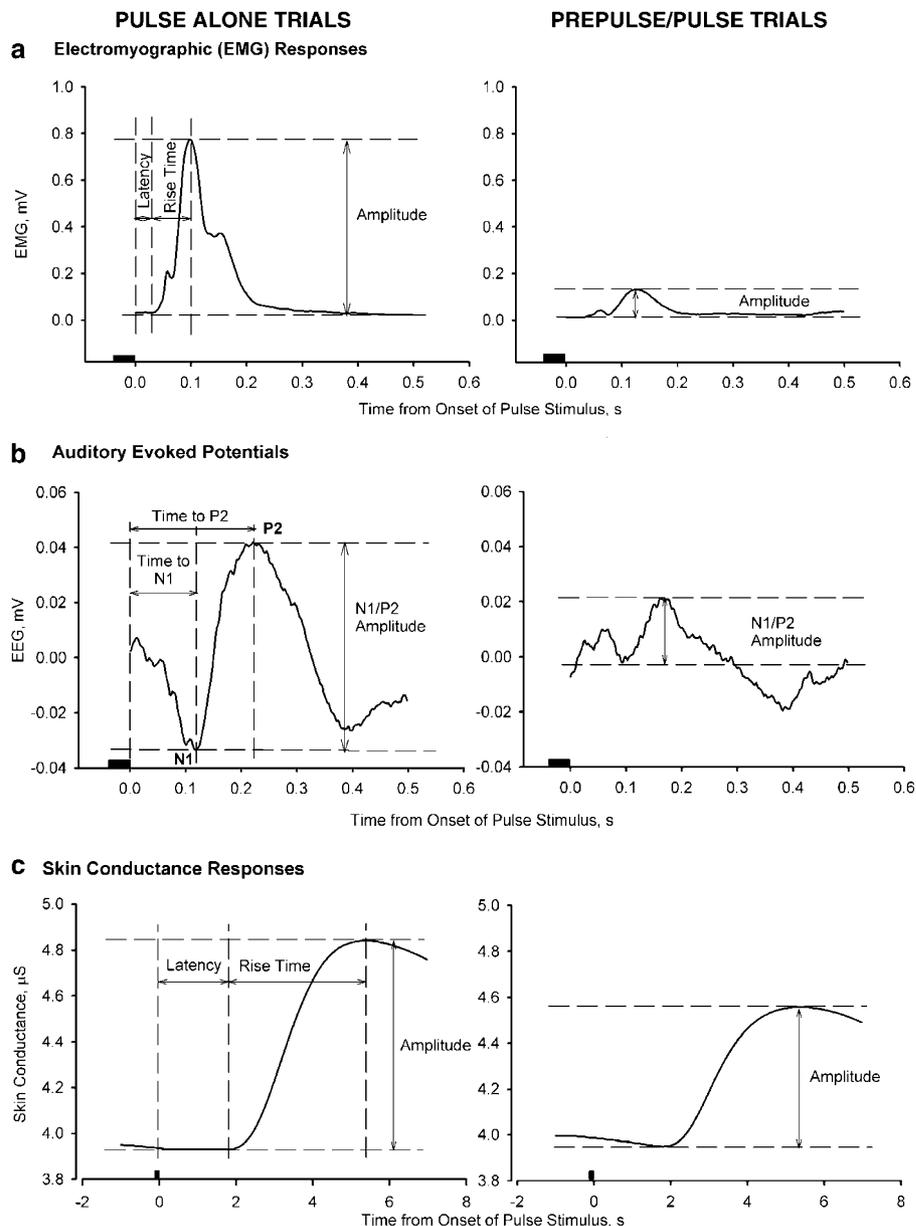


Figure 1 Examples of the averaged responses recorded over 12 pulse-alone trials (left-hand panels) and 12 prepulse/pulse trials (right-hand panels) for the acoustic startle response (a), the N1/P2 complex of the AEP (b), and the SCR (c). Stimulus presentation is represented by a black rectangle. The measurements derived from these recordings are labeled. The response amplitudes measured from these trials were used to calculate percentage PPI of the three responses (see text for details).

seconds), and amplitudes (maximal change in skin conductance, in micro-siemens) of the responses to the pulse alone and prepulse/pulse were obtained from the averaged responses and used for further analysis. Percentage PPI of the amplitude was calculated using the formula stated above (Abduljawad *et al*, 2001). A sample SCR trace is shown in Figure 1 (bottom).

Self-rated values of 'alertness', 'calmness', and 'contentedness' were derived from the VAS scores after weighting on these factors (Bond and Lader, 1974). Pretreatment/post-treatment differences for these ratings and for the CFFF values were calculated for further analysis. Pretreatment/post-treatment differences were also calculated for the PST parameters of power, PUI and pupil diameter, and these values were used for statistical analysis.

Pretreatment/post-treatment differences were analyzed for all autonomic measures with the exception of resting pupil diameter at different luminance levels, where the pretreatment and post-treatment data were analyzed separately. Pretreatment/post-treatment differences were not calculated for these pupillary measurements because the recordings were taken at different luminance levels, and calculating the difference would have eliminated the effect of luminance on the measures studied. All pupil data were averaged across the left and right eyes.

Effects of the treatments on the secretion of prolactin, TSH, and GH were analyzed using post-treatment plasma concentration values.

The data were initially checked for skew and subjected to a transformation where indicated. All data were analyzed using within-subject analysis of variance (ANOVA). Pretreatment values were analyzed to find any session effects within the results. No significant pretreatment session effects were found, and so pretreatment/post-treatment differences were taken as the dependent variable where appropriate (see above). Two-way within-subject ANOVA was used to analyze the EMG, AEP, and SCR data (drug condition, four levels; pulse condition, two levels) and resting pupil diameter data (drug condition, four levels; luminance level, four levels). Because the primary measure of interest was the effect of treatment condition on the acoustic startle response evoked by the presentation of the pulse alone, one-way within-subject ANOVA was conducted on pulse-alone data subsequent to the detection of a significant effect in the two-way ANOVA. One-way within-subject ANOVA (drug condition, four levels) was used to analyze the alertness, autonomic and endocrine measures, and PPI data. All significant main effects were further analyzed using Dunnett's corrected *t*-test ($df = 45$, $k = 4$): active treatment conditions were compared with placebo (criterion of significance $p < 0.05$).

RESULTS

All pretreatment values were initially analyzed using one-way ANOVA to determine the presence of any session effects. No significant effects were found within the pretreatment data ($p < 0.05$) and so pretreatment/post-treatment differences were taken as the dependent measure where appropriate (see above).

Acoustic Startle Response

The effects of the four treatment conditions on the three measures of EMG response (latency, rise time, amplitude) to the single pulse and the PPI of the amplitude are shown in Figure 2. Owing to technical reasons, one subject was excluded from the analysis of rise time, amplitude, and PPI. For latency, the two-way ANOVA (\log_{10} transformation) showed an effect of drug condition ($F_{3,45} = 3.88$, $p < 0.05$) and pulse condition ($F_{1,15} = 5.83$, $p < 0.05$) but no significant interaction ($F_{3,45} = 0.78$, NS). The effect of pulse condition was due to a reduction in latency to the presence of the prepulse compared to the presentation of the pulse alone. A one-way ANOVA (\log_{10} transformation) of drug condition on the pulse-alone data showed a significant effect of treatment ($F_{3,45} = 5.72$, $p < 0.01$), in which clonidine increased the latency of the EMG startle response. For rise time, the two-way ANOVA (square root transformation) showed no effect of pulse condition ($F_{1,14} = 0.56$, NS) or drug condition ($F_{3,42} = 1.16$, NS) and no significant interaction ($F_{3,42} = 0.54$, NS). For amplitude, the two-way ANOVA (square root transformation) showed effects of both pulse condition ($F_{1,15} = 13.25$, $p < 0.01$) and drug condition ($F_{3,45} = 2.81$, $p = 0.05$) but no significant interaction ($F_{3,45} = 2.30$, NS). The effect of pulse condition was due to suppression of amplitude in the presence of the prepulse compared to the presentation of the pulse alone. A one-way ANOVA (square root transformation) of drug condition on the pulse-alone data showed a significant effect of treatment ($F_{3,45} = 3.45$, $p < 0.05$), in which clonidine reduced the amplitude of the startle response. There was no effect of treatment on the PPI of the amplitude (square root transformation; $F_{3,42} = 0.73$, NS).

Auditory Evoked Potential

The effects of the four treatment conditions on the three measures of the AEP response (latency to N1 peak, latency to P2 peak, N1/P2 amplitude) and the PPI of the amplitude are shown in Figure 3. Owing to technical reasons, two subjects were excluded from the analysis of these measures. For latency to N1 peak, the two-way ANOVA showed a trend for an effect of drug condition ($F_{3,39} = 2.83$, $p = 0.051$) but no effect of pulse condition ($F_{1,13} = 0.92$, NS) and no significant interaction ($F_{3,39} = 1.83$, NS). A one-way ANOVA of drug condition on the pulse-alone data showed a significant effect of treatment ($F_{3,39} = 4.49$, $p < 0.05$), in which clonidine increased the time to the N1 startle response. For latency to P2 peak, the two-way ANOVA showed an effect of pulse condition ($F_{1,13} = 40.45$, $p < 0.001$) and drug condition ($F_{3,39} = 5.64$, $p < 0.05$) but no significant interaction ($F_{3,39} = 1.47$, NS). The effect of pulse condition was due to a reduction in time to reach the P2 peak in the presence of the prepulse compared with the presentation of the pulse alone. A one-way ANOVA of drug condition on the pulse-alone data showed a significant effect of treatment ($F_{3,39} = 3.96$, $p < 0.05$), in which modafinil reduced the latency to the P2 peak. For N1/P2 amplitude, the two-way ANOVA showed effects of both pulse condition ($F_{1,13} = 86.74$, $p < 0.001$) and drug condition ($F_{3,39} = 3.08$, $p < 0.05$) but no significant interaction ($F_{3,39} = 2.67$, NS). The effect of pulse condition was due to an attenuation of

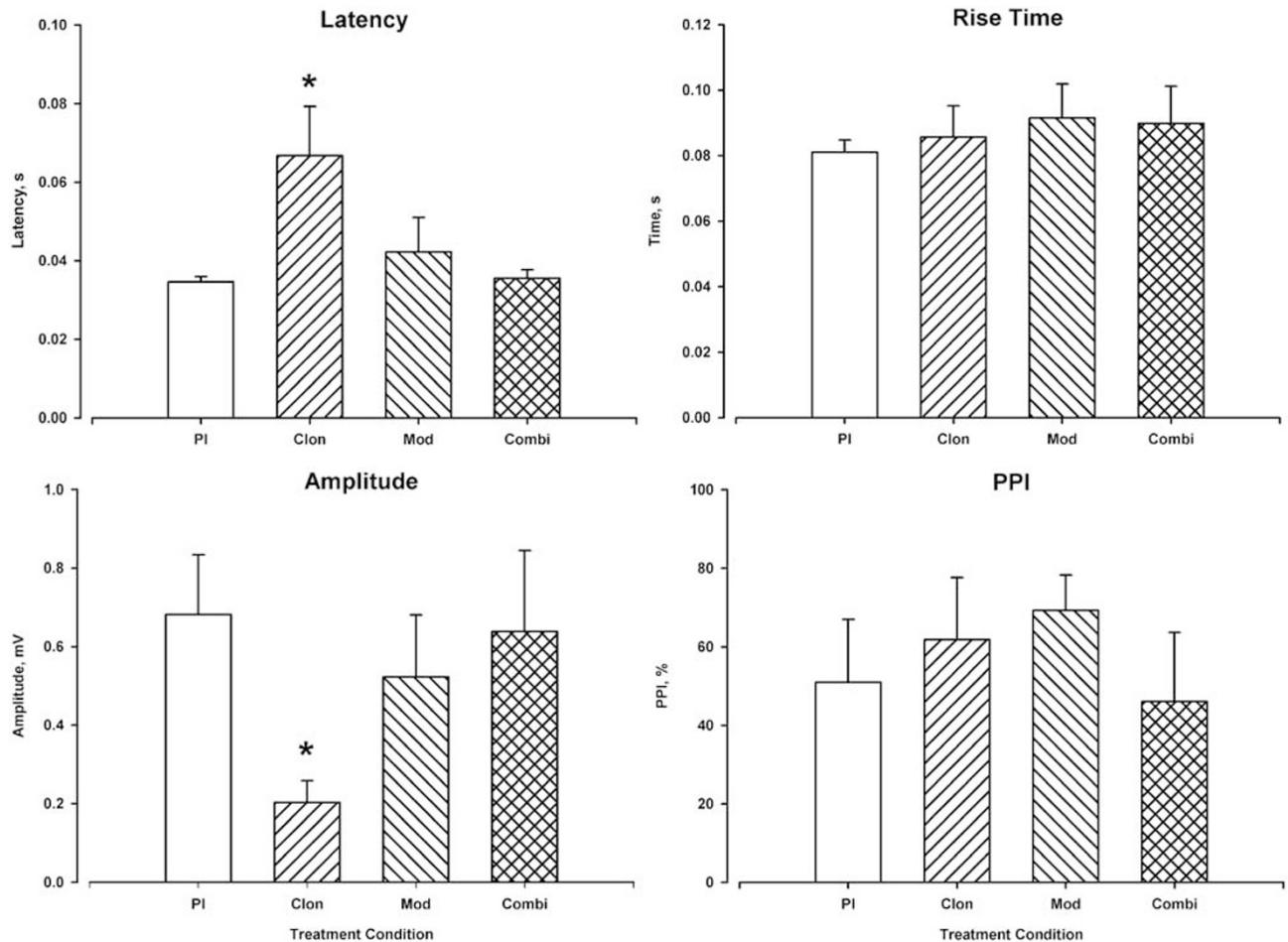


Figure 2 EMG responses of orbicularis oculi muscle to the 115-dB pulses in the pulse-alone trials: latency (s; top left), rise time (s; top right), amplitude (mV; bottom left) and PPI of the amplitude (%; bottom right). Columns correspond to mean values of the parameters for the group ($n = 16$); vertical bars represent SEM. Treatment condition is indicated at the bottom of the graphs: PI = placebo, Clon = 0.2 mg clonidine, Mod = 400 mg modafinil, Combi = combination of 0.2 mg clonidine and 400 mg modafinil. Clonidine significantly increased the latency and reduced the amplitude of the startle response: * $p < 0.05$ (Dunnett's test: comparison with placebo condition). The response amplitude was significantly reduced following a weak prepulse (PPI; see text for details).

the N1/P2 amplitude in the presence of the prepulse compared with the presentation of the pulse alone. A one-way ANOVA of drug condition on the pulse-alone data showed a significant effect of treatment (\log_{10} transformation; $F_{3,39} = 4.51$, $p < 0.01$), in which clonidine reduced the amplitude of the N1/P2 complex. There was no effect of treatment on the PPI of the amplitude (\log_{10} transformation; $F_{3,39} = 1.62$, NS).

Skin Conductance Response

The effects of the four treatment conditions on the three measures of the SCR (latency, rise time, amplitude) and the PPI of the amplitude are shown in Figure 4. For latency, the two-way ANOVA showed no effects of drug condition ($F_{3,45} = 0.79$, NS) or pulse condition ($F_{1,15} = 1.31$, NS) and no interaction ($F_{3,45} = 2.02$, NS). For rise time, the two-way ANOVA showed an effect of pulse condition ($F_{3,45} = 5.05$, $p < 0.05$) and a trend for an effect of drug condition ($F_{1,15} = 2.37$, $p = 0.08$), but no significant interaction ($F_{3,45} = 0.29$, NS). The effect of pulse condition was due to a reduction in the time to response peak in the presence of

the prepulse compared to the presentation of the pulse alone. A one-way ANOVA of drug condition on the pulse-alone data showed no effect of treatment compared to placebo ($F_{3,45} = 2.11$, NS). For amplitude, the two-way ANOVA showed an effect of pulse condition ($F_{1,15} = 16.15$, $p = 0.001$) and drug condition ($F_{3,45} = 7.38$, $p < 0.001$) but no significant interaction ($F_{3,45} = 0.52$, NS). The effect of pulse condition was due to an attenuation of amplitude in the presence of the prepulse compared to the presentation of the pulse alone. A one-way ANOVA of drug condition on the pulse-alone data showed a significant effect of treatment ($F_{3,45} = 8.48$, $p < 0.001$), in which both clonidine and the combination of clonidine and modafinil reduced the amplitude of the SCR. There was no effect of treatment on the PPI of the amplitude (\log_{10} transformation; $F_{3,45} = 0.45$, NS).

Alertness

Critical flicker fusion frequency. The effects of the four treatment conditions on the CFFF measurements are shown in Table 1. There was a significant effect of treatment

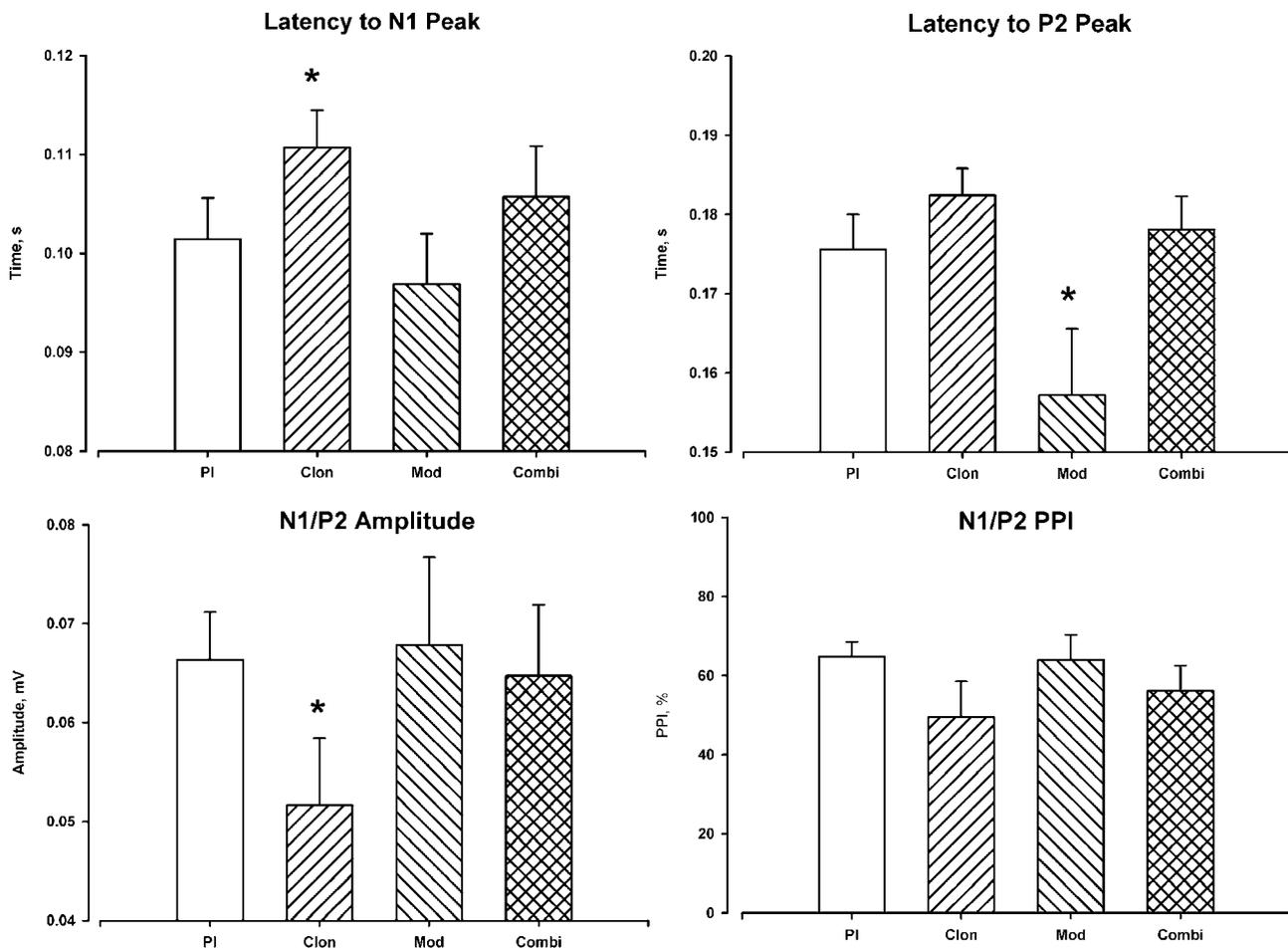


Figure 3 Auditory evoked potential evoked by 115-dB pulses in the pulse-alone trials: latency to N1 peak (s; top left), latency to P2 peak (s; top right), N1/P2 amplitude (mV; bottom left), and PPI of the amplitude (%; bottom right). Columns correspond to mean values of the parameters for the group ($n = 14$; data from two subjects were discarded for technical reasons); vertical bars represent SEM. Treatment condition is indicated at the bottom of the graphs: PI = placebo, Clon = 0.2 mg clonidine, Mod = 400 mg modafinil, Combi = combination of 0.2 mg clonidine and 400 mg modafinil. Clonidine significantly increased the latency to N1 peak and reduced the N1/P2 amplitude; modafinil significantly reduced the latency to P2 peak: * $p < 0.05$ (Dunnett's test: comparison with placebo condition). The response amplitude was significantly reduced following a weak prepulse (PPI; see text for details).

($F_{3,45} = 56.44$, $p < 0.001$), in which CFFF was reduced following both clonidine administration and the combination of clonidine and modafinil. The apparent increase in CFFF following modafinil just failed to reach significance.

Visual analogue scales. The effects of the four treatment conditions on the three VAS factors are shown in Table 1. There was a significant effect of treatment on alertness (\log_{10} transformation; $F_{3,45} = 14.09$, $p < 0.001$), in which alertness was reduced following the administration of both clonidine and the combination of clonidine and modafinil and increased following the administration of modafinil. The calmness factor showed a significant effect of treatment ($F_{3,45} = 3.56$, $p < 0.05$), in which calmness was reduced following modafinil administration. The contentedness factor showed a significant effect of treatment (\log_{10} transformation, $F_{3,45} = 4.89$, $p < 0.01$), in which contentedness was reduced following the administration of clonidine and the combination of clonidine and modafinil.

Pupillographic sleepiness test. The effects of the four treatment conditions on the PUI, total power of pupillary

fluctuations, and pupil diameter measurements from the PST are shown in Table 1. There was a significant effect of treatment on PUI ($F_{3,45} = 20.73$, $p < 0.001$), in which PUI was increased by the administration of both clonidine and the combination of clonidine and modafinil and was decreased by the administration of modafinil. Similarly, there was a significant effect of treatment on the total power of pupillary fluctuations ($F_{3,45} = 20.54$, $p < 0.001$), in which the administration of both clonidine and the combination of clonidine and modafinil increased the total power of the fluctuations, while the administration of modafinil reduced the total power. There was also a significant effect of treatment on pupil diameter ($F_{3,45} = 45.22$, $p < 0.001$), in which the administration of both clonidine and the combination of clonidine and modafinil decreased pupil diameter. The apparent increase in pupil diameter following modafinil administration just failed to reach significance.

Autonomic Functions

Pupil diameter. Figure 5 shows the effect of treatment condition on pretreatment (top) and post-treatment

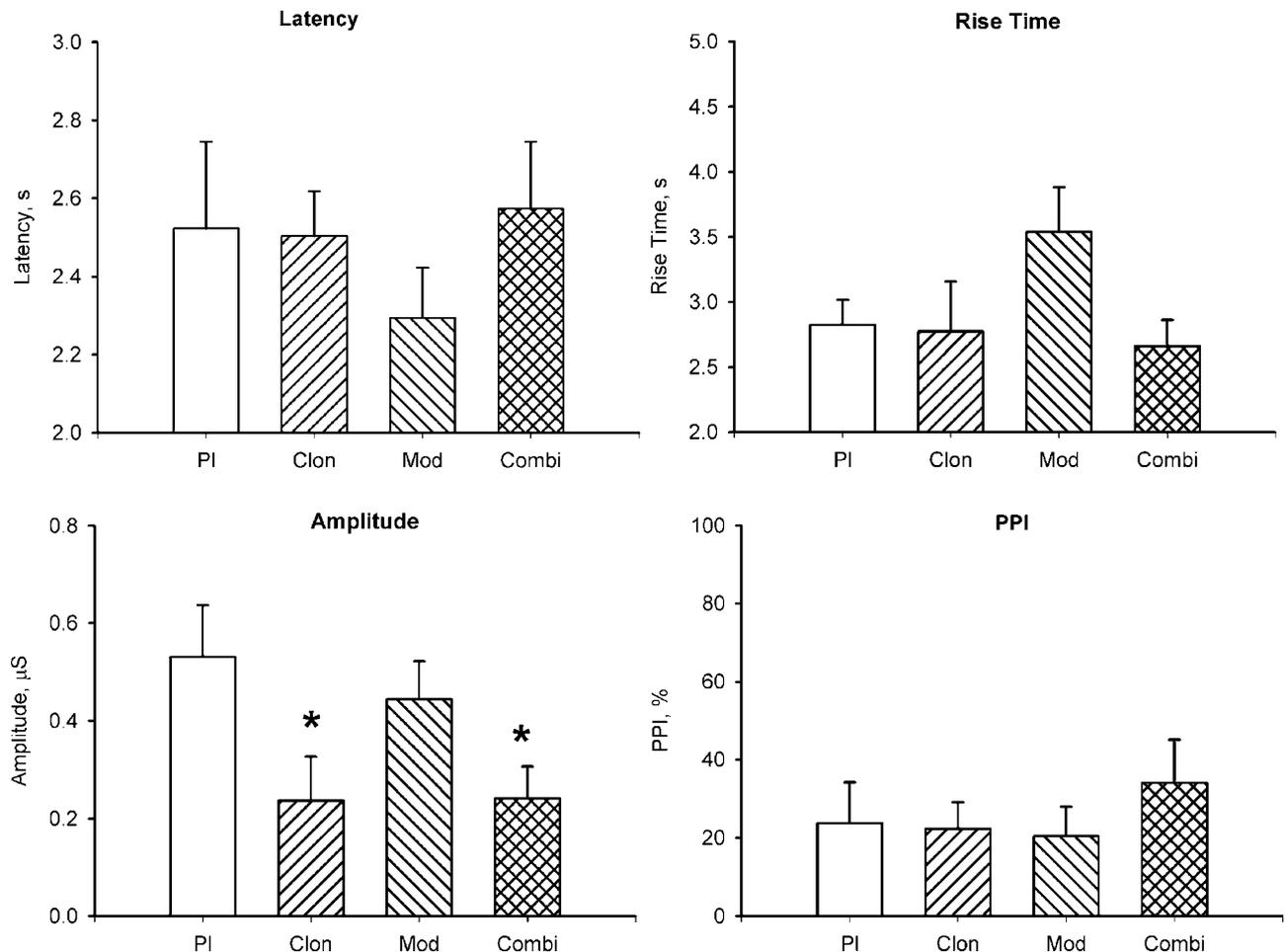


Figure 4 SCR evoked by 115-dB pulses in the pulse-alone trials: latency (s; top left), rise time (s; top right), amplitude (μS ; bottom left) and PPI of the amplitude (%; top right). Columns correspond to mean values of the parameters for the group ($n = 16$); vertical bars represent SEM. Treatment condition is indicated at the bottom of the graphs: PI = placebo, Clon = 0.2 mg clonidine, Mod = 400 mg modafinil, Combi = combination of 0.2 mg clonidine and 400 mg modafinil. Clonidine and the combination of clonidine and modafinil significantly reduced the amplitude of the startle response: $*p < 0.05$ (Dunnett's test: comparison with placebo condition). The response amplitude was significantly reduced following a weak prepulse (PPI; see text for details).

Table 1 Pre-treatment/Post-treatment Differences in Alertness (Mean \pm SEM)

	Placebo	Clonidine	Modafinil	Combination
<i>CFF</i> (Hz)				
VAS (mm)	0.06 \pm 0.21	-2.25 \pm 0.28*	0.61 \pm 0.21	-1.84 \pm 0.29*
Alertness	-1.59 \pm 1.99	-19.10 \pm 3.09*	2.05 \pm 3.24*	-12.07 \pm 3.29*
Calmness	1.18 \pm 3.22	4.29 \pm 2.43	-8.74 \pm 3.03*	0.70 \pm 2.55
Contentedness	-0.76 \pm 1.64	-5.78 \pm 1.23*	-0.78 \pm 2.42	-4.57 \pm 1.84*
<i>PST</i>				
PUI (mm min^{-1})	0.97 \pm 0.94	6.37 \pm 0.87*	-1.85 \pm 0.63*	4.69 \pm 0.98*
Power (arbitrary units)	270 \pm 235	1429 \pm 202*	-456 \pm 163*	1245 \pm 270*
Pupil diameter (mm)	-0.08 \pm 0.09	-1.38 \pm 0.16*	0.23 \pm 0.12	-1.27 \pm 0.21*

*Significant effect compared with placebo ($p < 0.05$).

(bottom) measures of pupil diameter in darkness and at three increasing luminance levels. A two-way ANOVA (treatment \times luminance) on the pretreatment data reveals an effect of luminance ($F_{3,45} = 190.01$, $p < 0.001$) but no

effect of treatment ($F_{3,45} = 0.14$, NS) and no significant interaction ($F_{39,135} = 1.44$, NS). A two-way ANOVA (treatment \times luminance) on the post-treatment data reveals effects of both luminance ($F_{3,45} = 261.32$, $p < 0.001$) and

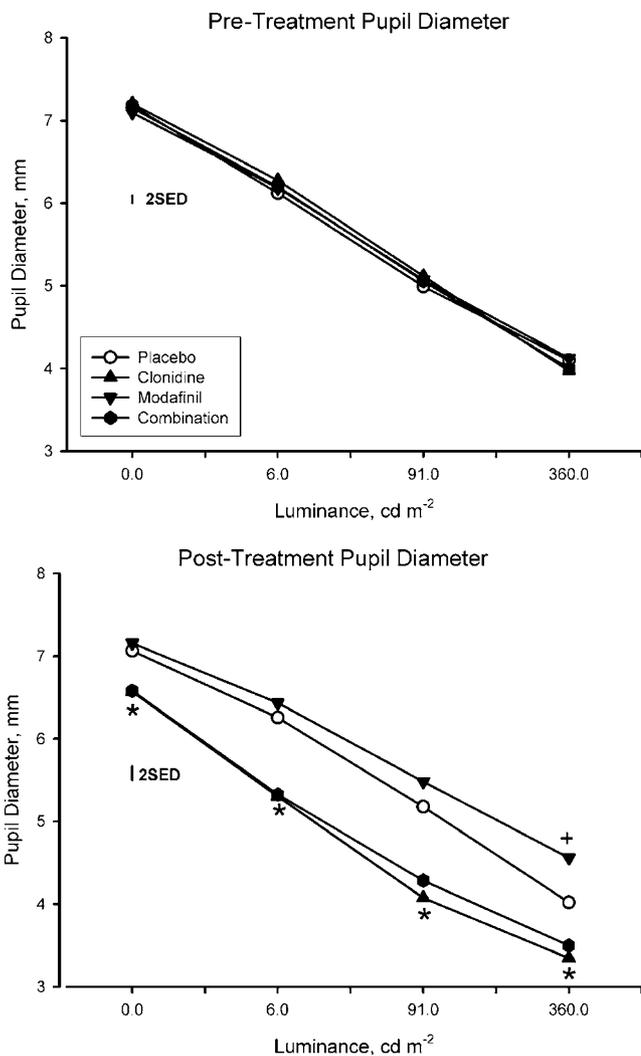


Figure 5 Resting pupil diameter: relationship between level of luminance and pupil diameter pretreatment (top) and post-treatment (bottom). The four treatment conditions are indicated by different symbols (see inset). Ordinate: absolute pupil diameter (mm); abscissa: level of luminance (cd m^{-2}). Each 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 ANOVA. Clonidine and the combination of clonidine and modafinil reduced pupil diameter at all luminance levels, $*p < 0.05$; modafinil increased pupil diameter at 360 cd m^{-2} , $+p < 0.05$ (Dunnett's test: comparison with placebo condition).

treatment ($F_{3,45} = 48.45$, $p < 0.001$) and a significant interaction ($F_{9,135} = 6.04$, $p < 0.001$). Analysis of pupil diameter at each luminance level separately, using one-way ANOVA, demonstrated an effect of treatment in darkness ($F_{3,45} = 17.17$, $p < 0.001$) and at 6 cd m^{-2} ($F_{3,45} = 38.06$, $p < 0.001$), 91 cd m^{-2} ($F_{3,45} = 33.12$, $p < 0.001$) and 360 cd m^{-2} ($F_{3,45} = 30.70$, $p < 0.001$). Multiple comparisons showed that the treatment effects were due to pupillary constriction produced by clonidine and the combination of clonidine and modafinil at all luminance levels as well as pupillary dilatation produced by modafinil at 360 cd m^{-2} .

Heart rate. The effects of the four treatment conditions on heart rate in the supine and standing conditions are shown

in Table 2. There was no effect of treatment on measures of heart rate in the standing or in the supine position. There was a significant effect of treatment on the orthostatic change in heart rate ($F_{3,45} = 5.80$, $p < 0.05$), in which clonidine administration increased this measure.

Blood pressure. The effects of the four treatment conditions on systolic and diastolic blood pressure in the supine and standing positions are shown in Table 2. There was a significant effect of treatment on systolic blood pressure in the supine ($F_{3,45} = 17.36$, $p < 0.001$) and standing ($F_{3,45} = 10.93$, $p < 0.001$) positions. The administration of clonidine reduced both supine and standing systolic blood pressure, while the administration of modafinil increased supine systolic blood pressure. The apparent increase in standing systolic blood pressure following modafinil just failed to reach significance. There was no effect on supine systolic blood pressure following the administration of the combination of clonidine and modafinil; standing systolic blood pressure was reduced following this treatment. There was a significant effect of treatment on diastolic blood pressure in the supine ($F_{3,42} = 11.17$, $p < 0.001$) and standing (\log_{10} transformation; $F_{3,45} = 12.72$, $p < 0.001$) positions. Administration of clonidine and the combination of clonidine and modafinil reduced diastolic blood pressure in both the supine and the standing positions. The apparent increase in diastolic blood pressure in both the supine and the standing positions following modafinil administration failed to reach significance. There was no effect of treatment on orthostatic change for either systolic or diastolic blood pressure.

Temperature. The effects of the four treatment conditions on core temperature are shown in Table 2. There was a significant effect of treatment on temperature measurements ($F_{3,45} = 5.48$, $p < 0.05$), in which the administration of the combination of clonidine and modafinil reduced core temperature.

Salivation. The effects of the four treatment conditions on salivation are shown in Table 2. There was a significant effect of treatment on salivation measurements ($F_{3,45} = 15.22$, $p < 0.001$), in which the administration of both clonidine and the combination of clonidine and modafinil reduced salivation.

Endocrine Functions

The effects of the four treatment conditions on the plasma concentrations of prolactin, TSH, and GH are shown in Figure 6.

Prolactin. There was a significant effect of treatment condition on plasma prolactin levels (\log_{10} transformation; $F_{3,45} = 19.59$, $p < 0.001$), in which modafinil and the combination of clonidine and modafinil reduced the concentration of prolactin

TSH. There was a significant effect of treatment condition on plasma TSH levels (\log_{10} transformation; $F_{3,45} = 3.13$, $p < 0.05$), in which clonidine increased the concentration of TSH.

Table 2 Pretreatment/Post-treatment Differences in Autonomic Function (Mean \pm SEM)

	Placebo	Clonidine	Modafinil	Combination
<i>Heart rate (bpm)</i>				
Supine	-9.81 \pm 1.96	-14.63 \pm 2.68	-4.81 \pm 2.63	-6.81 \pm 2.68
Standing	-11.56 \pm 2.96	-6.13 \pm 2.56	-8.63 \pm 3.42	-9.88 \pm 3.07
Orthostatic change	-1.75 \pm 2.33	8.50 \pm 2.18*	-3.81 \pm 2.45	-3.06 \pm 2.66
<i>Systolic BP (mm Hg)</i>				
Supine	-9.38 \pm 2.74	-22.13 \pm 2.13*	0.25 \pm 2.58*	-15.81 \pm 2.54
Standing	-4.75 \pm 2.76	-20.06 \pm 3.58*	3.81 \pm 2.28	-16.25 \pm 4.29*
Orthostatic change	4.63 \pm 1.21	2.06 \pm 1.50	3.56 \pm 1.07	-0.44 \pm 1.04
<i>Diastolic BP (mm Hg)</i>				
Supine	1.53 \pm 2.27	-8.73 \pm 1.60*	4.40 \pm 2.38	-5.80 \pm 1.49*
Standing	-0.44 \pm 1.75	-10.94 \pm 2.82*	7.44 \pm 1.79	-7.38 \pm 1.95*
Orthostatic change	-2.27 \pm 2.30	-1.13 \pm 2.72	2.67 \pm 2.67	-1.93 \pm 2.31
<i>Temperature ($^{\circ}$C)</i>				
	0.03 \pm 0.13	-0.21 \pm 0.06	0.21 \pm 0.09	-0.33 \pm 0.13*
<i>Salivation (g)</i>				
	-0.06 \pm 0.09	-0.40 \pm 0.05*	0.06 \pm 0.07	-0.45 \pm 0.05*

*Significant effect compared with placebo ($p < 0.05$).

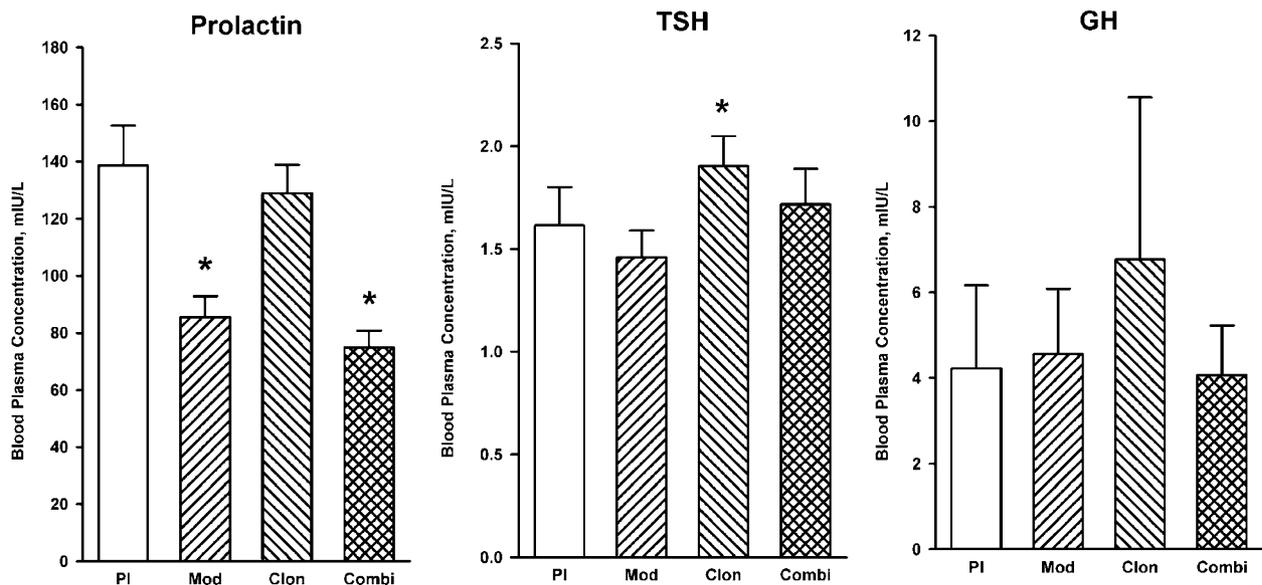


Figure 6 Post-treatment plasma concentrations of prolactin (left), TSH (center), and GH (right). Columns correspond to mean values of the parameters for the group ($n = 16$); vertical bars represent SEM. Treatment condition is indicated at the bottom of the graphs: PI = placebo, Clon = 0.2 mg clonidine, Mod = 400 mg modafinil, Combi = combination of 0.2 mg clonidine and 400 mg modafinil. The ordinate axis represents the hormone plasma concentration (mIU/L^{-1}). Clonidine significantly increased TSH concentration and modafinil significantly decreased prolactin concentration: * $p < 0.05$ (Dunnett's test: comparison with placebo condition).

GH. There was no significant effect of treatment condition on plasma GH levels (\log_{10} transformation; $F_{3,42} = 0.53$, NS).

DISCUSSION

In the present experiment, a single dose of 0.2 mg clonidine reduced both the subjective and the objective level of

alertness in healthy volunteers (see Table 1), in agreement with previous reports (Morley *et al*, 1991; Kumari *et al*, 1996; Abduljawad *et al*, 1997, 2001; Phillips *et al*, 2000; Hou *et al*, 2005, 2006). Alertness was robustly increased by a single dose of 400 mg modafinil, in contrast to previous reports using a 200-mg single dose where either a small effect (Hou *et al*, 2005, 2007) or no effect (Samuels *et al*, 2006) of modafinil in healthy non-sleep-deprived volunteers

was observed. In the latter study it was suggested that a sleep-deprivation protocol would be necessary to observe alertness-enhancing effects in healthy volunteers, because modafinil at a dosage of 200 mg has been effective in promoting alertness when healthy volunteers were sleep-deprived (Wesensten *et al*, 2002; Walsh *et al*, 2004). It has also been found to be alerting in patients with reduced baseline alertness levels (US Modafinil in Narcolepsy Multicentre Study Group, 1998, 2000; Pack *et al*, 2001; Rammohan *et al*, 2002; Szabadi *et al*, 2002; Rosenthal and Bryant, 2004). However, the present study has demonstrated that the larger dose of 400 mg is sufficient for the observation of an alerting effect in a small group of healthy volunteers. Despite the larger dose of modafinil used and the robust alerting effects of the drug administered alone, the administration of modafinil in combination with clonidine failed to antagonize the sedative effects produced by clonidine alone. Thus, the sedative effect of clonidine 0.2 mg appears to supersede the alerting effect of 400 mg modafinil. In addition to the opposite effects of clonidine and modafinil on subjectively rated alertness, the two drugs also had differential effects on the other two factors rated on the VAS: calmness was reduced by modafinil and unaffected by clonidine, and contentedness was reduced by clonidine and unaffected by modafinil. The reduction in calmness in response to modafinil may indicate a mild anxiogenic effect of this relatively high dosage of the drug, although it was not observed using the lower dosage level of 200 mg (Samuels *et al*, 2006). The reduction of subjective contentedness by clonidine indicates that the experience of sedation may be perceived as unpleasant.

The presentation of the auditory pulse stimulus produced a startle response in the orbicularis oculi muscle that was altered by the administration of clonidine: the latency to respond to the stimulus was increased and the amplitude of the response was reduced (see Figure 2). The effect on response amplitude is in agreement with previous reports on the effect of clonidine on the acoustic startle response amplitude (Kumari *et al*, 1996; Abduljawad *et al*, 1997, 2001) and also with previous reports on the effect of sedative drugs on the startle response amplitude (Phillips *et al*, 2000a; Graham *et al*, 2001, 2002, 2004; Scaife *et al*, 2005). The effect on latency is consistent with a reduced startle response to the acoustic stimulus.

Clonidine is known to act on presynaptic α_2 adrenoceptors in the LC and reduce neuronal activity (Szabadi and Bradshaw, 1996). The noradrenergic neurons of the LC, a major wakefulness-promoting nucleus, innervate the cerebral cortex (Jones *et al*, 1977; Robbins, 1984; Jones and Yang, 1985; Séguéla *et al*, 1990) and increase EEG signs of arousal (Berridge and Foote, 1991). Apart from directly activating the cerebral cortex, the LC also exerts an indirect alerting effect by inhibiting the ventrolateral preoptic nucleus (VLPO) of the hypothalamus (Nelson *et al*, 2002, 2003). It is possible that the sedative effects of certain drugs, including clonidine, may reflect an underlying reduction in the activity of the LC and thus a reduction in the arousal-enhancing outputs to the cortex. It is of interest that the LC also projects to the motoneurons in the facial nucleus (Jones and Yang, 1985), which innervates the orbicularis oculi muscle involved in the acoustic startle response, and that the application of noradrenaline enhances the excitatory

activity of these motoneurons (VanderMaelen and Aghajanian, 1980; Rasmussen and Aghajanian, 1990; White *et al*, 1991). Thus, the LC has a facilitatory influence on the facial nucleus, enhancing the tone of the orbicularis oculi muscle. It is therefore an intriguing possibility that a reduction in LC activity brought about by sedative drugs leads to a reduction in the tone of the orbicularis oculi muscle manifesting as a reduction in EMG startle response amplitude. We suggest that the suppression of startle response amplitude by sedative drugs may be due to a reduction in LC activity leading to the attenuation of the noradrenergic facilitation of the facial nucleus.

If our hypothesis is correct, it would have been expected that drugs enhancing LC activity would enhance startle reflex amplitude. In support of this hypothesis, it has been reported that the α_2 adrenoceptor antagonist yohimbine, which is known to enhance LC activity (Ivanov and Aston-Jones, 1995; Singewald and Sharp, 2000; Tanaka *et al*, 2000), enhances the amplitude of the acoustic startle response (Morgan *et al*, 1993). While modafinil on its own had no effect upon startle response latency or amplitude in the present experiment (see Figure 2), its administration in combination with clonidine resulted in an acoustic startle response that was not significantly different from the response obtained in the placebo condition. This indicates a functional antagonistic relationship between clonidine and modafinil, which in turn may reflect their opposite effects on LC activity (Hou *et al*, 2005).

The acoustic startle response amplitude was significantly suppressed by the presentation of a weak prepulse before the presentation of the startle-eliciting pulse, that is, there was significant PPI. This is in agreement with many previous reports (for example, Graham, 1975; Abduljawad *et al*, 1999; Phillips *et al*, 2000a, b; Graham *et al*, 2001, 2002, 2004; Bitsios *et al*, 2005; for reviews see also Davis, 1980; Swerdlow *et al*, 1992; Koch, 1999). None of the treatment conditions had any significant effect on PPI of the acoustic startle response (see Figure 2). For clonidine, this is in agreement with previous reports in healthy volunteers in which no effects on PPI were found (Abduljawad *et al*, 1997, 2001). In this respect it is of interest that, whereas the α_2 adrenoceptor agonist clonidine does not affect PPI in humans, the administration of α_1 -adrenoceptor agonists in rats (Carasso *et al*, 1998) and the absence of α_2 -adrenoceptors in transgenic mice (Sallinen *et al*, 1998) have been reported to lead to the disruption of PPI.

Like clonidine, modafinil was without any effect on PPI of the acoustic startle response. This negative finding is of special interest because the dopaminergic system has been implicated in the mode of action of modafinil and the dopaminergic system is known to play a key role in mediating PPI (Swerdlow *et al*, 1992; Koch and Schnitzler, 1997; Koch, 1999; Zhang *et al*, 2000). Indeed, it has been shown that amphetamine, a wakefulness-promoting drug acting via dopaminergic mechanisms (Bunney *et al*, 1973a, b; Calcagnetti and Schechter, 1992; Nishino *et al*, 1998; Paladini *et al*, 2001), disrupts PPI of the startle response in both rat (Mansbach *et al*, 1988; Swerdlow *et al*, 1990; Sills, 1999; Zhang *et al*, 2000; Swerdlow *et al*, 2003; Brunell and Spear, 2006) and human (Kumari *et al*, 1998; Hutchison and Swift, 1999; Swerdlow *et al*, 2003) startle paradigms. Although both modafinil and amphetamine

promote wakefulness, modafinil does not seem to share the psychostimulant and addictive potentials of amphetamine (Simon *et al*, 1995; Ferraro *et al*, 1997; Engber *et al*, 1998a, b; Jasinski, 2000; Deroche-Gamonet *et al*, 2002; Chapotot *et al*, 2003), which are known to be mediated via the mesoaccumbal dopaminergic system (Di Ciano *et al*, 1995; Zhang *et al*, 2000; Di Chiara *et al*, 2004). It is therefore tempting to speculate that modafinil may spare this dopaminergic projection while enhancing the activity of other dopaminergic pathways important in the maintenance of alertness. However, it should be noted that in studies in which amphetamine-induced PPI disruption in human volunteers was observed, the startle response was elicited using white-noise stimuli. In contrast, pure-tone stimuli were used in the present study. This may be of some relevance to the conclusions drawn from the lack of PPI disruption following modafinil, since a recent study found that disruptions in PPI in patients with schizophrenia were more easily detectable following the presentation of a white-noise startling stimulus compared to a pure-tone stimulus (Braff *et al*, 2001). It would be of interest, therefore, to compare the effects of modafinil and amphetamine on PPI in the same group of subjects using identical experimental conditions.

Presentation of the auditory pulse stimulus also resulted in an AEP, where the N1/P2 component reflects underlying cortical processing in the primary and secondary auditory cortices (Näätänen and Picton, 1987; Mauguière *et al*, 1995). Clonidine increased the time to the N1 peak (see Figure 3), suggesting that clonidine may slow the cortical processing of sensory information. Clonidine, similar to its effect on the EMG startle response amplitude, also reduced the amplitude on the N1/P2 component. This observation is in contrast to a previous report that, while describing the suppression of the EMG response of the ASR, failed to detect any change in the amplitude of the N1/P2 component of the AEP (Abduljawad *et al*, 2001). This suggests that suppression of the muscle response by clonidine is a more robust finding than suppression of the N1/P2 component of the AEP. Indeed, although a number of studies have shown that sedative drugs suppress the amplitude of the muscle response of the ASR (see above), many such drugs are without any effect on the amplitude of the N1/P2 component (clozapine: Graham *et al*, 2001; quetiapine: Graham *et al*, 2004; ketanserin: Graham *et al*, 2002; diphenhydramine: Curran *et al*, 1998; Scaife *et al*, 2005). On the other hand, the benzodiazepines have been reported with some consistency to be able to suppress the amplitude of the N1/P2 component (Lader, 1977; Pooviboonsuk *et al*, 1996; Curran *et al*, 1998; Abduljawad *et al*, 2001). The discrepancy between the effects of sedative drugs on the amplitudes of the muscle response and the N1/P2 component of the AEP suggests the involvement of different mechanisms. The mechanism in the case of the muscle response is likely to be the modulation of the tone of the orbicularis oculi muscle by the LC (see above), and this mechanism is obviously not involved in the AEP. Benzodiazepines may have an additional effect in modulating the generation of the AEP. The suppression of the amplitude of the N1/P2 component of the AEP by clonidine in the present experiment suggests that some other sedative drugs may also share this mechanism.

Interestingly, modafinil affected the N1/P2 component in an opposite direction to the effect of clonidine. Modafinil reduced the time to the P2 peak (see Figure 3), an effect that may reflect an increase in the speed of cortical processing of sensory stimuli. Although modafinil on its own had no effect on N1/P2 amplitude, it antagonized the suppressant effect of clonidine. It is tempting to speculate that the opposite effects of the two drugs reflect their opposite actions on arousal mechanisms.

Presentation of the weak prepulse before the startle-eliciting pulse produced significant PPI of the N1/P2 amplitude, in agreement with many previous reports on the N1/P2 component from our laboratory (Abduljawad *et al*, 1999, 2001; Phillips *et al*, 2000a, b; Graham *et al*, 2001, 2002, 2004) and on various AEP components by others (Perlstein *et al*, 1993, 2001; Schall *et al*, 1996; Bender *et al*, 1999). None of the treatment conditions had any significant effect on PPI of the AEP (see Figure 3). The lack of effect of clonidine on PPI of the N1/P2 component is in agreement with a previous report (Abduljawad *et al*, 2001). It is of interest that of a number of drugs tested (reboxetine, fluvoxamine, amitriptyline: Phillips *et al*, 2000a; haloperidol, clozapine: Graham *et al*, 2001; ketanserin: Graham *et al*, 2002; quetiapine: Graham *et al*, 2004), only two (amitriptyline and clozapine) have been reported to reduce PPI of the N1/P2 complex of the AEP. The lack of effect of clonidine (α_2 -adrenoceptor agonist), haloperidol (D_2 dopamine receptor and α_1 -adrenoceptor antagonist), reboxetine (noradrenaline reuptake inhibitor), ketanserin ($5HT_2$ receptor antagonist), and fluvoxamine (5HT uptake inhibitor) argues against the involvement of noradrenergic, dopaminergic and serotonergic mechanisms. A shared feature of amitriptyline and clozapine is high affinity for muscarinic cholinergic receptors, suggesting a possible role for the central cholinergic system in mediating PPI of the AEP.

The effect of the acoustic pulse stimulus could also be detected in the SCR, in agreement with previous reports (Turpin *et al*, 1999; Graham *et al*, 2005; Scaife *et al*, 2005). Clonidine, as well as the combination of clonidine and modafinil, reduced the amplitude of the SCR, whereas modafinil on its own had no effect on this response (see Figure 4). Because skin conductance is mediated via the sympathetic nervous system (Wallin, 1981), the reduction in the SCR by clonidine is concordant with its sympatholytic action, which in turn may reflect the ability of clonidine to 'switch off' LC activity (Szabadi and Bradshaw, 1996). This finding demonstrates that the reduction in the response to the startle-eliciting acoustic stimulus following the administration of clonidine can be observed not only in the EMG and AEP responses but also in the longer-latency autonomic component of the startle response. In contrast to clonidine, modafinil failed to modify the SCR, indicating that the sudomotor system is less sensitive to the sympathetic activating effect of modafinil than the pupillary and cardiovascular systems where modafinil evoked distinct effects consistent with sympathetic activation (see below). Furthermore, consistent with this finding, modafinil failed to antagonize the suppressant effect of clonidine on the SCR in contrast to its antagonistic effects on the EMG and AEP responses.

Presentation of the weak prepulse produced significant PPI of the SCR, an effect that has not previously been

investigated for this component of the startle response. It is interesting that a weak prepulse can inhibit the effect of a startling stimulus on this longer-latency response in addition to the well-documented effects on the faster responses in the EMG and AEP, and this demonstrates that sensorimotor gating has widespread physiologic consequences that include influences on autonomic nervous system activity. Neither clonidine nor modafinil affected PPI of the SCR (see Figure 4). This is in agreement with the effects of clonidine and modafinil on PPI of the EMG and AEP responses (see above) and suggests that the mechanism underlying PPI of these three responses is unaffected by noradrenergic modulation by either drug.

The effect of clonidine on autonomic responses is in agreement with previous reports. Clonidine reduced pupil diameter (Bitsios *et al*, 1996; Phillips *et al*, 2000c,d; Hou *et al*, 2005, 2006), reduced systolic and diastolic blood pressure in both the supine and the standing positions (Lal *et al*, 1975; Berlan *et al*, 1989; Morley *et al*, 1991; Bitsios *et al*, 1996; Kumari *et al*, 1996; Abduljawad *et al*, 1997, 2001; Arya *et al*, 1997; Phillips *et al*, 2000d; Hou *et al*, 2005, 2006), reduced salivation (Bitsios *et al*, 1996; Abduljawad *et al*, 1997, 2001; Arya *et al*, 1997; Phillips *et al*, 2000d; Hou *et al*, 2005, 2006), and reduced core temperature (Arya *et al*, 1997; Hou *et al*, 2006) (see Figure 5 and Table 2). The autonomic effects of clonidine are consistent with the stimulation of inhibitory α_2 -adrenoceptors on central neurons. The stimulation of autoreceptors on the noradrenergic cell bodies would 'switch off' the activity of the LC and other noradrenergic nuclei (ie A1/A5) leading to a central sympatholytic effect, manifesting as miosis, hypotension, and hypothermia, whereas the stimulation of postsynaptic receptors on salivatory neurons would result in a parasympatholytic effect, manifesting as hyposalivation (Szabadi and Bradshaw, 1996; Szabadi and Tavernor, 1999).

In contrast to the robust effects of clonidine on autonomic functions (see above), modafinil had relatively minor effects. The autonomic effects of modafinil were in opposite directions to those of clonidine, consistent with sympathetic activation, which in turn may be related to the activation of the LC (Hou *et al*, 2005; Taneja *et al*, 2005). Thus, modafinil caused a significant increase in pupil diameter at the highest luminance level, in agreement with a previous report using a 200-mg dose of modafinil (Hou *et al*, 2005, 2007). Modafinil also appeared to increase blood pressure, although only the change in supine systolic blood pressure reached statistical significance. This small increase is in agreement with previous reports on the effect of modafinil on blood pressure (200 mg: Makris *et al*, 2004; Turner *et al*, 2003; Samuels *et al*, 2006; >200 mg: Caldwell *et al*, 2000; Rush *et al*, 2002; Makris *et al*, 2004; Taneja *et al*, 2005). In addition, when clonidine and modafinil were administered together, modafinil antagonized the effect of clonidine on supine systolic blood pressure. In the present study, modafinil did not affect core temperature although previously modafinil has produced small increases in core temperature (Brun *et al*, 1998; Samuels *et al*, 2006).

The effects of clonidine and modafinil on the plasma concentrations of the hormones prolactin, TSH and GH, shown in Figure 6, were consistent with the complex catecholaminergic regulation of these pituitary hormones (see Figure 7 in Samuels *et al*, 2006) and showed that the

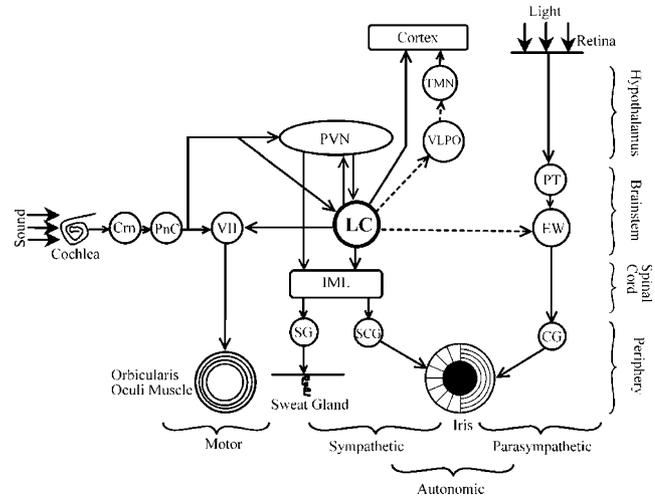


Figure 7 The central role of the LC in the regulation of arousal, autonomic function and motoneuron activity. Arousal: the LC sends an inhibitory output to the VLPO, a sleep-promoting area that inhibits the tuberomammillary nucleus (TMN). Both the LC and the TMN send major wakefulness-promoting excitatory outputs to the cerebral cortex to enhance alertness (Nelson *et al*, 2002, 2003; Hou *et al*, 2005). Autonomic function: the LC and the PVN of the hypothalamus are major pre-autonomic nuclei controlling sympathetic function. This is exemplified by pupillary and sweat gland activities. The LC is involved in the autonomic control of the pupil via projections to the preganglionic sympathetic neurons in the IML, which projects to the peripheral sympathetic superior cervical ganglion (SCG) that innervates the dilator muscle in the iris. The LC also inhibits the parasympathetic pathway controlling pupil diameter via a projection to the Edinger-Westphal nucleus (EW). The EW receives input from the pretectal nucleus (PT), which conveys light information from the retina, and projects to the ciliary ganglion (CG) that innervates the constrictor muscle in the iris (pupillary light reflex pathway) (Szabadi and Bradshaw, 1996). The cholinergic neurons in the sympathetic ganglia (SG) innervating sweat glands receive inputs from the IML, which in turn is under control from the PVN and LC. Motoneuron activity is facilitated by noradrenergic outputs from the LC. Both components (motor and autonomic) of the acoustic startle response are under noradrenergic modulation from the LC. The acoustic stimulus activates motoneurons in the facial nucleus (VII) and preautonomic neurons in the PVN and LC via relay neurons in the cochlea root nucleus (Cm) and caudal pontine reticular nucleus (PnC). The LC, apart from directly contributing to central sympathetic outflow, modulates facial motoneuron and PVN activity (see above) and thus the magnitude of the acoustic startle response (see text for details). Excitatory connections, solid lines; inhibitory connections, broken lines.

single doses used by us were also 'endocrinologically active.' Clonidine enhanced the plasma concentration of TSH but failed to affect the concentration of the other two hormones. The enhancement of TSH level by clonidine is in agreement with a previous report on the effect of medetomidine, another α_2 -adrenoceptor agonist (Kallio *et al*, 1988). The noradrenergic system exerts a stimulatory influence on TSH secretion (see Samuels *et al*, 2006), partly by enhancing stimulatory thyrotropin-releasing hormone secretion via excitatory α_1 -adrenoceptors and partly by reducing inhibitory somatotropin secretion via inhibitory α_2 -adrenoceptors. The stimulatory influence of clonidine on TSH secretion is likely to reflect the stimulation of postsynaptic α_2 -adrenoceptors on tuberoinfundibular neurons inhibiting the secretion of somatostatin. Although clonidine caused some increase in GH secretion, this increase failed to reach

statistical significance. Although the ability of clonidine to enhance GH secretion is well established (Lal *et al*, 1975; Lancranjan and Marbach, 1977; Gaspar *et al*, 1984; Murphy *et al*, 1984; Hunt *et al*, 1986), it is recognized that there are great individual differences in the GH-secretion-stimulating effect of the drug (Lal *et al*, 1975; Lancranjan and Marbach, 1977; Hunt *et al*, 1986; Vasconcellos and Spritzer, 2004). These variations in sensitivity, coupled with inter-individual variations in the time to peak of the response (Gaspar *et al*, 1984; Price *et al*, 1986; Cavallo *et al*, 1990), would have militated against the detection of a more robust effect in our relatively small sample. As in the case of TSH secretion, the stimulatory effect of clonidine on GH secretion is likely to be due to the attenuation of the inhibitory influence of somatostatin on the secretion of the hormone. Clonidine had no effect on the plasma concentration of prolactin, in agreement with a previous report (Lal *et al*, 1975). Modafinil reduced prolactin concentration, in agreement with our own previous report (Samuels *et al*, 2006), probably reflecting the enhancement of the noradrenergic stimulation of prolactin-release-inhibiting dopaminergic mechanisms in the arcuate nucleus. Modafinil had no effect on the plasma concentrations of TSH and GH, confirming previous observations (Brun *et al*, 1998; Samuels *et al*, 2006).

Although the present results *per se* do not allow us to infer the neuronal circuitry involved in the intimately linked regulation of arousal and startle responses, taken together with the published literature (eg Davis, 1980; De Sarro *et al*, 1987; Morgan *et al*, 1993; Sallinen *et al*, 1998; Hou *et al*, 2005; Samuels *et al*, 2006) they highlight the importance of the central noradrenergic system in controlling both functions. The LC may play a role in modulating both muscular and autonomic startle responses (see Figure 7). The noradrenergic facilitation of the activity of motor neurons is well documented, and attenuation of this facilitation by sedative drugs may underlie the inhibitory effect of these drugs on muscular startle responses. Furthermore, startle-eliciting acoustic stimuli lead to sympathetic activation manifesting as increases in BP (Baudrie *et al*, 1997; Holand *et al*, 1999; Girard *et al*, 2001) and sweat gland activity (present SCR results, see above), suggesting that the startle circuit impinges on central preautonomic neurons located in the paraventricular nucleus (PVN) of the hypothalamus and the LC, which in turn facilitate the activity of preganglionic sympathetic neurons in the intermediolateral (IML) cell column of the spinal cord. LC activation by an acoustic stimulus would be expected to be reflected in pupillary function because an increase in LC activity results in pupil dilatation and inhibition of the light reflex response (Szabadi and Bradshaw, 1996). The pupillary response to a startle-eliciting acoustic stimulus has not been studied so far, probably due to the difficulty of recording pupillary changes at the time of the eye-blink response.

In conclusion, the results of the present study have revealed several important points regarding the acoustic startle response paradigm: (i) Clonidine reduces the muscular startle response elicited in the orbicularis oculi muscle by a loud auditory stimulus, and this reduction can be antagonized by modafinil, although modafinil has no effect when administered alone, (ii) modafinil does not disrupt PPI of the muscular response, indicating that the

mechanism of action of modafinil is distinct from that of amphetamine, (iii) clonidine reduces the AEP response to the auditory stimulus, whereas modafinil facilitates the AEP response (shortening of latency), indicating reduced and increased speed, respectively, of cortical processing following drug administration, (iv) clonidine reduces the SCR elicited by the auditory stimulus, and (v) significant PPI of the SCR can be observed following the presentation of a weaker prepulse before the presentation of the pulse. A comprehensive framework for interpreting the effects of sedative and alerting drugs on startle response amplitude, physiological measures of arousal and autonomic activity has been proposed to explain these results. In addition, this study has demonstrated alerting effects of a 400-mg dose of modafinil in non-sleep-deprived volunteers. The autonomic and endocrine effects following clonidine and modafinil are largely consistent with previous reports and fit with the proposed mechanisms of action of both drugs.

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