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# Assessment of modafinil on attentional processes in a five-choice serial reaction time test in the rat

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## Abstract

It is well known that modafinil is an effective wake-promoting agent, but there is growing evidence to suggest that modafinil may also enhance some aspects of cognition. In man, modafinil has been shown to enhance vigilance in sleep-deprived and/or narcoleptic subjects and also to improve executive-type functioning (predominantly inhibitory response control processes) across a variety of human patient population groups. Preclinically, a delay-dependent improvement has been reported with modafinil in a mouse T-maze test of working memory. To investigate further the role of modafinil as a potential cognition enhancer, the effects of modafinil on attentional processes were assessed in the rat. The aim of the present study was to evaluate the potential of modafinil to enhance five-choice serial reaction time test (5-CSRT) performance. Lister Hooded rats received 32–128 mg/kg modafinil and 5-CSRT performance was assessed under standard and test parametric conditions in which the attentional load was increased, and also under conditions of scopolamine pre-treatment. Modafinil failed to significantly enhance 5-CSRT performance under standard conditions. Similarly, modafinil was unable to reverse the deficits in accuracy and/or increased omission errors induced by either parametric or pharmacological manipulations. Indeed, at higher doses, modafinil caused an increase in premature responding under certain test conditions, suggestive of increased impulsivity. The present findings suggest that, although modafinil may enhance vigilance in sleep-deprived human subjects, attentional processes in normal awake rats remain unaffected. No evidence was found to support a modafinil-induced improvement in response control; rather, under conditions of increased attentional load, modafinil appeared to facilitate impulsive responding. Finally, the failure of modafinil to improve a scopolamine-induced performance deficit suggests that modafinil does not act on the cholinergic system directly.

### Keywords

attention, 5-choice serial reaction time test, cognition, modafinil, rat, response control, scopolamine

## Introduction

Modafinil (diphenylmethyl sulphinyl-2-acetamide) was first identified as a novel wake-promoting agent for the treatment of narcolepsy, a disorder characterized by excessive daytime sleepiness. However, in addition to enhancing wakefulness, many recent sleep-deprivation studies also support a role for modafinil as a novel cognition enhancer. For example, modafinil was shown to enhance alertness and maintain the performance of sleep-deprived aviators in a flight simulator model (Caldwell *et al*., 2000), and to attenuate impairments in tests of perceptual judgement and mental addition in sleep-deprived volunteers (Baranski *et al*., 1997). Similarly, improvements in performance on a four-choice serial reaction time test have been demonstrated for up to 6 h when modafinil was administered after 47 h of sleep-deprivation, with the most pronounced effects observed during the early morning when fatigue was greatest (Pigeau *et al*., 1995). In the same study, logical reasoning and digit span performance were also enhanced by modafinil administration, in a comparable manner to that observed with amphetamine. Such effects have been replicated in a similar study where modafinil and caffeine were found to enhance alertness, mood and cognitive performance equally in a

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battery of tests, with the greatest effects again being observed during maximum fatigue, although the effect of modafinil appeared to be independent of dose (Wesensten *et al*., 2002). More recently, modafinil has been shown to both sustain wakefulness and to improve performance on the Wisconsin Card Sort Test in narcoleptic patients (Schwartz *et al*., 2004), and improve alertness, vigilance and aspects of executive functioning under conditions of simulated night shifts (Walsh *et al*., 2004).

Further investigations have focused on the wider potential of modafinil as a novel stimulant in non sleep-deprived subjects. Specifically, the effects of modafinil in normal healthy volunteers have been investigated. Modafinil was shown to significantly improve performance in tests of digit span, visual pattern recognition memory, delayed matching-to-sample, spatial planning and stop-signal reaction time at a 200 mg dose in a healthy young population (Turner *et al*., 2003). This improvement in performance was also accompanied by an increased latency to respond in many of the tasks, indicating that modafinil may enhance accuracy by causing an increase in the time taken to evaluate a problem before responding. Interestingly, these effects were not ubiquitous because modafinil failed to enhance performance in other tests from the CANTAB battery, namely spatial memory span, spatial working memory, rapid visual information processing, attentional set shifting or paired associate learning (Turner *et al*., 2003). Nevertheless, the data from this study suggest that modafinil may selectively enhance certain aspects of cognition in normal humans.

However, these findings are equivocal. In a separate study, modafinil was shown to have no effect on cognitive performance in tests of episodic memory, problem solving, sustained attention, mental flexibility and set shifting at a 200 mg (and 100 mg) dose in healthy young volunteers (Randall *et al*., 2003). Indeed, modafinil at 100 mg appeared to induce significantly higher ratings of 'somatic anxiety' compared to control-treated patients (similar to that reported by others, albeit at higher doses; see, for example, Caldwell *et al*., 2000). It is probable that the ability to detect an enhancement with modafinil may have been confounded as normal task performance appeared near ceiling. However, to address the possibility that the benefits of modafinil may only become apparent when there is some degree of impaired performance, healthy middle-aged subjects were utilized in a follow-up study, and were shown to be impaired in an attentional set-shifting task following modafinil treatment (Randall *et al*., 2004). This, combined with the finding that modafinil also significantly reduced the latency to complete a simple colour naming of dots (the control condition of the Stroop test), may be indicative that the impairment in cognitive performance induced by modafinil in this latter study may be due principally to an increase in speed of responding, a finding which is contrary to that published by Turner *et al*. (2003).

Preclinically, there is evidence to suggest that modafinil may improve some cognitive functions in normal mice. In a spontaneous alternation T-maze task, modafinil treatment induced a delay-dependent significant enhancement in spatial working memory (Béracochéa *et al*., 2000), and the authors hypothesized that modafinil may exert its effect by reducing the level of interference among trials or by enhancing the memory mechanisms involved in storing and sustaining information over short delay intervals. In addition, the same group demonstrated that administration of modafinil also induced a significant facilitation in acquisition of a T-maze serial spatial discrimination task (Béracochéa *et al*., 2002), which was dependent on the level of training (Béracochéa *et al*., 2003). In both cases, modafinil-treated mice required significantly fewer trials to reach the criterion level of performance compared to vehicle-treated controls, suggesting that modafinil is able to improve both learning and memory processes in rodents.

Overall, there would appear to be growing (albeit equivocal) evidence to support a role for modafinil in cognition, specifically in enhancing attentional processes in sleep-deprived human subjects, facilitating aspects of executive-type functions in non sleep-deprived humans, and in improving mnemonic function in mice. However, the effects of modafinil on attentional processes, specifically in rodents, remain undetermined. To address this issue, the present study investigated the effects of modafinil on performance in the five-choice serial reaction time test (5-CSRT). This is a test of visuo-spatial attention analogous to the continuous performance task in humans. It has been used extensively in rats for measuring the effects of systemic and central pharmacological manipulations on various aspects of attentional performance, including selective, divided and sustained attention (or vigilance), and executive or response control (Carli *et al*., 1983; Robbins, 2002; Chudasama and Robbins, 2004). The effects of modafinil were evaluated under both standard conditions and under test conditions where the attentional load was increased (e.g. by reducing the stimulus duration and/or intensity). Previous studies have demonstrated that such manipulations induce a robust deficit in accuracy (Stolerman *et al*., 2000; Robbins, 2002). A secondary aim of the present study was to examine whether modafinil may exert an effect on attentional processes via the cholinergic system. Previous studies have shown that cholinergic dysfunction can induce deficits in 5-CSRT performance (Muir *et al*., 1992; Jones and Higgins, 1995), which can be reversed by acetylcholinesterase inhibitors such as donepezil and tacrine (Kirkby *et al*., 1996) and physostigmine (Muir *et al*., 1992). Consequently, the present study also compared the effects of modafinil with that of physostigmine following scopolamine pre-treatment in the 5-CSRT. Finally, before conducting the behavioural experiments, a pharmacokinetic study was undertaken to confirm an optimal dose and administration regime for modafinil.

## Methods

### *Pharmacokinetic study*

***Subjects*** A total of 72 male adult Lister Hooded rats (Harlan, Oxon, UK) were used. The rats were group-housed four per cage under a 12 : 12 h light/dark cycle (lights on at 07.00 h) under controlled temperature (21 ± 2 °C) and humidity (55 ± 5%). The rats weighed 240–270 g on the day of the experiment. Food was withheld overnight before dosing, but water was freely available at all times. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986.

***Procedure*** Animals were divided into three groups and were dosed with either 32, 64 or 128 mg/kg modafinil p.o. Three rats from each drug group were then culled by stunning and decapitation at time points of 15, 30, 45, 60, 75, 90, 120 and 240 min post-drug administration and samples of plasma and brain were taken. Each plasma sample (50 µl) was prepared by addition of an internal standard (10 µl of 1 ng/µl diazepam) and dimethyl sulphoxide (DMSO) (10 µl) followed by precipitation of proteins with acetonitrile (150 µl). After mixing and centrifugation, 25 µl of supernatant was mixed with 125 µl of 25 mM ammonium formate, pH 3. The resulting supernatants were analysed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Brains were homogenized in four volumes of pH 7 saline, and then processed as for the plasma samples. Aliquots of control plasma or brain homogenate spiked with modafinil (10 µl of appropriate concentration in DMSO) were prepared in the same way to generate calibration and quality control samples. The calibration ranges for the plasma and brain assays were 2–40 000 ng/ml and ng/g, respectively. In each case, quality controls samples ($n = 8$ per level) at 40, 400 and 4000 ng/ml or ng/g gave coefficients of variation of < 10% and accuracy within 11% of the expected concentration. These procedures were performed on 96-well plates using an automated method on a Biomek 2000 robot. For LC-MS/MS a Kromasil KR100 5C18 (HiChrom, Berks, UK) (5 cm × 3.2 mm i.d.) column was used, with gradient elution employing acetonitrile and 25 mM ammonium formate, buffered to pH 3. An injection volume of 10 µl was used, and a Micromass Quattro LC mass spectrometer (Waters Ltd, Herts, UK) was used with MRM detection. The transitions used were: modafinil (sodium adduct) 296 → 129; diazepam (proton adduct) 285 → 193. To minimize degradation of modafinil to modafinil acid, plasma samples and brains were thawed at 4 °C and brains were homogenized on ice. Plasma samples and brain homogenates were maintained on ice during sample preparation. Statistical analysis of the pharmacokinetic data was not appropriate because only three animals per group were dosed.

## *5-CSRT experiments*

***Subjects*** A total of 64 male adult Lister Hooded rats (Harlan, Oxon, UK) were trained to perform the 5-CSRT task. The rats were group-housed four per cage under a 12 : 12 h light/dark cycle (lights on at 07.00 h) under controlled temperature (21 ± 2 °C) and humidity (55 ± 5%). The rats weighed 270–300 g at the start of training and were food-deprived to 85% of their free-feeding weight throughout testing, with water freely available. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986.

***Apparatus*** The test apparatus consisted of 16, 25 × 25 cm test aluminium chambers housed within sound-insulated and ventilated enclosures. The rear wall of the chamber contained five 2.5 $cm^2$ apertures, 5 cm deep and 2.5 cm above the level of the floor. Each aperture had a 3 W bulb located at the rear to provide the stimulus light, and a photocell across the front which enabled a nose-poke to be detected. On the front wall of the chamber, a food hopper was covered by a hinged plexi-glass panel connected to a micro-switch. Noyes 45 mg food pellets (Noyes, Middlesex, UK) were automatically dispensed into the food hopper following a correct response. The chamber was illuminated by a central house-light in the roof. The apparatus and data collection were controlled by an Acorn RISC computer (Pace Micro Technology, Yorks, UK) and a Paul Fray controller unit (Paul Fray Ltd, Cambridge, UK).

***Behavioural procedure*** The training procedure was similar to that described previously (e.g. Carli *et al*., 1983; Muir *et al*., 1992). Rats were trained over approximately 3 months to respond with a nose poke to a brief light stimulus presented pseudo-randomly in one of the five apertures in the test chamber. A nose poke made into the correct aperture when the light was on (stimulus duration, SD) or within a 5-s period afterwards (limited hold, LH) was recorded as a correct response and a food pellet was automatically dispensed into the food hopper. When the rat retrieved this food reward by pushing the hopper panel, the inter-trial interval (ITI) was initiated. A nose poke made into an incorrect aperture (incorrect response), or after the limited hold period (omission), or during the ITI (premature response), resulted in a timeout period (TO) and the house-light was extinguished for 5 s. Each daily training session consisted of 100 trials or was terminated after 30 min. During training, the stimulus duration and limited hold were progressively shortened and the inter-trial interval and timeout were increased until target parameters of SD = 0.5 s, LH = 5 s, ITI = 5 s and TO = 5 s were reached (standard conditions). Training was continued until a stable performance of > 80% correct responses and < 20% omissions had been achieved on two consecutive sessions, after which the percentage correct scores from those two sessions were used to assign individual animals into balanced test groups. Animals that failed to reach the criterion level of performance were omitted from the test session.

Performance in the task was calculated using the following behavioural measures:

*Percentage of correct responses*: The proportion of correct responses as a percentage of the total number of trials minus the number of omitted trials as a measure of performance accuracy.

*Percentage of omission errors*: The number of trials that were initiated, but to which no response was made during the SD or LH period as a measure of failure to attend or motivational state.

*Number of premature responses*: The number of responses made into an aperture during the ITI as a measure of anticipatory or impulsive behaviour.

*Latencies*: The correct latency was defined as the time between presentation of the light stimulus and the rat initiating a response into the correct aperture. The reinforcement latency was defined as the time between recording a correct response and collection of the food pellet. Both latency scores provide a measurement of speed of responding.

### *5-CSRT experiments*

The effects of modafinil (Sequoia Research Products, Oxon, UK) on 5-CSRT performance were tested under four separate experiments.

In Experiment 1, the ability of modafinil to improve performance under standard conditions without causing confounding adverse effects *per se* was investigated. Modafinil (32–128 mg/kg) or vehicle (0.5% methyl cellulose) was administered p.o. with a pre-treatment time of 30 min.

In Experiments 2 and 3, the effect of modafinil under conditions of increased attentional load was investigated. Specifically, in Experiment 2, a combined reduced stimulus duration and reduced stimulus intensity (rSDSI) manipulation was employed, where the stimulus duration (SD) was reduced from 0.5 s to 0.25 s and the stimulus intensity (SI) was reduced to one-tenth brightness. Modafinil (32–128 mg/kg) or vehicle was administered p.o. with a pre-treatment time of 30 min.

Finally, to determine further the potential mode of action, the effects of modafinil on the cholinergic system were investigated (Experiment 4). Scopolamine hydrobromide (Sigma, St Louis, MO, USA) (0.075 mg/kg s.c., 30-min pre-treatment time) was combined with random white noise (WN, 102 dB) as a distractor. Modafinil (64 mg/kg p.o., 30-min pre-treatment time) or physostigmine (Sigma) (0.1 mg/kg i.p., 20-min pre-treatment time) or vehicle was administered subsequently. A within-subject design was adopted in which rats were dosed on Tuesdays and Fridays in a pseudo-latin square design with all animals receiving all treatments.

### *Statistical analysis*

***Experiments 1–3*** Data for each performance measure were subjected to a one-way analysis of variance (ANOVA) using the BMDP statistical package (Statistical Solutions, Cork, Ireland). Post-hoc comparisons were made using Tukey's test.

***Experiment 4*** Data for each performance measure were subjected to a one-way analysis of variance (ANOVA) with repeated measures and planned contrasts. $p \leq 0.05$ was considered to be statistically significant.

## Results

### *Pharmacokinetic study*

The mean plasma and brain concentrations of 32, 64 and 128 mg/kg modafinil were determined over a time course from 15 min to 4 h post-drug administration. Modafinil levels in both plasma and brain samples were found to peak between 30 and 60 min post-drug administration for all doses tested, after which the concentration of modafinil decreased rapidly (Table 1). Therefore, a pre-treatment time of 30 min and p.o. dosing route was considered to be optimal for testing modafinil in the 5-CSRT as it gave maximum exposure to drug.

**Table 1** Plasma and brain concentrations of modafinil at timepoints between 15 min and 4 h (240 min) following p.o. administration ($n = 3$) presented as mean and standard deviation (SD)

| Dose (mpk) | Time (min) | Mean plasma (ng/ml) | Mean brain (ng/g) | Mean brain : plasma | Plasma (SD) | Brain (SD) | Brain : plasma (SD) |
|---|---|---|---|---|---|---|---|
| 32 | 15 | 645 | 257 | 0.40 | 47 | 79 | 0.15 |
| 32 | 30 | 1057 | 689 | 0.63 | 450 | 335 | 0.06 |
| 32 | 45 | 668 | 566 | 0.85 | 72 | 67 | 0.08 |
| 32 | 60 | 1090 | 1134 | 1.00 | 924 | 1014 | 0.11 |
| 32 | 75 | 325 | 370 | 1.25 | 244 | 207 | 0.26 |
| 32 | 90 | 221 | 163 | 0.74 | 11 | 24 | 0.15 |
| 32 | 120 | 266 | 266 | 1.64 | 317 | 232 | 1.08 |
| 32 | 240 | 23 | 18 | 0.76 | 23 | 20 | 0.12 |
| 64 | 15 | 1908 | 752 | 0.39 | 585 | 328 | 0.05 |
| 64 | 30 | 6223 | 2539 | 0.50 | 3809 | 593 | 0.28 |
| 64 | 45 | 3973 | 2277 | 0.67 | 2677 | 795 | 0.22 |
| 64 | 60 | 853 | 1056 | 1.24 | 260 | 329 | 0.13 |
| 64 | 75 | 1162 | 1203 | 1.11 | 692 | 525 | 0.19 |
| 64 | 90 | 1349 | 1382 | 1.10 | 1436 | 1446 | 0.29 |
| 64 | 120 | 795 | 364 | 1.21 | 1125 | 274 | 0.99 |
| 64 | 240 | 16 | 15 | 0.89 | 2 | 8 | 0.41 |
| 128 | 15 | 8413 | 3434 | 0.41 | 4054 | 1823 | 0.09 |
| 128 | 30 | 11779 | 5245 | 0.45 | 1798 | 357 | 0.10 |
| 128 | 45 | 6263 | 4653 | 0.76 | 3913 | 2796 | 0.08 |
| 128 | 60 | 8448 | 7127 | 0.84 | 3449 | 2940 | 0.01 |
| 128 | 75 | 3702 | 3197 | 0.96 | 2035 | 1246 | 0.34 |
| 128 | 90 | 967 | 1327 | 1.55 | 699 | 693 | 0.39 |
| 128 | 120 | 1392 | 2083 | 1.83 | 1077 | 940 | 1.07 |
| 128 | 240 | 144 | 242 | 1.62 | 30 | 120 | 0.49 |

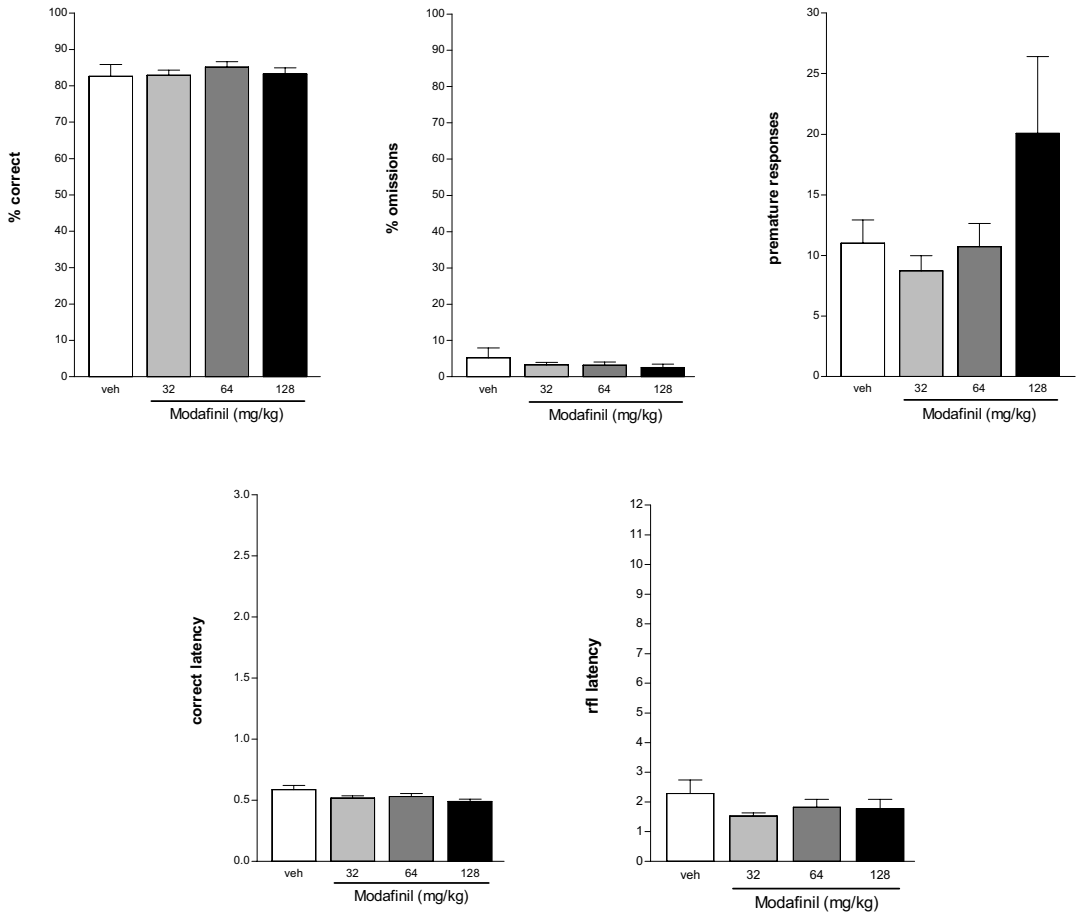


**Figure 1** The effect of modafinil under standard test conditions in the five-choice serial reaction time test. Modafinil 32–128 mg/kg administered p.o., 30 min before testing. Percentage correct responding, percentage omissions, premature responding and correct and reinforcement latencies are shown. Data are shown as group mean ± SEM ($n$ = 10–15)

## *5-CSRT experiments*

***Experiment 1: effects of modafinil under standard conditions***

The effects of modafinil in the 5-CSRT under standard conditions are shown in Figure 1. Under standard conditions, there was no main effect of treatment group on percentage correct responding [$F(3,47) = 0.45$, $p = 0.7167$], percentage omissions [$F(3,47) = 0.54$, $p = 0.6580$], premature responding [$F(93,47) = 2.27$, $p = 0.0931$] or either reinforcement or correct latencies [$F(3,47) = 0.54$, $p = 0.6552$] and [$F(3,47) = 1.98$, $p = 0.1294$], respectively. These data demonstrate that modafinil pre-treatment did not improve accuracy of responding in rats trained to a high level of performance, nor adversely affect baseline performance.

***Experiment 2: effects of modafinil under combined rSDSI***

Reducing both the stimulus duration and stimulus intensity (rSDSI) had a profound effect on 5-CSRT task performance (Fig. 2). There was a significant main effect of treatment group on percentage correct responding [$F(4,47) = 62.52$, $p < 0.0001$], correct latency [$F(4,47) = 6.02$, $p = 0.0005$], reinforcement latency [$F(4,47) = 3.45$, $p = 0.0149$] and premature responding [$F(4,47) = 8.01$, $p < 0.0001$]. There was no significant main effect of treatment group on the percentage of omitted trials [$F(4,47) = 1.01$, $p = 0.4126$]. However, although Tukey's post-hoc tests revealed that the rSDSI manipulation induced a significant decrease in percentage correct responding compared to rats performing under standard conditions ($p < 0.01$), pre-treatment with modafinil (32–128 mg/kg) failed to attenuate these deficits in accuracy. Similarly, the increase in the correct latency induced by the rSDSI manipulation compared to the control group ($p < 0.01$) was also not reversed by modafinil at any of the doses tested. Interestingly, although the rSDSI manipulation alone had no effect on premature responding, in rats challenged with this manipulation under higher doses of modafinil (64–128 mg/kg), a significant increase in premature responding compared to vehicle-treated controls ($p < 0.01$) was observed. These data demonstrate that, although modafinil failed to reverse performance deficits induced by the rSDSI manipulation in the 5-CSRT, there was an increase in premature responding in modafinil-treated rats, indicative of an increase in impulsive behaviour.

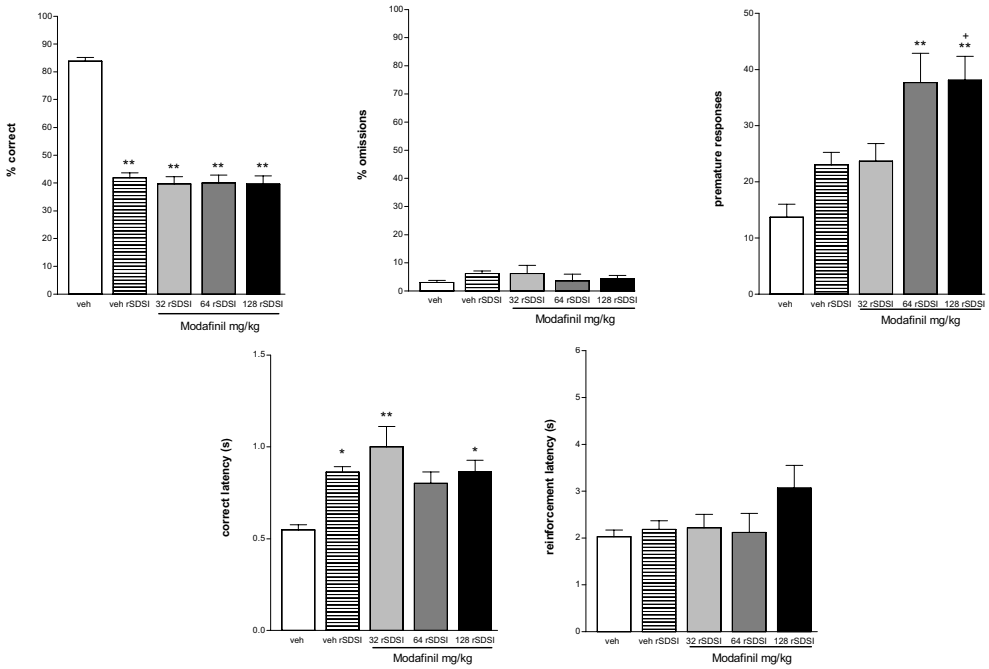


**Figure 2** The effects of modafinil vs. reduced stimulus duration and intensity (rSDSI) in the five-choice serial reaction time test. Modafinil 32–128 mg/kg administered p.o., 30 min before testing. Percentage correct responses, percentage omissions, number of premature responses, correct response latency and reinforcement latency are shown. Stimulus duration = 0.25 s; stimulus intensity = 1/10th brightness. Data are shown as group mean ± SEM ($n$ = 10–11). *$p$ < 0.05 significantly different from vehicle-treated controls under standard conditions ($n$ = 10–11); **$p$ < 0.01 significantly different from vehicle-treated controls under standard conditions; +$p$ < 0.01 significantly different from vehicle-treated controls under rSDSI conditions

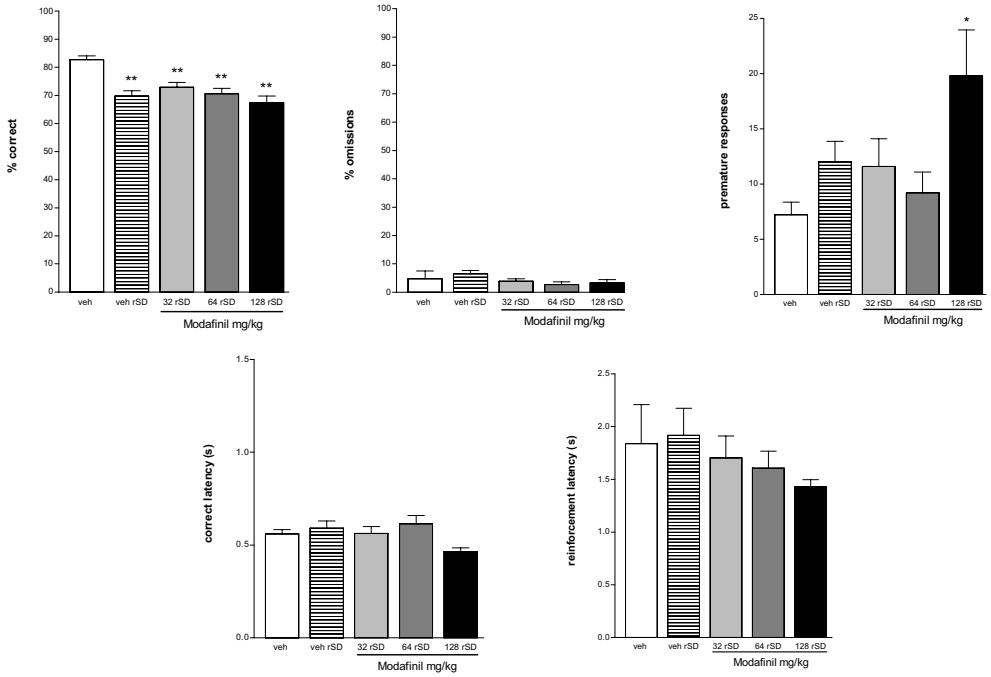


**Figure 3** The effects of modafinil versus reduced stimulus duration (rSD) in the five-choice serial reaction time test. Modafinil 32–128 mg/kg administered p.o. 30 min before testing. Percentage correct responses, percentage omissions, number of premature responses, correct response latency and reinforcement latency are shown. Stimulus duration = 0.25 s. Data are shown as group mean ± SEM ($n$ = 9–10). *$p$ < 0.05 compared to vehicle-treated controls under standard conditions; **$p$ < 0.01 significantly different from vehicle-treated controls under standard conditions

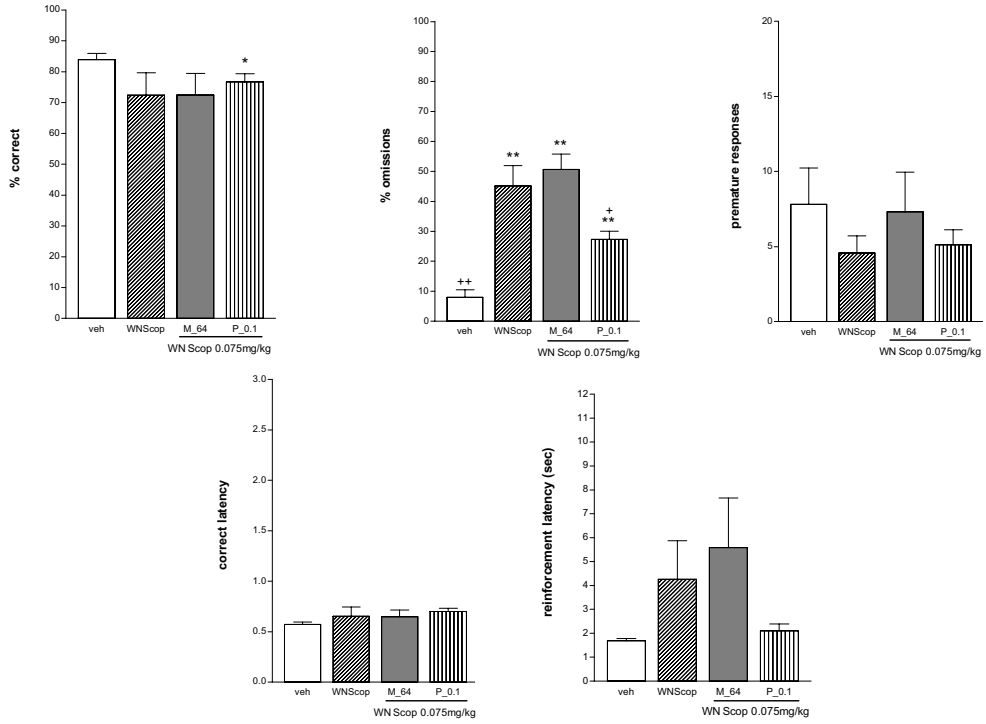


**Figure 4** The effects of modafinil 64 mg/kg p.o. on pharmacological deficits in the five-choice serial reaction time test. Percentage correct responding, percentage omissions, number of premature responses, correct response latency and reinforcement latency under conditions of combined white noise (102 dB) and scopolamine (0.075 mg/kg s.c.) (WNScop) are shown. Physostigmine 0.1 mg/kg i.p. was used as a positive control. Data are shown as group mean ± SEM ($n$ = 16 in all groups) (within-subject experimental design). *$p$ < 0.05 compared to vehicle-treated controls under standard conditions; **$p$ < 0.01 compared to vehicle-treated controls under standard conditions; +$p$ < 0.05 compared to WNScop treatment group, ++$p$ < 0.01 compared to WNScop treatment group

***Experiment 3: effects of modafinil under rSD alone*** The lack of effect induced by modafinil in Experiment 2 may have been attributable to the overly detrimental effect on performance induced by the combined rSDSI manipulation. Therefore, the effects of modafinil were tested under a reduced stimulus duration (rSD) alone manipulation (Fig. 3). Under these conditions, there was a significant main effect of treatment group on percentage correct responding [$F(4,43) = 9.22$, $p < 0.0001$], premature responding [$F(4,43) = 3.45$, $p = 0.0156$] and correct latency [$F(4,43) = 2.90$, $p = 0.0326$]. However, there was no significant main effect of treatment group on either percentage omissions [$F(4,43) = 0.99$, $p = 0.4236$] or reinforcement latency [$F(4,43) = 0.72$, $p = 0.5821$]. Although Tukey's post-hoc tests showed that task accuracy was significantly impaired under rSD conditions ($p < 0.01$) compared to standard conditions, this was to a lesser degree than that induced by the combined rSDSI manipulation (percentage correct trials: 69.74 ± 1.89% compared to 41.84 ± 1.76% for rSDSI). Nevertheless, pre-treatment with modafinil again failed to reverse the performance deficit at any of the doses tested. Furthermore, similar to Experiment 2, although rSD alone had no effect on premature responding, in animals challenged with the manipulation and pre-treated with modafinil (128 mg/kg), premature responding was significantly increased ($p < 0.05$) compared to controls. Therefore, although modafinil was unable to reverse even modest performance deficits in the 5-CSRT, the increase in premature responding again observed under parametric manipulation conditions strongly suggests that modafinil may increase impulsive behaviour.

***Experiment 4: effects of modafinil under a combined scopolamine pre-treatment and random white noise manipulation*** To reduce pharmacological variation and to increase robustness in the scopolamine pre-treatment manipulation, a within-subject experimental design was adopted. Although scopolamine (0.075 mg/kg s.c.) combined with random bursts of white noise failed to induce a significant deficit in percentage correct responding [$F(1,15) = 2.31$, $p = 0.1494$), there was a significant effect on the percentage of omitted trials [$F(3,45) = 19.59$, $p < 0.0001$] (Fig. 4). Planned contrasts analysis demonstrated that scopolamine pre-treatment significantly increased the percentage omissions compared to vehicle-treated controls [$F(1,15) = 24.07$, $p = 0.0002$], and that this effect was reversed by the acetylcholinesterase inhibitor physostigmine (0.1 mg/kg i.p.) [$F(1,15) = 6.18$, $p = 0.0252$] but not by modafinil [$F(1,15) = 0.4$, $p = 0.5361$) (Fig. 4). There was no significant main effect of treatment group on premature responding [$F(3,45) = 0.73$, $p = 0.5417$], or on reinforcement and correct latencies [$F(3,45) = 2.11$, $p = 0.1127$ and [$F(3,45) = 0.94$, $p = 0.4280$], respectively.

## Discussion

The results of the present study demonstrate clearly that modafinil does not enhance attentional processes *per se* in a normal awake rat as measured using the 5-CSRT. Under baseline conditions, 32–128 mg/kg modafinil had no effect on any of the parameters measured (i.e. accuracy, omission errors, response latencies or premature responding). Under modified test conditions in which the stimulus duration and/or stimulus intensity was reduced, 128 mg/kg modafinil was shown to significantly increase premature responding without affecting accuracy, omission errors or response latencies. Finally, modafinil also failed to attenuate a scopolamine-induced performance deficit, suggesting that modulation of the cholinergic pathway is unlikely as a potential mechanism of action.

The results from the dosing study enabled the pharmacokinetic profile of modafinil in rats to be determined. At 64 mg/kg p.o. with a 30-min pre-treatment time, plasma and brain levels of modafinil in rats were comparable to those achieved following a 200 mg dose in healthy human subjects (McClellan and Spencer, 1998) namely 6223 ng/ml and 2539 ng/g respectively. However, it should be noted that there remain differences across species. In humans, the peak plasma concentrations of modafinil are reached more slowly compared to rats (2.3–2.5 h after a single 200 mg dose), and the half-life is also longer (between 9 and 14 h) (McClellan and Spencer, 1998). These differences demonstrate the importance of determining the pharmacokinetic profile of modafinil before conducting the 5-CSRT studies, to aid interpretation of the behavioural data.

In humans, a 200 mg dose of modafinil has been shown to enhance wakefulness in sleep-deprived subjects (Caldwell *et al*., 2000) and to improve performance in a range of cognitive tests in normal volunteers (Turner *et al*., 2003), although this finding appears equivocal (Randell *et al*., 2003). In the present preclinical study, which focussed specifically on attentional processes in the rat, modafinil failed to improve performance in the 5-CSRT under all test manipulations at all doses tested. It would be reasonable to suggest that the lack of an attention-enhancing effect by modafinil under standard conditions in the 5-CSRT might be attributable to a perceived 'optimal' level of responding in animals trained to criterion. Indeed, stimulants such as nicotine have also failed to enhance accuracy of responding under standard test conditions, most likely due to the subtlety of the drug effect and the small 'window' for improvement (Stolerman *et al*., 2000). By contrast, Robbins (2002) proposed that, even when rats are trained to a high level of accuracy (> 80% correct trials), performance may not be at ceiling; for an example, see Winstanley *et al*. (2003). Consequently, there remains a possibility to detect improvement, primarily due to the belief that the less than perfect level of performance in the 5-CSRT is attributable to an inability to maintain attention, rather than to effectively learn the task rule. Given this, it may still have been possible to detect a modafinil-induced enhancement in 5-CSRT performance, regardless of high baseline accuracy. Nevertheless, although the present study did not detect such facilitation in performance under standard conditions, neither did modafinil appear to induce a detrimental effect on performance. High doses of modafinil have been reported to induce hyperactivity in rats (Simon *et al*., 1996; McClellan and Spencer, 1998) and nausea in human subjects (McClellan and Spencer, 1998). It would be predicted that side-effects such as hyper-locomotion are likely to affect the rat's ability to respond to the light stimulus, whereas nausea would reduce motivation for the food reward. However, such adverse effects were not evident in the present study at the doses tested, as demonstrated by the lack of effect of modafinil on accuracy and on the correct and reinforcement latency measures.

As expected, increasing the attentional load by manipulating the task parameters induced a robust deficit in performance. The results obtained from reducing the stimulus duration and/or stimulus intensity were comparable with those previously recorded in our laboratory (Bright and Dias, 2001) and from other research groups (Robbins, 2002; Stolerman *et al*., 2000). However, modafinil failed to reverse the deficits in performance induced by either manipulation. Interestingly, the findings of the present study are almost identical to those reported by Milstein *et al*. (2003). Here, modafinil was tested in a modified Latin-Square drug design at doses ranging from 3 to 100 mg/kg i.p. Under baseline and reduced stimulus duration conditions, response latency and accuracy both remained unaffected by modafinil treatment. Overall, these data strongly suggest that modafinil does not exert its cognition enhancing effects simply by increasing stimulus detection or control of divided attentional processes.

The present findings also suggest that modafinil does not affect sustained attention or vigilance mechanisms beneficially, although this could be addressed in further detail by assessing the effects of modafinil under conditions of a vigilance performance decrement with time. Such a task manipulation has been utilized previously to demonstrate the attention-enhancing effects of nicotine, amphetamine and caffeine (Grottick and Higgins, 2002). Certainly, sustained attention can be improved by modafinil in adult patients with attention deficit hyperactivity disorder (ADHD), but not in normal healthy human subjects (Turner *et al*., 2004a), suggesting that modafinil may only have the capacity to improve vigilance mechanisms when baseline performance is impaired. A time-related decrement versus a reduced stimulus duration and/or intensity induced impairment may prove to be more sensitive in detecting the potential beneficial action of modafinil on sustained attention specifically.

As mentioned previously, the 5-CRST not only taxes divided and sustained attention, but also response control. However, the present findings suggest that modafinil does not exert a positive effect on response control mechanisms as measured in the 5-CSRT test. In both the present study and in Milstein's study (2003), modafinil had no effect on premature responding under standard conditions, and actually increased premature responding under conditions of greater attentional load. This latter finding is reminiscent of observations following amphetamine administration (Cole and Robbins, 1987; Evenden, 1999; Robbins, 2002), and is highly indicative of a deficit in inhibitory response control.

Intriguingly, this observed increase in impulsivity appears to be in direct contrast to clinical findings that support a positive role for modafinil in affecting impulse control. For example, modafinil has been shown to: (i) significantly improve the inhibition of prepotent responding in healthy adults and in adult patients with ADHD (Turner *et al*., 2003, 2004a); (ii) significantly slow response times

on tests such as spatial planning in normal, ADHD and schizophrenic patients (Turner *et al*., 2003, 2004a, b); and (iii) ameliorate the attentional set-shifting impairment in schizophrenic patients (Turner *et al*., 2004b). Taken together, these clinical findings suggest a predominant role for modafinil in both inhibitory control and cognitive flexibility in man. However, the apparent discrepancy that exists between the preclinical and clinical observations regarding the role of modafinil in response control functions remains unclear. One possibility may be that the enhancements observed in disorders such as ADHD and schizophrenia could be attributable in some degree to improvements in general fatigue or mood state. However, although such an explanation should not be dismissed entirely, it would appear unlikely given the cognitive specificity of the improvements induced by modafinil in these studies.

Finally, the precise neural and pharmacological mechanisms by which modafinil exerts its potential cognition enhancing effects remains undetermined. Using 2-deoxyglucose autoradiography, modafinil has been shown to increase glucose utilization in the hippocampus and the central nucleus of the amygdala (Engber *et al*., 1998). Similarly, modafinil treatment has been shown to increase c-Fos expression in the striatum and the cingulate cortex (Scammel *et al*., 2000). Furthermore, electroencephalogram studies in rats (Sebban *et al*., 1999) and functional magnetic resonance imaging studies in both healthy volunteers and narcoleptic patients (Ellis *et al*., 1999) have demonstrated that modafinil modulates prefrontal cortex activity. Neurochemically, several studies have shown that many different brain systems appear to be involved in regulating the wake-promoting effects of modafinil, including the hypocretin neurones (Beuckmann and Yanagisawa, 2002), the noradrenergic, serotonergic and dopaminergic systems (De Saint Hilaire *et al*., 2001) and the GABAergic and glutamatergic systems (Ferraro *et al*., 1997). To date, there is no published evidence to suggest that modafinil modulates the cholinergic system directly. However, one proposal for the mechanism of action of modafinil stems from the finding that modafinil increases histamine release substantially (Ishizuka *et al*., 2003). With preliminary evidence suggesting that $H_3$ receptors may modulate the activity of cholinergic neurones (Blandina *et al*., 1996), it is possible that modafinil may modulate the cholinergic system indirectly to exert its effects on specific cognitive domains. However, although support for this hypothesis is provided by the observation that an $H_3$ autoreceptor antagonist improved accuracy in the 5-CSRT (Ligneau *et al*., 1998), the same class of compound failed to reverse a scopolamine-induced attentional dysfunction in the same 5-CRST (Kirkby *et al*., 1996). Certainly our findings, which clearly demonstrate the inability of modafinil to attenuate a scopolamine-induced performance deficit in the 5-CSRT, do not provide support for a role of modafinil in modulating the cholinergic system to exert a cognition-enhancing effect.

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