Investigating the Cognitive Enhancing Effects of Modafinil in Rats using a novel Self-Ordered Sequencing Touchscreen Task

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ABSTRACT. Reports of the cognitive enhancing effects of modafinil have been inconsistent across human and rodent literatures. Cognitive enhancements are typically observed on specific tasks above a certain difficulty threshold, being more pronounced in subjects with impaired baseline performance. The current study employs a novel touchscreen task in rodents analogous to Cambridge Neuropsychological Test Automated Battery (CANTAB)'s self ordered spatial working memory task (SWM) in humans, where pro-cognitive effects of modafinil were observed (Müller et al. 2013). Consistent with previous literature in humans, results showed modafinil selectively enhanced performance on the more difficult trial types. This improvement was unaccompanied by changes in correct or error latency measures. The current touchscreen paradigm proves to be more sensitive to modafinil's enhancing effects on spatial working memory than other current existing pre-clinical tests in rodents. This task can be combined with lesion and neurochemical assays in the future to narrow down the neural basis and molecular pathways underpinning modafinil's cognitive enhancing effects. Its close resemblance to CANTAB SWM touchscreen task used to test human patients makes it a useful preclinical tool to measure self-ordered spatial working memory.

1. Introduction

Modafinil is a wake-promoting agent licensed to treat narcolepsy, with putative cognitive enhancing effects. In humans, modafinil has yielded enhancements on tasks of attention, working memory and executive control in non-sleep-deprived healthy adults, and in neuropsychiatric populations such as ADHD and schizophrenia patients (Müller et al., 2013; Turner et al., 2003; Turner et al., 2004). However, others have failed to either replicate attentional enhancements in healthy adults, or have yielded heterogeneous results (Randall, Fleck, Shneerson, & File, 2004; Randall, Shneerson, Plaha, & File, 2003; Müller, Steffenhagen, Regenthal, & Bublak, 2004; Randall et al., 2005). The rodent literature is similarly inconsistent, with some positive findings on cognitive enhancement of abstract rule learning (Béracochéa et al., 2001; Béracochéa, Celerier, Peres, & Pierard, 2003; Béracochéa, 2002; Ward, Harsh, York, Stewart, & McCoy, 2004), sustained attention, executive control (Chudasama & Robbins, 2004; Morgan, Crowley, Smith, LaRoche, & Dopheide, 2007), and object recognition (Redrobe, Bull, & Plath, 2010), while others showed increased impulsivity and worsening of performance by reducing sustained attention (Liu, Tung, Lin, & Chuang, 2011; Waters, Burnham, O'Connor, Dawson, & Dias, 2005), or differential findings depending on baseline performance level of subjects (Eagle, Tufft, Goodchild, & Robbins, 2007).

Three main factors may be contributing toward this mixed profile of modafinil's cognitive enhancing effects. First, enhancement may be subject dependent. Greater cognitive-enhancement is typically observed in poor-baseline-performers, in both healthy young human adults (Esposito et al., 2013), and ADHD patients (Turner, Clark, Dowson, et al., 2004). Eagle et al. (2007) mirrored this finding in rats using a stop-signal-reaction-task (SSRT), and found modafinil at 10 and 30mg/kg improved performance of rats with slow baseline SSRT, and not those with fast baseline SSRT. This suggests baseline performance may determine modafinil's potential cognitive enhancing effects.

Second, enhancement may be task dependent, with key factors being task specificity and task difficulty. Randall et al. (2005) assessed whether previous discrep-

ancies between their negative findings and Turner et al. (2003, 2004)'s positive findings was partly due to inconsistent use of selection of Cambridge Neuropsychological Test Automated Battery (CANTAB) tests. They replicated Turner et al.'s positive results only by using the same tasks (Digit-Span, Pattern Recognition Memory) at 100mg and 200mg doses of modafinil, suggesting only certain tasks in non-sleep-deprived young healthy adults are sensitive to effects of modafinil. Moreover, Müller et al. (2013) found that modafinil enhanced performance of healthy volunteers on CANTAB self ordered spatial working memory task (SWM) by reducing error-rates only at their newly implemented, more difficult, 10-box and 12-box levels. This result suggests that Turner et al. (2003)'s failure to detect modafinil's enhancement on SWM may have been a ceiling effect without using these more difficult levels. Similarly, Morgan et al. (2007) found a dose dependent improvement in response accuracy and impulsivity control in rats on three choice visual discrimination task only when sustained attention load was increased by making the task more difficult with unpredictable stimulus onset and duration. However, modafinil induced enhancements related to task difficulty may interact with task specificity as pro-cognitive effects have not been observed in versions of the 5 choice serial reaction time task for rats, irrespective of task difficulty.

Third, the relationship between modafinil's cognitive enhancing effects and impact on impulsivity remains unclear. Turner et al. (2003) found increased correct response and reduced error rates were accompanied by increased response latency, suggesting modafinil affected speed accuracy trade off. Slowed response latency was also observed in ADHD (Turner, Clark, Dowson, et al., 2004) and schizophrenia patients (Turner, Clark, Pomarol-Clotet, et al., 2004), implicating modafinil reduces response impulsivity. In contrast, Müller et al. (2013) used a novel 6 move solutions version of Stockings of Cambridge-task and found modafinil's improvement on accuracy was unaccompanied by any effect on latency, suggesting speed error trade off is not sufficient to explain modafinil's cognitive enhancing effect. In addition, some rodent literature have shown that modafinil actually increased impulsivity, whether it improved response accuracy (Chudasama & Robbins, 2004) or not (Waters et al., 2005). Therefore further investigation on modafinil's effect on impulsivity and how this affects response accuracy and error rate is needed.

The current study employs a novel self ordered sequencing touchscreen task to investigate modafinil's cognitive enhancing effects in young healthy non-sleep-deprived rats. It aims to pharmacologically validate this novel preclinical test by attempting to emulate and extend the cognitive enhancing effects of modafinil observed in humans (Muller et al. 2013). Whereas previous rodent research mostly used various maze (Béracochéa et al., 2001; Béracochéa et al., 2003; Béracochéa, 2002; Piérard et al., 2006) or odours discrimination tasks (Goetghebeur & Dias, 2009), a touchscreen task bears closer resemblance to the human CANTAB tasks, facilitating translation to clinical studies (Bussey et al., 2012). A validated preclinical test of cognition is important as it can be used in conjunction with lesion (not possible in humans) and biochemical assays, to gain further insight into the mechanistic workings of modafinil. The use of touchscreen ensures close proximity between the animal and the visual stimulus on screen, and maximizes performance despite the rat's poor visual acuity (1.0 cycle/degree) (Bussey et al., 2008). It is more ethical, using appetitive not negative (Ward et al., 2004) reinforcement, and minimizes handling stress during testing which may add variability to results (Piérard et al., 2006). It is ecologically valid as it exploits rats' natural tendency to explore novelty (Horner et al., 2013). Computer automation increases range and precision of parameters measured (Bussey et al., 2012).

It also attempts to address the three previously stated sources of inconsistencies underlying modafinil's cognitive enhancing effects. First, this task was developed because it is analogous to the SWM CANTAB task used by Müller et al. (2013), where positive findings were obtained. Whereas Müller et al. (2013) manipulated task difficulty by introducing new 10- and 12-box levels, the current study divides difficulty into four levels by using 2- or 3-stimulus trials, and each subdivided into delay of stimulus onset and no-delay trials. The hypothesis based on previous literature is modafinil should selectively enhance performance on more difficult trials only (3-stimulus, delay). Second, to assess whether observable differences in modafinil effects is affected by pre-treatment baseline performance, rats were grouped based on training performance into good and poor performers. The hypothesis is modafinil should have a greater cognitive-enhancement-effect in poor performers. Third, latencies are analysed to assess Turner et al (2003)'s hypothesis of modafinil's cognitive-enhancing-effect being mediated by reducing speed of responding. This predicts any performance enhancement should be accompanied

by increased latencies.

2. Method

Subjects

20 male Lister Hooded rats (Charles River, UK) were kept on a reverse day/night cycle (lights on 7pm-7am) and housed four per cage, in a room maintained at constant temperature (20-24°C) and humidity (55±10%). Rats were fed on a restricted diet (standard rat chow, Special-Diets-Services) to maintain 85% of their free-feeding weight, with water provided ad libitum. Prior to training, handling and habituation to the facility took place. Testing was conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986.

Apparatus

Each animal was tested in an automated touchscreen operant chamber (Campden Instruments, Med Associates) . The touchscreen (15-inch), located at the front of the chamber, used optical IT sensors to register responses. The magazine was at the rear of chamber. Illumination of the magazine light paired with a click produced by the tone generator signaled the delivery of a 45mg Noyes pellet (Sandown Scientific). An IR camera above the chamber looked through the transparent lid, enabling monitoring of animal behaviour on computers outside the testing room to minimize interference during testing. The touchscreens were controlled and data collected by Whisker control system (Version 3.6.1, Cardinal and Aitken 2001), using the OWM program written by A. Mar.

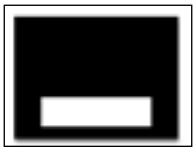
Shaping

Animals learned the basic operation of touchscreen chamber through a series of training stages. Figure 1 shows stimulus and training procedure during shaping trials.

Self-ordered sequencing task

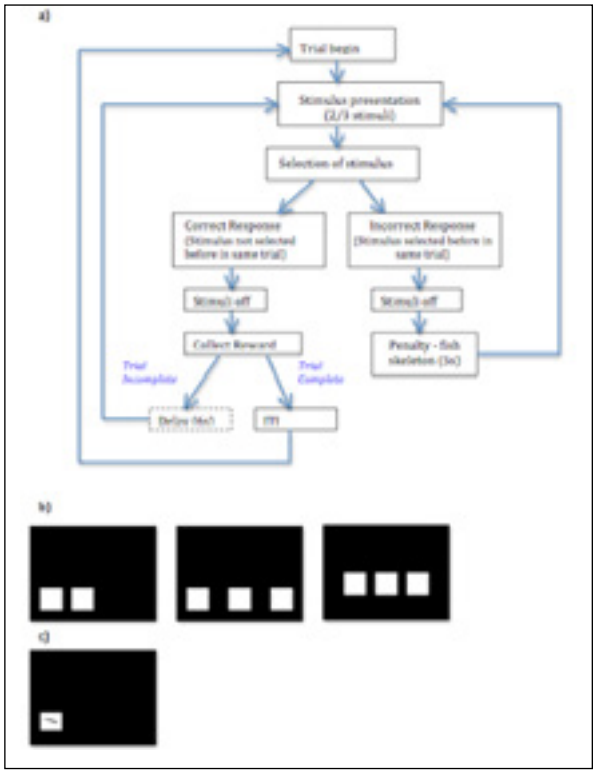
Figure 2a outlines the proceedings of each trial. Each session began with a free pellet; signaled by a click and illumination of magazine. Trial one was self-initiated upon collection of this free pellet, resulting in stimuli presented on screen (Figure 2b). A correct attempt required the animal to select a stimulus it had not selected before within the same trial. It resulted in removal of all stimuli from the screen followed by reward delivery. Re-presentation of the stimuli immediately followed reward collection from magazine on no-delay trials, or after 6-seconds on delay trials. An incorrect attempt occurred when the animal revisited a previously selected stimulus in the same trial (Figure 2c), resulting in a 5-second penalty before re-presentation of stimuli and no rewards. The trial was completed when all stimuli were selected, and a perfect trial if each stimulus was selected once only. An inter-trial-interval (ITI) (25, 30, 35, 40, 45s) was randomly

Figure 1. Stimulus and training procedure during shaping trials.



Note. For shaping sessions, a solid white rectangular stimulus (10 cm-long x 2.5 cm wide) was presented on the screen for each trial. Touching the stimulus resulted in a click and illumination of magazine, followed by reward delivery. Light was switched off upon collection of reward from magazine, and next trial initiated. Each session lasted 100 trials or 60 minutes, whichever elapsed first. Criterion of completing all 100 trials within 60 minutes was reached in 3-4 sessions.

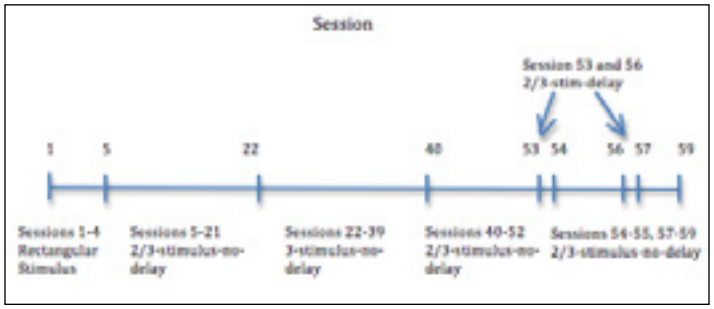
Figure 2. Trial proceedings, stimuli, and response penalty



Notes. (a) Flowchart overview of the self-ordered-sequencing procedure. Dotted line for delay indicates 6-second delay is only present on delay trials, and ITI length is variable. (b) stimuli presentation on touchscreen. Depending on trial type, either 2 (left) or 3 stimulus (middle/right) would be simultaneously presented on the screen. On 3-stimulus trials, stimuli were presented either close together - ≤8cm apart (middle)/far apart - ≥8cm apart (right) distribution of stimuli on screen. Each stimulus was a white square (3cm×3cm) (c) Incorrect response penalty results in fish skeleton presented at the wrong position touched on that attempt.

selected and initiated upon collection of last reward from magazine on any given trial.

Figure 3. Timeline of training schedule prior to modafinil administration



Note. All training sessions consisted of 80 trials or 60 minutes, whichever elapsed first.

Training Schedule

Sessions 1-4 = shaping sessions, training rats to learn the basic operation of a touchscreen chamber
Sessions 5-21 = training using 2/3 stimulus-no-delay sessions. After eighteen sessions, below chance performance (22.2%) on 3-stimulus trials across cohort observed. In order to ensure criterion level performance on 3-stimuus trials could be reached under time constraints before modafinil administration, a 3-stimulus-no-delay trial only stage was added to facilitate learning of 3-stimulus task.
Sessions 22-39 = training using 3-stimulus-no-delay sessions. Eighteen sessions were completed with the last three consecutive sessions showing stabilized above criterion-level performance.
Sessions 40-52 = training using 2/3 stimulus-no-delay sessions. Thirteen sessions were completed, which led to half the cohort reaching stabilised above-criterion level performance for both 2-stimulus (50% perfect) and 3-stimulus trials (22.2% perfect).
Session 53 = probe session 1 using 2/3-stimulus-delay (6-seconds)
Session 54-55 = 2 sessions using 2/3-stimulus-no-delay trials to re-baseline performance before probe session 2
Session 56 = probe session 2 using 2/3-stimulus-delay (6-seconds)
Session 57-58 = 2 sessions using 2/3-stimulus-no-delay trials to re-baseline performance before modafinil study

Drug administration and testing schedule

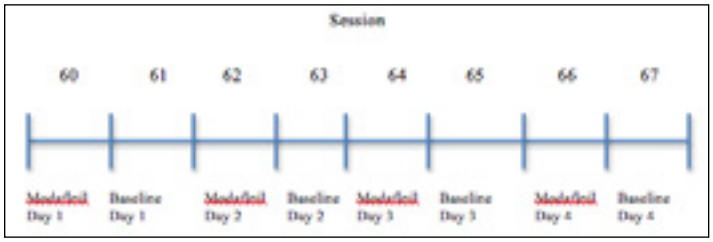
Modafinil was administered using a Latin-Square design (TABLE 1). Modafinil (Eli Lilly – milled) was prepared on each drug day in 10% sucrose suspension and administered orally at doses of 8mg/kg, 16mg/kg, 32mg/kg, or vehicle (10% sucrose suspension). Vigorous shaking of the modafinil suspension prior to dosing was performed, maximising even distribution of drug in solution. Each animal was weighed prior to drug administration, to calculate volume (1ml/kg) needed to reach the specified dose. FIGURE 4 shows modafinil dosing schedule. Latin-Square was completed using once/daily sessions, over eight days.

Table 1. Latin Square (4 x 4) used for modafinil administration.

Group	Day 1 Dose (mg/kg)	Day 2 Dose (mg/kg)	Day 3 Dose (mg/kg)	Day 4 Dose (mg/kg)
1	Vehicle	8	16	32
2	8	32	Vehicle	16
3	16	Vehicle	32	8
4	32	16	8	Vehicle

Note. Rates were divided into four groups, matched for performance based on training. All animals received all doses across four alternating days in a randomized manner.

Figure 4. Timeline of training schedule during modafinil study (post-rtraining).



Note. Modafinil day testing began thirty minutes after modafinil administration, and used 2/3 stimulus-delay sessions with 80 trials or 75 minutes, whichever elapsed first. On drug dosing sessions, session length was increased from 60 to 75 minutes to maximize number of trials completed per session. A drug free 2/3 stimulus-no-delay session (80 trials, 60 minutes) baseline day followed each drug day. This allowed recovery of performance and reduced any potential cumulative drug effects that may confound data interpretation. In total, modafinil study lasted for 8 days, with 4 drug days and 4 baseline days to complete the Latin Square.

Statistical analysis

Performance in the task was calculated using the following measures:

Percentage of perfect trials: number of perfect trials divided by total trials completed for each trial type.
Errors: First-mistake on 3-stimulus trials was broken down by attempt (expressed as a percentage of first mistakes made on attempt two or three); and by where on the screen the mistake was made (left, middle, right). A perseverative error is any mistake made after the first mistake on any attempt. Error rate was number of perseverative-errors expressed as a percentage of the total number of completed imperfect trials of that type.
Latencies: Correct response latency was the time taken for the first correct response on all except first attempts. Error latency was the time taken for a mistake to be made on any attempt. Reward collection latency was the time taken to enter the magazine for reward collection.
Splitting subjects into groups: Based on the hypothesis that

modafinil's cognitive enhancing effects are greater in subjects with more impaired baseline performance, group-split was based on stable performance across training sessions 40-52, prior to drug testing (See Table 2).

Table 2. Group average performance across training sessions 40-52.

Group	% Perfect - 2 stimulus trials	% Perfect - 3 stimulus trials
1 (n=10, Good)	57.93±3.48	28.5±2.04
2 (n=8, Poor)	52.10±2.67	13.5±0.71

Note. Data are shown as group mean ± SEM (n=10 in good group, 8 in poor group). Efforts were made to maximize difference in performance on both 2-stimulus and 3-stimulus trials between the groups, using median of percentage of perfect trials across sessions 40-52 as a variable for splitting. The uneven group size was because groups were determined pre-Latin-Square, based on total of 19 rats with 9 rats in poor group. One rat from poor group failed to complete Latin-Square, thus reducing group size to 8.

For all analyses, trial types was broken down by stimulus-number (2/3) and delay (0/6sec), each analysed at two levels, and group was used as a between-subject variable. Two rats were excluded. One did not complete training due to illness. The other failed to complete the Latin-Square due to insufficient drug to make up the correct dose on the last day of testing. Data of the remaining 18 subjects were analysed.

Statistical analysis was carried out using Windows versions of SPSS (Version 15, SPSS, Chicago), using repeated-measures factorial Analysis of Variance (ANOVA). Skewness and range of values for each variable were checked using SPSS descriptive statistics, and given n is equal across all drug groups, homogeneity of variance was not violated. Normality was checked using Whisker-box-plots for any extreme outliers. Sphericity was checked using Mauchly's test. When violated ($p<0.05$), if Greenhouse-Geisser estimate of sphericity (Epsilon factor) is <0.75, Greenhouse-Geisser correction was used; if Epsilon factor is >0.75, Huynh-Feldt correction was used. No serious violations of any assumptions were found. $p\leq0.05$ was considered to be statistically significant. However, $0.05\leq p\leq0.1$ were also discussed if it helped to interpret any drug effects observed. Dunnett's test was used as a post-hoc analyses for any significant effects of ANOVA of dose, comparing each dose against vehicle. Tukey's HSD was used as a post-hoc analyses investigating between-dose or non-dose related significant outputs of ANOVA.

3. Results

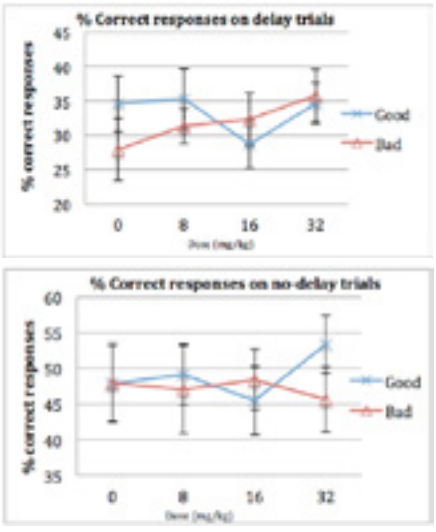
Percentage perfect trials

Across the entire cohort, ANOVA revealed main effects of stimulus-number ($F(1,17)=379.717$; $p<0.001$) and delay ($F(1,17)=38.21$; $p<0.001$), with lower percentage-perfect on delay and 3-stimulus trials relative to no-delay and 2-stimulus trials. Dunnett's confirmed delay introduced a significant reduction in percentage-perfect ($p<0.05$). This suggests both delay and stimulus-number variations successfully manipulated task difficulty, as expected.

ANOVA revealed a stimulus-number × delay interaction ($F(1,17)=17.769$; $p<0.001$), Dunnett's post hoc tests showed delay had a greater impact on performance of 3-stimulus than on 2-stimulus trials ($p<0.01$), in line with the prediction that delay would increase working memory load to a greater extent for 3-stimulus trials compared to 2-stimulus trials.

One comparison was to see if modafinil selectively improved performance of the poor-baseline-performers more than the good-baseline-performers. Using group as a between-subject variable, ANOVA revealed a non-significant trend of dose×delay×group interaction ($F(3,51)=2.596$; $p=0.063$) (FIGURE 5). Linear contrast comparison revealed no significant linear trends ($p>0.05$).

Figure 5. Effect of modafinil on % of correct responses on groups divided by baseline performance.



Note. Data are shown as group mean ± SEM (within-subject experimental design). Factorial ANOVA did not reveal any significant interaction when group was used as a between-subject factor, although there is a near-significant trend ($p=0.063$) of modafinil's differential effect on % of correct responses between the two groups. The trend is interesting on harder delay trials, where modafinil had little enhancement on good group, though improved poor group's performance dose-dependently. This is consistent with the hypothesis that modafinil has greater cognitive-enhancing-effect on poor-baseline-performers on more difficult trial types.

Error Analyses

First-mistakes. For analysis of which attempt in time in a 3-stimulus trial the first-mistakes were made, there were main effects of delay ($F(1,17)=12.437$; $p=0.003$), attempt ($F(1,17)=56.196$; $p<0.01$) and a delay×attempt interaction ($F(1,17)=13.699$; $p=0.002$). All were confirmed by linear contrast comparison indicating significant linear relationships ($p<0.05$). Tukey's post-hoc tests revealed that both delay and attempt had a significant effect on number of first mistakes made, and the interaction was because on delay trials more first-mistakes were made on attempt two, but on no-delay trials, more were made on attempt three.

Factorial ANOVA of which position on the screen first-mistakes in a 3-stimulus trial revealed a delay×position effect ($F(2,34)=7.001$; $p=0.003$). Using group as a between-subject variable, there was a delay×position×group effect ($F(2,34)=3.481$; $p=0.043$). Tukey's post-hoc tests revealed on delay trials, both groups made more first-mistakes on the middle stimulus. In contrast, Tukey's tests found on no-delay trials, whereas the good group made more first-mistakes on the right and fewest on the left, the poor group made more on the left and fewest in the middle. There was no significant difference between sequencing for both groups between baseline and drug days ($p>0.05$). Sequencing data on drug days (TABLE 3) revealed that the poor group, on delay trials, preferred to start in the middle (39.91%-MRL+MLR), but on no-delay trials, preferred to start on the left (48.01% - LMR+LRM). Given first-mistake is the first time a rat revisits a previously visited location, the starting position is more likely to be revisited on following attempts. This shift in starting position preference from middle (delay) to left (no-delay) may account for the difference in number of first mistakes made at each of these locations on different trial types.

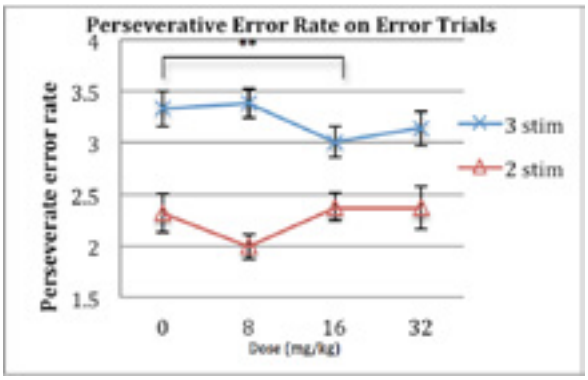
Table 3. Sequencing analysis.

Trial Type	Group	L-M-R (%)	L-R-M (%)	M-R-L (%)	M-L-R (%)	R-L-M (%)	R-M-L (%)
Delay	1 (Good)	15.74 (4.11)	9.96 (2.81)	16.15 (2.54)	17.97 (2.53)	17.16 (3.96)	23.02 (3.82)
	2 (Poor)	24.43 (5.69)	8.76 (1.70)	25.24 (2.74)	14.67 (2.23)	13.42 (2.55)	13.49 (3.14)
No Delay	1 (Good)	10.75 (2.29)	16.46 (3.67)	19.04 (5.15)	24.71 (2.9)	11.38 (2.43)	17.67 (2.59)
	2 (Poor)	21.45 (2.48)	26.56 (3.67)	7.39 (2.02)	21.27 (4.63)	11.48 (3.89)	11.84 (4.76)

Notes. L=left, M=middle, R=right position on screen, in order of tapping of touchscreen. Data are shown as group mean with SEM in brackets.

Preservative mistakes error rate. This was calculated by dividing number of mistakes (excluding first mistakes) by number of imperfect trials. ANOVA showed significant main effect of delay ($F(1,17)=5.298$; $p=0.034$). Linear contrast comparison found a significant linear trend of delay ($p=0.034$), and post-hoc Dunnett's tests revealed a significant effect of delay increasing perseverative-mistake-error-rate ($p=0.034$). ANOVA also found a dose×stimulus-number interaction ($F(3,51)=2.96$; $p=0.041$), Dunnett's test revealed a significant ($p=0.007$) difference between 16mg/kg and vehicle (FIGURE 6). Using group as a between-subject variable, there was no significant interaction of dose×stimulus-number ($F(3,51)=2.009$; $p=0.176$).

Figure 6. Effect of modafinil on perseverative error rate.

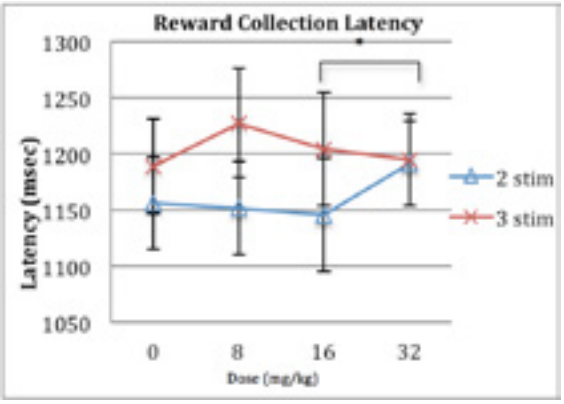


Note. Data are shown as group mean ± SEM (n=18) (within-subject experimental design). Factorial repeated measures ANOVA found a significant dose×stimulus-number interaction ($F(3,51)=2.96$; $p=0.041$), Dunnett's post-hoc found a significant difference at dose 16mg/kg compared to vehicle ($p=0.007$), though not at any other doses ($p>0.05$). ** denotes the significant difference ($p<0.01$). The graph reveals a differential effect of modafinil across 2 and 3-stimulus. On 3-stimulus trials, 16mg/kg significantly reduced perseverative error rate (lowest at 16mg/kg). No significant effects were found for 2-stimulus trials.

Latency Analyses

There were no significant main effects of drug or drug×stimulus-number for either correct-response-latency or error-latency ($p>0.05$). ANOVA revealed a significant stimulus-number×drug interaction for reward-collection-latency ($F(3,51)=2.784$; $p=0.005$) (FIGURE 7). Dunnett's post-hoc did not reveal any significant difference between any doses and vehicle ($p>0.05$). Tukey's revealed a significant difference between 16mg/kg and 32mg/kg ($p=0.019$). ANOVA found no significant main effect of drug when group was used as a between-subject variable ($p=0.07$).

Figure 7. Effect of modafinil on reward collection latency.



Notes. Data are shown as group mean ± SEM (n=18) (within-subject experimental design). Dunnett's post-hoc tests revealed reward collection latency at none of the doses for either stimulus number were significantly different from vehicle ($p>0.05$). Tukey's post-hoc test revealed there was a significant difference between 16mg/kg and 32mg/kg ($p=0.019$), denoted by *. The graph shows whereas 32mg/kg decreased reward collection latency on 3-stimulus trials, it increased reward collection latency on 2-stimulus trials. For 2-stimulus trials, a relatively stable latency was observed across low doses, with a sudden increase at 32mg/kg. In contrast, 3-stimulus trials showed greatest latency at 8mg/kg, whereas at higher doses the latency was comparable to vehicle.

4. Discussion

The main finding of this study was that modafinil significantly reduced error-rate selectively on the more difficult 3-stimulus trials, mirroring the effect found in humans by Müller et al (2013). There was also a near significant increase in percentage-perfect, only on the more difficult delay trials in poor baseline group. Neither was accompanied by any changes in correct/error latencies. There was a significant effect of modafinil on reward collection latency, which may reflect changes in motivation. The effects of modafinil in this study are discussed from both a psychological perspective by assessing its implications for spatial working memory, as well as from a neurobiological perspective by hypothesizing the neural substrates underlying this cognitive enhancing effect.

The finding of reduced error rate only on the harder 3-stimulus trials is consistent with Müller et al (2013)'s finding in humans, where a reduced error-rate was only found on the more difficult 10- and 12-box levels of CANTAB SWM task. From a psychological perspective, this suggests modafinil's pro-cognitive effects may only be observed when cognitive load on spatial working memory is above a critical threshold. This has been found in animal literature using T-maze (Beracochea et al., 2001, 2002, 2003) and Morris Water Maze (Shuman, Wood, & Anagnostaras, 2009). However, these previous studies only found enhanced performance at doses of modafinil (64mg/kg or 75mg/kg) higher than the present study, suggesting the current task may be more sensitive for detecting modafinil's effect on spatial working memory. The significant reduction in error rate found by Müller et al (2013) was by using a clinically relevant plasma concentration of modafinil in humans (200mg), which translates to around 10mg/kg in rodents (Yu et al. 2000), close to the significant effect observed at 16mg/kg in the current study. This suggests the current novel touchscreen study is a successful translation of the CANTAB SWM task in humans for use in preclinical animal studies, and its improved sensitivity to modafinil's cognitive-enhancing-effect makes it superior to existing paradigms assessing spatial working memory in rodents.

Two interesting findings also emerged when comparing modafinil's effect on error-rate and percentage perfect measures. First, the cognitive enhancing effect for each measure was dependent on a different type of difficulty manipulation. Error rate on stimulus-number, percentage perfect on delay. Second, subject's baseline performance only seemed to predict modafinil's effect on percentage-perfect, reflected by a near significant interaction between dose, delay and group, where modafinil improved poor group's performance on harder delay trials more than good performers. Baseline performance did not affect modafinil's effect on error rate. Orthogonality between these two measures suggests they may reflect two different components of spatial-working-memory. Spatial working memory is a complex cognitive process involving many components, such as planning and selection of most efficient strategy mediated by frontal lobe, and the self ordered sequencing nature of the task would have also required efficient mnemonic processing and accurate sequencing encoding mediated by subcortical areas such as hippocampus. Therefore, the two performance measures may reflect different aspects of spatial working memory, with percentage perfect reflecting efficiency of executive planning and error rate reflecting quality of mnemonic encoding. This dissociation suggests each measure may be assessing the efficiency of a different node in neural network underlying spatial working memory. The fact that modafinil improved performance assessed by both measures may imply that it improved overall efficiency of neural network, rather than a single node.

Functional imaging in rats supports modafinil's enhancement of this neural network. Gozzi et al. (2012) used pharmacological magnetic resonance imaging to map circuitry activated by modafinil at 10mg/kg admin-

istered intravenously in rats. They found stimulation of fronto-cortical areas involved in higher cognitive function, and activation of other subcortical areas (e.g. thalamus and hippocampus), suggesting modafinil does act on the neural substrates associated with spatial working memory. Note the dosage chosen is clinically relevant and can be easily translated to human studies.

Neurobiological effects of modafinil on spatial working memory has also been assessed in human patients with frontal lobe lesions, temporal lobe lesions and amygdalo-hippocampectomy, using SWM task with 4/6/8 boxes (Owen, Sahakian, Semple, Polkey, & Robbins, 1995). Whereas frontal lobe patients displayed impairment across all difficulty levels due to failure to adopt the most efficient sequencing strategy, temporal and amygdalo-hippocampectomy groups only showed impairment at the most challenging level, and was unrelated to strategy selection but rather reflect a more fundamental disruption of mnemonic processing. This study also suggested a spatial working memory network consisting of cortical and subcortical nodes. Top-down control exerted by prefrontal cortex is responsible for selecting the most efficient strategy to solve a problem, and the efficiency of each strategy depends on bottom-up feedback of mnemonic processing by subcortical regions. Therefore, selective enhancement observed on harder 3-stimulus trials in the current study suggests that modafinil perhaps enhanced the efficiency of subcortical nodes to improve mnemonic processing, and improved coding accuracy of more complex sequences on harder trials, improving quality of bottom-up feedback.

Owen, Downes, Sahakian, Polkey, & Robbins (1990), using an automated Tower of London task, found patients with frontal lobe lesion could solve the most difficult versions but required more moves per problem than matched controls, suggesting inefficient planning and selection of sequencing strategy. Therefore modafinil could have also improved frontal lobe's top-down control to plan and select the most efficient sequencing strategy to minimize load on spatial working memory, and reduced error rate on harder 3-stimulus trials. Owen et al. (1990) also found that frontal lobe patients did not show an increase in latency before first move on any trial, but there was an increase in latency accompanying correct responses on subsequent moves. The authors interpreted this as increased impulsivity to respond before forming an efficient sequencing plan, therefore, to ensure subsequent responses are correct, more time is spent thinking per move to compensate for lack of pre-planning.

Perhaps the difference in baseline performance between the groups reflected a difference in their frontal lobe executive planning efficiency. Poor baseline group may require more thinking time before beginning of next attempt to ensure a correct response. If this increased planning of the next move can take place during delay and complete before the re-presentation of stimuli for the next attempt in any trial, it could account for why near significant effect of increased percentage perfect is observed only across delay trials, and why this was unmatched by any changes in correct response latency because thinking was completed during the delay. However, this account predicts that on no delay trials, this increased thinking should result in an increase in correct response latency, but this was not observed. This suggests modafinil's cognitive enhancing effect is more complex than simply increasing frontal lobe's planning efficiency, and given that baseline performance did not affect modafinil's effect on error rate, it implies modafinil did not act solely on frontal lobe, but also on subcortical areas in the network underlying spatial working memory.

Finally, enhancement in performance was not accompanied by any changes in correct/error latency measures, inconsistent with Turner et al. (2003)'s proposal of modafinil shifting speed accuracy trade off. However, perhaps speed accuracy trade-off was not as important a factor in optimizing performance for the current non-speeded spatial working memory task, compared to reaction tasks (e.g. Stop-Signal) used by Turner et al. (2003) where subjects were under greater time pressure. Modafinil did affect reward collection latency. This was not a change in general motor readiness or feeding, which should have affected all latencies across all trial types consistently, but was not observed in the present study. In addition, the suggested anorexigenic effect of modafinil (Nicolaidis & De Saint Hilaire, 1993) lacks replication (Morgan, Crowley, Smith, LaRoche, & Dopheide, 2007), making it an unlikely explanation. Perhaps higher doses made the harder 3-stimulus trials more enjoyable and increased motivation. Müller et al (2013) found participants rated the task as more enjoyable after taking modafinil, although subjective ratings are not easy to assess in rats. One potential measure for general motivation is the number of trials completed within each session, although this analysis was not possible as nearly all subjects completed all trials within each session.

The current study did not include any biochemi-

cal assays to investigate modafinil induced neurochemical changes, but some potential underlying neurotransmitter pathways are discussed. Catecholamines are involved in regulation of arousal, and stimulants (e.g. amphetamine) often work by increasing presynaptic release of dopamine and noradrenaline. In contrast, modafinil has been proposed to work post-synaptically as a putative noradrenergic receptor agonist, binding to and enhancing activity of alpha-1 receptors (Akaoka, Roussel, Lin, Chouvet, & Jouvet, 1991) and beta receptors (Lin et al., 1992). Modafinil's effect on dopaminergic system is more controversial. Some animal studies suggested it had little impingement on dopaminergic activity (Redrobe, Bull, & Plath, 2010; Waters, Burnham, O'Connor, Dawson, & Dias, 2005), has low abuse potential and less effect on extrapyramidal motor activities compared to amphetamine, making it safer to use. However, modafinil may modulate dopaminergic activity indirectly, such as by influencing enzymatic activity involved in formation of dopamine from Levo-Dopa (Murillo-Rodríguez, Haro, Palomero-Rivero, Millán-Aldaco, & Drucker-Colín, 2007). Studies using dopamine receptor antagonists such as SCH23390 (D1-receptor-antagonist) and raclopride (D2-receptor-antagonist) have generated mixed findings. Some, using knockout mice models, argued modafinil enhanced dopamine activity by directly binding to D1 and D2 receptors (Young, Kooistra, & Geyer, 2011; Young, 2009). Others suggested indirect activity by inhibiting dopamine transporters (DAT), mimicking the effect of GBR12909 (selective DAT inhibitor) (Young & Geyer, 2010). The latter has been replicated in humans using clinically relevant doses of modafinil (Volkow et al., 2009). Dopamine influences motivation, Young & Geyer (2010) found an increased motivation using modafinil at 16, 32, and 64mg/kg, similar to doses used in the current study.

If faster reward collection latency indicated higher motivation, then the present study only partially supports modafinil's role in enhancing motivation since latency decreased only for 3-stimulus trials, and increased for 2-stimulus trials. Perhaps increased dopamine transmission led to aberrant salience attribution to irrelevant cues in the environment (Kapur, 2003), which distracts focused attention. This may be more problematic on easier 2-stimulus than harder 3-stmulus trials, as the former has lower cognitive load so the subject may be more easily distracted. An alternative hypothesis is rats may have experienced increased anxiety, distracting from limited executive resources for controlling sustained attention (Eysenck, 1979), and human subjects high in trait anxiety were prone to greater distraction by task-irrelevant stimuli (Deffenbacher & Hazaleus, 1985; Forster, Elizalde, Castle, & Bishop, 2013). This seems less plausible than the aberrant salience account, since modafinil has not been shown in animal studies to have anxiogenic effects (Simon, Panissaud, & Costentin, 1994), and it is unlikely that modafinil would selectively increase anxiety on 2-stimulus trials but not on 3-stimulus trials within the same session. However, a biochemical assay assessing modafinil's effect on dopamine transmission is needed to test these speculations.

Limitations

Within-subject design minimised limitations of small sample size on statistical power. However, when group was used as a between-subject variable, sample size was halved which reduced statistical power, so larger sample size would have been favourable. To use difference in number of trials completed as a measure of motivation, either session length should be decreased or number of trials increased to prevent completion of all trials within each session. A time-out should be added so a response made outside a certain time frame is counted as incorrect. This requires subjects to respond quickly to maximize performance, analogous to human reaction-time tasks (e.g. Stop-Signal) used by Turner et al. (2003), allowing better assessment of modafinil's effect on speed-accuracy-trade-off.

Future Experiments

Two further experiments are proposed based on current data interpretation. To assess the contribution of cortical and subcortical structures to performance and whether modafinil affects each region differently, a lesion study can be used, with frontal lobe lesion and hippocampus lesion compared against sham lesioned animals. Receptor antagonists to noradrenergic and dopaminergic receptors can also be used in combination to central microinfusions of modafinil into the prefrontal cortex to investigate the neurobiological specificity underlying the mechanisms of modafinil.

A second experiment modifies the touchscreen task to assesses whether any differences observed in performance on 2 and 3-stimlus trials is attributable to a difference in working memory load imposed by trial type, or executive function governing flexible encoding of positions poked to always minimize working memory load.

To succeed in the current self-ordered-sequencing-task, subject can either choose to encode the position poked or unpoked within a given trial. For 2-stimulus trials, both strategies require memory of one location with no difference on memory load. However, for 3-stmulus trials, different strategies generate different working memory load. A correct attempt two can be achieved by either remembering one previously poked location, or two unpoked locations, the former minimises working memory load. The reverse is true for attempt three. Therefore, subjects who flexibly change their strategy to always minimize their working memory load across attempts within the same trial may perform better on harder 3-stimlus trials, and across delays. The difference between good and poor performers may not be one of working memory but of executive control, suggesting modafinil may enhance executive control by increase flexibility in coding strategy in poor baseline performers.

To assess if this flexible encoding affect performance, a 4-stimulus trial type can be introduced. Attempt three in the 4-stimulus trial always requires encoding of two locations no matter what coding strategy used, which serves as a baseline for comparison since attempts two and four require encoding of either one (easier than attempt three) or three locations (harder than attempt three). None of the attempts within a 3-stimulus trial can be used as a baseline, and therefore it is necessary to use 4-stimulus trials. If animals can flexibly change their coding strategy to always minimize working memory load, then error-rate on attempts two and four should be less than for attempt three (always encoding one rather than two locations), suggesting the task assesses executive function. If animals do not change coding strategy and the task is purely assessing working memory, then depending on whether the animals chooses to encode places poked or unpoked, more errors should be made on attempts four and two, relative to attempt three, respectively. This predicted difference in pattern of error rate enables further specification of the cognitive processes underlying this task.

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