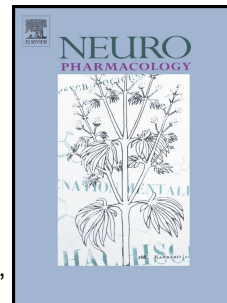


Accepted Manuscript

Title: The psychostimulant modafinil facilitates water maze performance and augments synaptic potentiation in dentate gyrus

Authors: Marian Tsanov, Declan G. Lyons, Sally Barlow, Rodrigo E. González Reyes, Shane M. O'Mara



PII: S0028-3908(10)00086-9

DOI: [10.1016/j.neuropharm.2010.03.010](https://doi.org/10.1016/j.neuropharm.2010.03.010)

Reference: NP 3895

To appear in: *Neuropharmacology*

Received Date: 31 July 2009

Revised Date: 16 February 2010

Accepted Date: 19 March 2010

Please cite this article as: Tsanov, M., Lyons, D.G., Barlow, S., González Reyes, R.E., O'Mara, S.M. The psychostimulant modafinil facilitates water maze performance and augments synaptic potentiation in dentate gyrus, *Neuropharmacology* (2010), doi: 10.1016/j.neuropharm.2010.03.010

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**The psychostimulant modafinil facilitates water maze performance
and augments synaptic potentiation in dentate gyrus.**

Marian Tsanov^{a,b,1}, Declan G. Lyons^{a,b,1}, Sally Barlow^{a,b,1}, Rodrigo E. González Reyes^{a,b} and Shane M. O'Mara^{a,b,CA}

^a Trinity College Institute of Neuroscience, Trinity College Dublin, Ireland

^b School of Psychology, Trinity College Dublin, Ireland.

^{CA} Shane M. O'Mara:

Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland

Fax: +353-1-896 3183; Tel: +353-1-896 8447; E-mail: smomara@tcd.ie

¹ These authors contributed equally to the study

Running title: Modafinil enhances hippocampal learning and synaptic plasticity.

Keywords: Modafinil, Water Maze, Theta oscillation, Long-Term Potentiation, Dentate Gyrus

Abstract

Modafinil is a psychostimulant drug used widely for the treatment of narcolepsy, which also has additional positive effects on cognition. Here, we investigate the effects of modafinil on behavioral performance and synaptic plasticity in rats. Improved acquisition of water maze task was observed for animals that underwent chronic treatment with modafinil. We found that the distance travelled and escape latency were reduced after the first day in chronically-treated rats, compared to controls. Importantly, the swim velocity was similar for both groups, excluding pharmacological effects on motor skills. We also found that modafinil increases synaptic plasticity in the dentate gyrus of urethane-anaesthetized rats; modafinil induced a robust augmentation of the population spike, evident after application of 2 bursts of 200Hz, high-frequency stimulation. Furthermore, the modafinil-dependent enhancement of postsynaptic potentials correlated selectively with theta rhythm augmentation. We propose that modafinil may facilitate spatial orientation via increased theta-related hippocampal plasticity.

Introduction

Modafinil [(diphenyl-methyl)-sulfinyl-2-acetamide] is a psychostimulant used for the treatment of excessive sleepiness associated with narcolepsy (Moldofsky et al., 2000; Mignot et al., 2002; Mignot and Nishino, 2005), Parkinson's disease (Ferraro et al., 1998; Nieves and Lang, 2002) and multiple sclerosis (Kraft and Bowen, 2005). In contrast to traditional psychostimulants, modafinil has relatively few side-effects and a lower risk of adverse effects on organ systems such as the cardiovascular system (Scammell and Matheson, 1998; Jasinski and Kovacevic-Ristanovic, 2000; Cox and Pappagallo, 2001; Deroche-Gamonet et al., 2002; Minzenberg and Carter, 2007). Modafinil appears to bind directly and inhibit the dopamine and noradrenaline transporters (Madras et al., 2006; Korotkova et al., 2007).

Modafinil promotes enhanced alertness and sustaining wakefulness and also is effective in sustaining and restoring executive functions in sleep-deprived subjects (Schwartz et al., 2004; Wesensten, 2006). Furthermore, in non sleep-deprived healthy subjects, modafinil has a positive effect on visual pattern recognition memory, spatial planning and stop-signal reaction time (Turner et al., 2003). Preclinical studies have also revealed positive effects on neurocognitive performance. Modafinil treatment in normal mice induced a significant improvement in learning and memory, compared to vehicle-treated controls, using spontaneous alternation and serial spatial discrimination T-maze tasks (Beracochea et al., 2001; Beracochea et al., 2003), although it appears to be without effect on the five-choice serial reaction time in rats (Waters et al., 2005).

Finally, modafinil treatment in mice and rats is not associated with an increase in anxiety, stereotyped behavior (Duteil et al., 1990; Simon et al., 1994) or visual discrimination (Morgan et al., 2007).

In order to evaluate the influence of modafinil on rats' spatial performance, we have explored in this study the performance of modafinil-treated rats in the water maze (Morris, 1984). The hippocampal region is believed to play a key role in the formation of episodic memories (in particular spatial memory), and long-term synaptic plasticity is generally accepted as the mechanism underlying experience-dependent alterations in hippocampus. Thus, the second objective of the present work was to examine the treatment effect of modafinil on hippocampal synaptic plasticity, using extracellular recording from the dentate gyrus. Synaptic plasticity and the formation of memory traces have been linked to particular states of the hippocampal network, determined by theta rhythm (Buzsáki, 2002; Buzsáki and Draguhn, 2004). Therefore, we have also explored how oscillatory activity changes in response to modafinil application.

Materials and methods

Animals. Experiments were conducted in accordance with European Community directive, 86/609/EC, and the Cruelty to Animals Act, 1876, and followed Bioresources Ethics Committee, Trinity College, Dublin, Ireland, and international guidelines of good practice. Male (15 week-old) Wistar rats (Harlan, UK) were triple housed and maintained on 12 : 12 h light : dark cycles with food and water provided *ad libitum*.

Morris Water Maze training. The Morris water maze procedure (Morris, 1984), designed to test spatial reference memory, utilized a black circular tank (of 150 cm inner diameter, and 40cm height) filled with water to a depth of 30cm (Cowley et al., 2008). This circular arena was divided into four quadrants: North East (NE), North West (NW), South East (SE) and South West (SW), defined relative to the experimenter's position, with all eight cardinal compass points marked on the tank. A submerged platform (9.5cm in diameter) was located in the centre of the NE quadrant, at a depth of 2cm under the surface of the water, concealing its position. The whole apparatus was isolated from the experimental room (and the experimenter) by floor-to-ceiling black curtains, on which distinctive visual cues were affixed above the Northern and Western points. Low-level lighting was provided by three 40-Watt desk lamps arrayed at regular intervals around the base of the tank. Water temperature was maintained at constant degree of 20 - 21°C.

Testing in the water maze began 7 days following the start of drug treatment. For each trial the subject rat was placed into the tank facing the wall. The start position for each trial was chosen randomly, from among the eight compass points. The rat was then allowed to explore the tank by swimming. If the platform was reached the rat was allowed to remain there for 15 seconds before being removed to a waiting area, outside the curtains, for a 30 second inter-trial period. If after 60 seconds in the tank the rat had not reached the platform, he was gently guided there and left for 15 seconds before removal to the waiting area for 30

seconds. Each rat was given 5 trials per day over 5 consecutive days (Days 1-5), after which no tests were carried out for 2 days before being re-tested for 2 further days (Days 8 and 9). The order in which the rats were tested was counterbalanced from day-to-day. The velocity (cm/s), distance traveled (cm), and latency to find the platform (escape latency, s) for each rat was recorded. Thirty minutes after the last trial of the last animal on day 9, each rat underwent a probe trial. For this trial the platform was removed from the tank and the rats were all allowed the maximum trial-time of 30 seconds. Analysis of each rat's performance was carried out using video tracking software (Ethovision 3.1, Noldus Information Technology, Wageningen, Netherlands).

Analysis of Morris Water Maze Data. A 2-way repeated-measures ANOVA was used to assess differences in the latency trials (with treatment group and trial day the independent variables). A 1-way ANOVA followed by a Tukey Test was used to assess differences between the treatment groups on each day. In cases where groups weren't normally distributed, a Kruskal-Wallis test by ranks was employed, followed by Dunn's Multiple Comparison test.

For the probe trial, velocity and distance were analysed by the use of a 1-way ANOVA with a Tukey post-hoc test with treatment group as the independent variable. In cases where the data was not normally distributed, a Kruskal-Wallis test by ranks was employed, followed by Dunn's Multiple Comparison test. All

statistical tests were carried out using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, California, USA)

Surgical preparation. Under urethane anesthesia (ethyl carbamate: 1.5 g/kg, i.p.), the animals underwent implantation of a monopolar recording electrode (RNEX-300, Kopf Instruments) in the granular layer of the dentate gyrus and bipolar stimulating electrodes in the medial perforant pathway. For the recording electrode, a drill hole was made (1-mm in diameter), 1.9 mm posterior to bregma and 3.1 mm lateral to the midline, corresponding to the dentate gyrus in the rat (Kruger et al., 1995). A second drill hole was made for a bipolar stimulating electrode with coordinates targeting the medial perforant pathway (6.9 mm posterior to bregma, 4.1 mm lateral to midline). The dura was pierced through both holes using a needle. A recording electrode was lowered 2.5–3.0 mm from the dural surface to reach the dentate gyrus. A stimulating electrode was positioned in the medial perforant pathway ipsilateral to the hemisphere from which dentate recordings were obtained. The depth was 2.3–2.7 mm from the dural surface (Kruger et al., 1995). Final positions of the stimulating and recording electrodes were determined by maximizing the amplitude of the field potential recorded in the dentate gyrus in response to electrical stimulation of perforant path. Monopolar recordings from dentate gyrus were made relative to ground and reference screws inserted into the contralateral parietal and frontal bones. Once verification of the location of the electrodes was complete, recordings were allowed to stabilize for 10 min prior to the experiment.

Measurement of evoked potentials. Signals were filtered between 0.1 Hz and 1 kHz, and then amplified (DAM-50 differential amplifier; World Precision Instruments, Hertfordshire, UK). Recordings were digitized online using a PC connected to a CED-1401 plus interface and analyzed using “Spike 2” software (CED, Cambridge, UK). The field excitatory postsynaptic potential slope (fEPSP) was used as a measure of excitatory synaptic transmission in the dentate gyrus. To obtain these measurements, an evoked response was generated in the medial perforant pathway by stimulating at low frequency (0.025 Hz) with single biphasic square wave pulses of 0.1 msec duration per half wave, generated by a constant current isolation unit. For each time-point measured during the experiments, five records of evoked responses were averaged. The fEPSP slope was measured as the intermediate 90% of the slope value between the first negative and the first positive deflections of the fEPSP. The population spike (PS) amplitude represents the absolute difference between the value of the first positive and the value of the first negative deflections of the fEPSP.

By means of input–output (IO) curve determination, the maximum fEPSP was found, and during experiments all potentials used as baseline criteria were evoked at a stimulus intensity that produced 40% of this maximum (100–400 μ A). Baseline responses were collected for 30 min before the application of the high-frequency stimulation (HFS) protocol. HFS consisted of two bursts of 15 stimuli with pulse duration of 0.2 ms with an interstimulus interval of 5ms and interburst interval of 10 s. HFS protocols with one, three and four bursts were also tested.

In all cases, the stimulus amplitude was that used for recordings. For the experiments that measured the direct effect of modafinil application on fEPSP, an injection with the drug was applied after 30 min of baseline recording. The stimulation intensity of the applied current was: 120 – 240 μ A for the HFS control group (n = 4), 100 – 250 μ A for the HFS modafinil group (n = 4), 130 – 240 μ A modafinil injection group (n = 7) and 110 – 270 μ A for the baseline group (n = 5).

Analysis of electrophysiological data. The baseline fEPSP data were obtained by averaging five sweeps at 40s intervals evoked by stimulation of the perforant path. The baseline tests-pulses were conducted every 5 min over a period of 30 min. Electrophysiological data were then expressed as mean percentage of baseline fEPSP \pm standard error of the mean (S.E.M.). Statistical significance was estimated by using factorial analysis of variance (ANOVA) and post-hoc Student Newman-Keuls test. Using factorial ANOVA, we estimated the effects of stimulation protocol and time on the field potential values, compared to the baseline period, defined as the first six time points. The probability level interpreted as statistically significant was $p < 0.05$.

Analysis of network activity. The intracortical electroencephalogram (EEG) was obtained via recordings from the dentate gyrus. EEG was sampled at 0.5 kHz and stored for further off-line analysis. In order to evaluate delta (1.5-3.0 Hz), low theta (3.0-5.5 Hz), high theta (5.5-11 Hz), alpha (11-13.5 Hz), beta (13.5-30 Hz) and gamma (30-80 Hz) oscillatory activity during the course of experiment, 4-s

long epochs 1 s after each test-pulse were selected. Fourier analysis of artefact-free epochs was performed with the Hanning window function using “Spike2” software (Cambridge Electronic Design). The absolute values of spectral power for each individual animal were transformed into relative ones that were then used further for statistics. For each time-point, the results of Fourier analysis of five epochs were averaged. Theta activity was measured by the ratio between the relative values of theta and delta spectral powers (T-ratio). The statistical treatment and analysis of data included the calculation of descriptive statistics (mean, S.E.M.) and ANOVA. For the comparison of 30 min T-ratio periods, we used Student’s t-test and for the correlation analyses, we used Pearson’s correlation coefficient. EEG signal frequency analysis was done using MATLAB’s Signal Processing Toolbox (MATLAB Natick, MA) where the power was calculated by extracting the short-time Fourier transform from a signal (hanning window of 2s, overlap of 1 s, sampling frequency 100 Hz) and interpolated into a continuous power spectrum aligned to the haemodynamic traces. Information was displayed as the magnitude of the time-dependent Fourier transform versus time in a colour gradient graph.

Post-mortem verification of electrode site. At the end of the study, brains were removed for histological verification of electrode localization. The animals underwent transcardial perfusion with 0.1 M PBS followed by 10% formol-saline. The brains were postfixed in 10% formol-saline and then transferred to 25% sucrose overnight. Brain sections (16 μ m) were embedded in paraffin, stained

according to the Nissl method using 1% toluidine blue, and then examined using a light microscope. Brains in which incorrect electrode localization was found were discarded from the study.

Drug Treatment. Modafinil (Provigil; Cephalon Inc. Frazer, Pennsylvania) tablets (100 mg Modafinil/tablet) were thoroughly pulverised by sonication in distilled water, and maple syrup was subsequently added to this suspension to form the administered treatment mixture. This mixture was administered p.o., by feeding each rat the appropriate quantity via a 1 ml syringe. Each rat had been administered 0.2 ml of maple syrup daily for 4 days prior to the first drug administration. This served the dual purpose of habituating the rats to the procedure of feeding from a syringe and of familiarizing them to the flavour of the maple syrup and hence the drug vector preparations based upon it. Consequently, the rats fed avidly upon these drug preparations, and the risk of variable dosing due to the rats not swallowing the vector was minimized. Before each administration of the drug preparation, it was thoroughly vortexed to ensure homogeneity, as modafinil is only sparingly soluble in aqueous solution (Minzenberg and Carter, 2007). We have previously found (Della-Chiesa et al., 2008) that this method of administration is both reliable and avoids the stresses associated with gavage.

The drug preparations were administered once daily, at a regular time, at least 1 hour before the commencement of any of the behavioural tasks, because acute administration of modafinil for behavioural tasks involved drug administration

approximately 30 minutes before the procedures (Waters et al., 2005). The drug preparations were delivered for a total of 16 consecutive days, commencing 7 days before the first water-maze trial, and continuing until the end of the water-maze protocol. The target doses were 10 mg/kg and 64 mg/kg, which reflect concentrations of low-end effective dose and a high dose, respectively, for humans (Wong et al., 1999b). The testing on the water-maze began 7 days following the start of drug treatment.

For the electrophysiological experiments, modafinil tablets (as described above) were dissolved using an ultrasound sonicator for 15 minutes and dissolved in 6 ml of normal 0.9% saline solution with a final concentration of 150 mg/kg (Ishizuka et al., 2003). The effect of drugs that activate monoamine transporters depends on the mode of administration; i.p. injection of modafinil has diminished effect in comparison to its equivalent amphetamine concentration administered i.v (Engber et al., 1998a; Florence et al., 2000). In addition the effect of the anaesthesia alters modafinil pharmacokinetics (Florence et al., 2000), unlike the freely-behaving animals (Engber et al., 1998a; Engber et al., 1998b). Therefore the target of our bolus dose of modafinil was to overcome the decreased drug distribution of i.p. administration under urethane anaesthesia compared to the chronic 64 mg/kg administration of behaving rats. We also limited the i.p. dose to 150 mg/kg, avoiding the dose-dependent, secondary effects on the glutamate and GABA that require doses over 200mg/kg (Tanganelli et al., 1994; Tanganelli et al., 1995; Ferraro et al., 1997; Ferraro et al., 1998). Modafinil was injected

(i.p.) after the initiation of the anaesthesia and 120 min before the baseline recording. After 30 minutes of baseline recording under modafinil treatment a 2 burst (with 10 seconds interval) protocol to induce potentiation was used and a 120 minutes follow-up period was recorded. In the second type of recordings – drug injection instead of HFS, a 30 minutes baseline was followed by modafinil injection (150 mg/kg i.p.) with a follow-up recording period of 120 minutes.

Results

Chronic treatment with modafinil improves water maze performance

We observed a significant main effect due to treatment group on the distance travelled measure (ANOVA, $F_{(2, 702)} = 3.572$, $p < 0.05$, $n = 8$); path lengths in the modafinil-treated groups were shorter compared to controls (Fig 1A). The effect of modafinil-group by day interaction was also significant (ANOVA, $F_{(12, 702)} = 4.102$, $p < 0.001$, $n = 8$). Additional analysis (Tukey's test following One-Way ANOVA) revealed that these differences were statistically significant on day 2 (Tukey's, $p < 0.05$ for 10 mg/kg modafinil versus control groups; $p < 0.001$ for 64 mg/kg modafinil versus control groups, Fig 1A) and also on day 3 (Tukey's, $p < 0.05$ for 10 mg/kg modafinil, $p < 0.001$ for 64 mg/kg modafinil group versus controls, Fig 1A).

Similarly, a significant overall main effect of modafinil treatment on the latency of escape was found in the acquisition trials (ANOVA, $F_{(2, 702)} = 3.110$, $p < 0.05$). Concurrently, the effect of modafinil-group by day interaction for latency of escape demonstrated significant value (ANOVA, $F_{(12, 702)} = 2.327$, $p < 0.01$, $n =$

8). The mean escape latency was reduced with the time to find the platform being significantly shorter on day 2 (Tukey's, $p < 0.05$, for 10 mg/kg modafinil and $p < 0.01$, for 64 mg/kg modafinil versus controls, Fig 1B) and on day 3 (Tukey's, $p < 0.01$ for 10 mg/kg modafinil and $p < 0.05$ for 64 mg/kg modafinil versus controls).

For an estimation of whether modafinil induces motor effects which could influence water-maze performance, we analyzed the swim velocity throughout the experiment. No significant differences were found in the mean swim velocities of each group per day (ANOVA, $F_{(2, 702)} = 0.552$, $p > 0.05$, $n = 8$ Fig 1C).

A probe trial was used to assess the spatial reference memory in the animals. For this purpose thirty minutes after the last trial of the last animal on day 9, the platform was removed from the tank and the rats were allowed to explore for a trial time of 30 seconds (Fig 1D). We compared the time spent in each quadrant and found no significant differences between groups in the proportion of the trial time that they spent in each of the quadrants, including the target (North-East) quadrant (Tukey's test following One-Way ANOVA, $p > 0.05$, $n = 8$, Fig 2A). Additionally, no differences were present in the average swim velocity (Dunn's multiple comparison test following Kruskal-Wallis ANOVA by ranks, $p > 0.05$, $n = 8$, Fig 2B) or in the number of entries into each quadrant (Dunn's test, $p > 0.05$, $n = 8$, Fig 2C). Although the 10 mg/kg modafinil group did reveal a longer mean distance traveled during the trial, compared to the control group (Dunn's test, $p <$

0.05, $n = 8$, Fig 2D), no significant change was observed for distance traveled in the 64 mg/kg modafinil group, compared to controls (Dunn's test, $p > 0.05$, $n = 8$, Fig 2D).

Modafinil enhances dentate gyrus LTP induced by 200Hz, 2 bursts stimulation to medial perforant pathway

Under urethane anaesthesia male rats underwent implantation of a recording electrode in hippocampal dentate region and stimulation electrode in medial perforant pathway. 30 min of baseline recording was followed by high-frequency stimulation (HFS, see methods). We first attempted to evoke stable and long-lasting synaptic plasticity at an intermediate level, which would allow a pharmacological modulation to increase or decrease the degree of potentiation. In order to find the optimal protocol for such HFS we tested differing numbers of 200Hz bursts (Fig. 3). One burst failed to evoke consistent long-term potentiation of the fEPSP slope (Fig. 3A) and population spike (PS) amplitude (Fig. 3B). Three bursts lead to saturation of plasticity response about 60 min after the stimulation, whereas four bursts reached the maximal point of plasticity immediately after the stimulation. Two bursts evoked small amplitude, but long-lasting potentiation, for both fEPSP slope (Fig. 3A) and PS (Fig. 3B).

Next, we evaluated the effect of modafinil on the 2 burst protocol. The rats were injected with a single dose of modafinil (150 mg/kg i.p.), 120 min prior to tetanization (timing was adjusted to allow modafinil absorption, which reaches

plasmatic peak levels 2 to 4 hours after oral administration with a half-life of 15 hours (Wong et al., 1999a; Wong et al., 1999b). Modafinil-treated animals expressed higher values of fEPSP slope after HFS, compared to controls (Fig. 4A, ANOVA, $F_{(1, 29)} = 2.912$, $p < 0.05$, $n = 4$). Even more robust was the increase of population spike (PS) amplitude in the modafinil group (Fig. 4B, Supplementary fig S1A,B; ANOVA, $F_{(1, 29)} = 8.443$, $p < 0.001$, $n = 4$). The observed augmentation for both fEPSP slope and PS continued throughout the 120 min post-tetanic recording.

Modafinil increases baseline dentate gyrus fEPSP

The differences in LTP in modafinil-treated rats raised the question of whether the field response was increased by the modafinil application before the stimulation protocol. Thus, we explored the changes in the fEPSP of rats, which were injected with modafinil (i.p. 150 mg/kg) after 30 min of baseline recording (Fig. 5). We measured fEPSP slope, PS and field EEG recordings to modafinil application. 30 min after injection the fEPSP slope gradually increased and reached a plateau 60 min after injection. The slope augmentation was subtle but consistent, reaching a level of 110% 90 min after drug application (Fig. 5A, black circles, ANOVA, $F_{(1, 29)} = 3.891$, $p < 0.05$, $n = 7$). The control animals that did not undergo modafinil treatment revealed no long-term alteration of their fEPSP slope (Fig. 5A, white circles, $n = 5$). The increase in PS was more robust at about 160% (Fig. 5B, Supplementary fig S1D; black circles, ANOVA, $F_{(1, 29)} = 5.152$, $p < 0.01$, $n = 7$) compared to the control group (Fig. 5B, Supplementary fig S1C;

white circles, $n = 5$). Importantly, the delayed increase of PS and slope modulation reflects the timing of modafinil pharmacokinetics (Wong et al., 1999a). The modafinil application did not result in detectable reduction of the anaesthesia depth as the toe pinch reflex and tail pinch reflex revealed consistent lack of response throughout the entire recording session.

Modafinil drives selective elevation of theta rhythm

Paralleling the fEPSP slope and PS measurements, we recorded changes in dentate spectral power (Fig. 6). We found an increase in theta spectral power (Fig. 6 A,B, 3.0-11.0 Hz, black line), compared to the slower delta range (Fig. 6 A,B, 1.5-3.0 Hz, grey line). More robust changes after modafinil injection occurred in the low band of theta rhythm (3.0-5.5 Hz). The slower spectral profile of theta oscillation (Fig. 6A), compared to freely-moving recordings (Buzsáki, 2002), is a characteristic feature of peripherally-evoked limbic theta rhythm when recorded under urethane anaesthesia (Holscher et al., 1997; Manns et al., 2003). To compare changes in oscillatory mode, we plotted theta-ratio values in 30 min periods after modafinil injection (white bars, Fig. 6C). Theta-ratio represents the ratio between the relative values of theta and delta spectral powers (Harris et al., 2002). Importantly, the change of theta-ratio also follows modafinil pharmacokinetics, with a significant increase 60-90 min after injection (Fig. 6C, Student t -test, $p < 0.05$, $n = 7$) and higher values for 90-120 min (Fig. 6C, Student t -test, $p < 0.01$, $n = 7$). In contrast, the theta ratio did not change significantly for the control animals (black bars, $n = 5$, Fig 6C).

Detailed analysis revealed parallel changes of low theta and delta during the recording sessions (Fig. 7A). The major factor for theta-ratio increases was the gradual elevation of low theta (Fig. 7A, black symbols, ANOVA, $F_{(1, 29)} = 2.974$, $p < 0.05$, $n = 7$), whereas delta did not change (Fig. 7A, white symbols, ANOVA, $F_{(1, 29)} < 1$, $p > 0.05$, $n = 7$). **The power of the very slow oscillations (0.1-1.5 Hz), which are prominent feature of the hippocampal EEG under urethane anaesthesia during non-theta states, were not affected by the modafinil injection (ANOVA, $F_{(1, 29)} < 1$, $p > 0.05$, $n = 7$).** Compared to the modafinil group, the oscillatory activity in control animals revealed concurrent patterns for both theta (black symbols) and delta (white symbols) spectral powers ($n = 5$, Fig 7B). The EEG of the animals under urethane anaesthesia revealed a slow-onset gradual increase of low theta and delta, reaching a level of 150%, although the ratio between them was kept constant throughout the recording session (Fig 6C). Interestingly, the other high-frequency ranges such as high theta (5.5-11 Hz, Fig. 7C, black symbols, ANOVA, $F_{(1, 29)} < 1$, $p > 0.05$, $n = 7$) and gamma (30-80 Hz, Fig. 7C, white symbols, ANOVA, $F_{(1, 29)} < 1$, $p > 0.05$, $n = 7$) did not change their power after drug application.

In order to gain a better understanding of the time course of the oscillatory patterns before and after modafinil application we have analysed the theta alterations for each individual experiment (Fig 8). This approach revealed a cyclic alternation of the theta band and this fluctuation was paralleled by increased amplitude of the absolute theta power after the modafinil injection (Fig 8A). At the

same time delta spectral power did not undergo any apparent change (Fig 8B). Exploration of shorter time-epochs from the same case demonstrated that the theta episodes tend to occur with longer duration (Fig 8C). The cyclic shifts between theta and delta after modafinil injection favour oscillatory activity with higher frequency and higher amplitude (Fig 8E), compared to the baseline period (Fig 8D). These data reveal the selective impact of modafinil on the low theta spectral power in rats under urethane anaesthesia. An intriguing issue was to estimate the degree of concurrency between oscillatory and plastic alterations due to modafinil action. Low theta power highly correlated with fEPSP slope (Fig. 9, Pearson, $n = 7$, $p < 0.0001$, $r = 0.8118$), demonstrating a parallel elevation of theta epochs and synaptic potentiation. Similarly, low theta power positively correlated with PS (Pearson, $n = 7$, $p < 0.0001$, $r = 0.8077$, data not shown); thus spectral power change and postsynaptic excitability are revealed as co-occurring processes.

Discussion

Our data demonstrate that the behavioural and plasticity effects of modafinil are expressed by facilitated performance in the water maze and increased hippocampal long-lasting potentiation, respectively. Furthermore, modafinil is able to augment both postsynaptic responsiveness and theta rhythm in dentate gyrus after a single application.

Effects of modafinil on water maze performance

Modafinil treatment enhances working memory performance in mice (Pierard et al., 2006) and rats (Ward et al., 2004). In contrast, modafinil did not alter the performance on five-choice serial reaction time in rats (Waters et al., 2005). Our first intention was to test the behavioural effect of modafinil by evaluating the performance of drug-treated rats on a spatial learning task. We found that modafinil-treated rats travel a significantly shorter distance to the hidden platform for the second and the third day of water-maze, associated with a decreased latency of escape. The reduced escape latency is not due to a change of the swim velocity, suggesting that a cognitive, hippocampal-associated process might mediate this performance. The probe trial showed no differences in spatial reference memory between the modafinil-treated and control groups. This, in combination with the latency of escape trials on days 1-5, suggests that modafinil enhances the acquisition of the water maze task, without a concurrent effect on peripheral motor activity and without biasing the exploration strategy of the animal. The poor spatial performance of the animals from the 64mg/kg group in the probe trial could reflect the ability of modafinil to promote wakefulness (Dinges et al., 2006), thus interfering with the sleep-dependent stages of memory consolidation (Hobson and Pace-Schott, 2002). Our data demonstrate facilitated water maze performance, although the decreases in path lengths or escape latencies could reflect differences in non-spatial aspects of the performance.

A recent study has shown enhanced probe trial performance in the absence of differential escape latency in training trials relative to control in modafinil treated mice (Shuman et al., 2009). Our data display an alternate pattern, with reduced

escape latency in the training trials and no alteration of performance in the probe trial for modafinil-treated rats. Nonetheless, we arrive at a similar conclusion, suggesting that modafinil enhances the acquisition of water maze task, without a concurrent facilitation of retrieval or locomotor performance in the task. The effects of modafinil may not be specific to spatial learning, but they must reflect the increased monoaminergic activity, which was the next target of our study.

The potentiation of monoamine transporters drives enhanced plasticity

Extensive research has been conducted to establish the contribution of LTP to spatial learning (McNaughton and Morris, 1987; Castro et al., 1989; Barnes, 1995; Moser, 1995; Morris and Frey, 1997). For memory to occur, the changes in neuronal efficacy must be long-lasting in accordance with synaptic plasticity and memory (SPM) hypothesis, which states that the activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation (Martin et al., 2000). The hippocampal formation plays a central role in spatial learning and has also been identified through imaging studies as one of the major targets for modafinil action (Ellis et al., 1999). As hippocampal long-lasting synaptic plasticity is proposed to be the cellular mechanism underlying the formation of spatial learning (Barnes, 1995; Morris and Frey, 1997) our next goal was to examine whether modafinil also influences plasticity-related processes in hippocampus. Catecholaminergic pathways can induce or reinforce long-term plasticity in dentate gyrus (Almaguer-Melian et al., 2005), providing the physiological link for modafinil's action on LTP. Modafinil targets the

noradrenergic transporter as well as the dopamine transporter, acting as a dopaminergic agonist (Madras et al., 2006; Korotkova et al., 2007). LTP in hippocampus is strongly dependent on dopamine: the blockade of the dopaminergic D1 receptor, which is positively coupled to the adenylate cyclase, impairs LTP in vitro (Frey et al., 1990; Frey et al., 1991; Frey et al., 1993; Huang et al., 1995; Bach et al., 1999) and in vivo (Swanson-Park et al., 1999; Lemon and Manahan-Vaughan, 2006). Depotentiation in the dentate gyrus of freely-moving rats is modulated by D1/D5 dopamine activity (Kulla and Manahan-Vaughan, 2000). The antagonist flupenthixol used to block the dopaminergic neurotransmission acts on dopamine released from dopaminergic axons by the LTP-inducing stimuli (Frey et al., 1990; Morris et al., 2003). Importantly, the antagonism of D1/D5 receptors impairs the object-configuration learning (Lemon and Manahan-Vaughan, 2006), while the promotion of dopamine activity in the hippocampus of behaving animals results in positive cognitive effects. Intrahippocampal injection of D1 agonists improved the acquisition of an 8-arm radial maze (Packard and White, 1991) and enhanced memory retention (Bernabeu et al., 1997). Our data support the behavioural and electrophysiological findings of dopaminergic modulation on hippocampal function. Here we show for the first time that a single application of modafinil (i.p., 150 mg/kg), 120 min prior HFS, modifies the LTP induced by a 200Hz, 2 bursts stimulation protocol. The major component affected by the drug treatment was the population spike (PS) amplitude of the postsynaptic field potential, which was 250%, compared to the 150% LTP for the control group. The fEPSP slope was

also significantly increased from 110% for controls to 120% for the modafinil-injected rats. The greater amplitude of LTP in the drug-treated group is proposed to be due to the direct action of modafinil on granule cell postsynaptic responsiveness. A single i.p. injection of modafinil of 150 mg/kg (Ishizuka et al., 2003) elevated neuronal responsiveness in the dentate gyrus to 175% for PS amplitude and 75% for the fEPSP slope, compared to the baseline, pre-injection period. Importantly, the time course of the observed changes is congruent with the rate of modafinil absorption (Wong et al., 1999a; Wong et al., 1999b).

Modafinil increases the duration and the power of theta rhythm

Long-term synaptic potentiation is optimal when the time interval between stimuli is approximately 200ms due to activation of NMDA receptor-mediated inward current or removal of the inactivation of T-type Ca^{2+} channels followed by a rebound depolarization after 100–200ms (Gustafsson et al., 1987). This timing corresponds with the synchronized depolarization during theta oscillation, which considers the theta cycle (125ms) as an information quantum (Buzsáki et al., 1994; Eichenbaum et al., 1999). **One of the proposed links between theta rhythm and LTP involves the use of theta-frequency stimulation for the induction of long-lasting plasticity** (Buzsaki, 1988; Larson and Lynch, 1988). LTP is more effectively induced in the dentate gyrus when a tetanic stimulus is delivered on positive phases of theta (Pavlidis et al., 1988). The EEG theta band is considered to be a necessary feature for the acquisition of new information in the hippocampus (Buzsáki et al., 2002). Coordinated oscillation of cell groups

during theta activity is believed to ensure the temporal encoding of spatial cues subsequently stored as sequences in the hippocampal network (Kamondi et al., 1998; Dragoi and Buzsáki, 2006). Exploration of individual EEG traces in our recordings revealed cyclic shifts of theta and delta rhythms, in agreement with recent findings, demonstrating that urethane-anaesthesia shares similar cyclic patterns with natural sleep (Clement et al., 2008). Importantly, the shape of this oscillatory fluctuation was modified by an increase of the absolute theta power after the modafinil injection. We demonstrate that modafinil alone augmented hippocampal theta spectral power, which is restricted to the low theta range (3.0-5.5 Hz). 60 minutes after the drug injection a clear dissociation between the amplitude of theta and delta powers occurred. In contrast, the control group revealed that under urethane anaesthesia theta and delta spectral powers undergo a concurrent, gradual tendency of augmentation over time. The increase of the ratio between theta and delta (theta-ratio) in modafinil group followed the same slow onset as the fEPSP parameters. The ratio was significantly increased for the period 60 to 90 min after the injection with even higher value, occurring from 90-120 min. Furthermore, point-by-point comparisons between theta spectral power and fEPSP values revealed high degree of correlation between low theta and fEPSP slope. Similarly, PS amplitude changes correlated positively with the low theta augmentation. The increase of PS amplitude and theta power could reflect subtle changes of anaesthesia depth. Although modafinil does not interfere competitively with urethane's action on CNS (Tonner et al., 1992; Hara and Harris, 2002) we should consider modafinil-evoked anaesthesia reduction

due to dopaminergic effect on wakefulness (Dzirasa et al., 2006). The dopaminergic modulation of sleep/wake cycle (Abbott, 2005; Adler, 2005) might account for the augmentation of theta power amplitude and for the increased timing of theta epochs in our experiments.

Modafinil affects preoptic hypothalamic areas of the brain (Lin et al., 1992), influencing thermoregulation (Bratincsak et al., 2008). Brain temperature changes might interfere with short-term fEPSP fluctuations in hippocampal plasticity (Moser et al., 1994; Andersen and Moser, 1995), so here we explored the long-lasting effects that persist after the drug application. Our approach of low dose chronic administration for the behavioural experiments negated any putative thermoregulatory effects; indeed we found similar swim velocities in the modafinil and control groups, most probably that excluding alterations of the negative reinforcing properties of the water temperature of the maze as a confounding variable.

Conclusions

The present study describes hippocampal-dependent behavioral and electrophysiological effects of modafinil. **Our investigation focused on the long-lasting changes in neuronal activity resulting from modafinil application that might alter behavioural performance. It is important to note that the improved performance is not necessarily directly linked to improved learning of the task, as it is possible that modafinil has effects on performance that are independent of effects on learning. Further**

investigation of the systems involved in the effects of monoamine modulation on learning and performance will be of great interest in this regard. The findings presented here reveal the physiological basis for pharmacological modulation of monoamine transporters as a potential method for the treatment of age-related cognitive disorders.

Acknowledgements

This work was supported in part by a Health Research Board of Ireland grant to Shane M. O'Mara. We thank Dr Cathal Walsh of the School of Statistics and Computer Science, Trinity College, Dublin, for assistance with statistical analyses.

References

- Abbott, A., 2005. Neuroscience: while you were sleeping. *Nature* 437, 1220-1222.
- Adler, C. H., 2005. Nonmotor complications in Parkinson's disease. *Mov Disord* 20 Suppl 11, S23-29.
- Almaguer-Melian, W., Rojas-Reyes, Y., Alvare, A., Rosillo, J. C., Frey, J. U., Bergado, J. A., 2005. Long-term potentiation in the dentate gyrus in freely moving rats is reinforced by intraventricular application of norepinephrine, but not oxotremorine. *Neurobiol Learn Mem* 83, 72-78.
- Andersen, P., Moser, E. I., 1995. Brain temperature and hippocampal function. *Hippocampus* 5, 491-498.
- Bach, M. E., Barad, M., Son, H., Zhuo, M., Lu, Y. F., Shih, R., Mansuy, I., Hawkins, R. D., Kandel, E. R., 1999. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc Natl Acad Sci U S A* 96, 5280-5285.
- Barnes, C. A., 1995. Involvement of LTP in memory: are we 'searching under the street light'? *Neuron* 15, 751-754.
- Beracochea, D., Cagnard, B., Celerier, A., le Merrer, J., Peres, M., Pierard, C., 2001. First evidence of a delay-dependent working memory-enhancing effect of modafinil in mice. *Neuroreport* 12, 375-378.

- Beracochea, D., Celerier, A., Peres, M., Pierard, C., 2003. Enhancement of learning processes following an acute modafinil injection in mice. *Pharmacol Biochem Behav* 76, 473-479.
- Bernabeu, R., Bevilaqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., Izquierdo, I., Medina, J. H., 1997. Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. *Proc Natl Acad Sci U S A* 94, 7041-7046.
- Bratincsak, A., Kovacs, Z. I., Palkovits, M., 2008. Direct neuronal projection from a brainstem thermosensitive cell group to the preoptic thermoregulatory center. *Neuroscience* 156, 966-972.
- Buzsáki, G., 1988. Polysynaptic long-term potentiation: a physiological role of the perforant path-CA3/CA1 pyramidal cell synapse. *Brain Res* 455, 192-195.
- Buzsáki, G., 2002. Theta oscillations in the hippocampus. *Neuron* 33, 325-340.
- Buzsáki, G., Csicsvari, J., Dragoi, G., Harris, K., Henze, D., Hirase, H., 2002. Homeostatic maintenance of neuronal excitability by burst discharges in vivo. *Cerebral Cortex* 12, 893-899.
- Buzsáki, G., Draguhn, A., 2004. Neuronal oscillations in cortical networks. *Science* 304, 1926-1929.
- Buzsáki, G., Llinás, R., Berthoz, A., Christen, Y., 1994. *Temporal Coding in the Brain*. Springer-Verlag, Berlin.
- Castro, C. A., Silbert, L. H., McNaughton, B. L., Barnes, C. A., 1989. Recovery of spatial learning deficits after decay of electrically induced synaptic enhancement in the hippocampus. *Nature* 342, 545-548.
- Clement, E. A., Richard, A., Thwaites, M., Ailon, J., Peters, S., Dickson, C. T., 2008. Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia. *PLoS One* 3, e2004.
- Cowley, T. R., Fahey, B., O'Mara, S. M., 2008. COX-2, but not COX-1, activity is necessary for the induction of perforant path long-term potentiation and spatial learning in vivo. *Eur J Neurosci* 27, 2999-3008.
- Cox, J. M., Pappagallo, M., 2001. Modafinil: a gift to portmanteau. *Am J Hosp Palliat Care* 18, 408-410.
- Della-Chiesa, A., Wang, B., Hok, V., Irving, E., Virley, D., Upton, N., O'Mara, S. M., 2008. Acute and chronic rosiglitazone administration enhances hippocampal-associated learning in middle-aged, but not young, rats. . *ICAD*, Abstr P1-008.
- Deroche-Gamonet, V., Darnaudery, M., Bruins-Slot, L., Piat, F., Le Moal, M., Piazza, P. V., 2002. Study of the addictive potential of modafinil in naive and cocaine-experienced rats. *Psychopharmacology (Berl)* 161, 387-395.
- Dinges, D. F., Arora, S., Darwish, M., Niebler, G. E., 2006. Pharmacodynamic effects on alertness of single doses of armodafinil in healthy subjects during a nocturnal period of acute sleep loss. *Curr Med Res Opin* 22, 159-167.
- Dragoi, G., Buzsáki, G., 2006. Temporal encoding of place sequences by hippocampal cell assemblies. *Neuron* 50, 145-157.
- Duteil, J., Rambert, F. A., Pessonnier, J., Hermant, J. F., Gombert, R., Assous, E., 1990. Central alpha 1-adrenergic stimulation in relation to the behaviour stimulating effect of modafinil; studies with experimental animals. *Eur J Pharmacol* 180, 49-58.
- Dzirasa, K., Ribeiro, S., Costa, R., Santos, L. M., Lin, S. C., Grosmark, A., Sotnikova, T. D., Gainetdinov, R. R., Caron, M. G., Nicolelis, M. A., 2006. Dopaminergic control of sleep-wake states. *J Neurosci* 26, 10577-10589.
- Eichenbaum, H., Dudchenko, P., Wood, E., Shapiro, M., Tanila, H., 1999. The hippocampus, memory, and place cells: is it spatial memory or a memory space? *Neuron* 23, 209-226.

- Ellis, C. M., Monk, C., Simmons, A., Lemmens, G., Williams, S. C., Brammer, M., Bullmore, E., Parkes, J. D., 1999. Functional magnetic resonance imaging neuroactivation studies in normal subjects and subjects with the narcoleptic syndrome. Actions of modafinil. *J Sleep Res* 8, 85-93.
- Engber, T. M., Dennis, S. A., Jones, B. E., Miller, M. S., Contreras, P. C., 1998a. Brain regional substrates for the actions of the novel wake-promoting agent modafinil in the rat: comparison with amphetamine. *Neuroscience* 87, 905-911.
- Engber, T. M., Koury, E. J., Dennis, S. A., Miller, M. S., Contreras, P. C., Bhat, R. V., 1998b. Differential patterns of regional c-Fos induction in the rat brain by amphetamine and the novel wakefulness-promoting agent modafinil. *Neurosci Lett* 241, 95-98.
- Ferraro, L., Antonelli, T., O'Connor, W. T., Tanganelli, S., Rambert, F., Fuxe, K., 1997. The antinarcoleptic drug modafinil increases glutamate release in thalamic areas and hippocampus. *Neuroreport* 8, 2883-2887.
- Ferraro, L., Antonelli, T., O'Connor, W. T., Tanganelli, S., Rambert, F. A., Fuxe, K., 1998. The effects of modafinil on striatal, pallidal and nigral GABA and glutamate release in the conscious rat: evidence for a preferential inhibition of striato-pallidal GABA transmission. *Neurosci Lett* 253, 135-138.
- Florence, G., Bonnier, R., Plagnes, D., Pierard, C., Satabin, P., Peres, M., Lagarde, D., 2000. Effect of modafinil on cerebral blood flow of anaesthetised rats. Comparison with amphetamine. *Exp Brain Res* 135, 552-556.
- Frey, U., Huang, Y. Y., Kandel, E. R., 1993. Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 260, 1661-1664.
- Frey, U., Matthies, H., Reymann, K. G., Matthies, H., 1991. The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. *Neurosci Lett* 129, 111-114.
- Frey, U., Schroeder, H., Matthies, H., 1990. Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. *Brain Res* 522, 69-75.
- Gustafsson, B., Wigstrom, H., Abraham, W. C., Huang, Y. Y., 1987. Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. *J Neurosci* 7, 774-780.
- Hara, K., Harris, R. A., 2002. The anesthetic mechanism of urethane: the effects on neurotransmitter-gated ion channels. *Anesth Analg* 94, 313-318, table of contents.
- Harris, K. D., Henze, D. A., Hirase, H., Leinekugel, X., Dragoi, G., Czurko, A., Buzsáki, G., 2002. Spike train dynamics predicts theta related phase precession in hippocampal pyramidal cells. *Nature* 417, 738-741.
- Hobson, J. A., Pace-Schott, E. F., 2002. The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. *Nat Rev Neurosci* 3, 679-693.
- Holscher, C., Anwyl, R., Rowan, M. J., 1997. Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can be depotentiated by stimulation on the negative phase in area CA1 in vivo. *J Neurosci* 17, 6470-6477.
- Huang, Y. Y., Kandel, E. R., Varshavsky, L., Brandon, E. P., Qi, M., Idzerda, R. L., McKnight, G. S., Bourchouladze, R., 1995. A genetic test of the effects of mutations in PKA on mossy fiber LTP and its relation to spatial and contextual learning. *Cell* 83, 1211-1222.
- Ishizuka, T., Sakamoto, Y., Sakurai, T., Yamatodani, A., 2003. Modafinil increases histamine release in the anterior hypothalamus of rats. *Neurosci Lett* 339, 143-146.
- Jasinski, D. R., Kovacevic-Ristanovic, R., 2000. Evaluation of the abuse liability of modafinil and other drugs for excessive daytime sleepiness associated with narcolepsy. *Clin Neuropharmacol* 23, 149-156.

- Kamondi, A., Acsady, L., Wang, X. J., Buzsáki, G., 1998. Theta oscillations in somata and dendrites of hippocampal pyramidal cells in vivo: activity-dependent phase-precession of action potentials. *Hippocampus* 8, 244-261.
- Korotkova, T. M., Klyuch, B. P., Ponomarenko, A. A., Lin, J. S., Haas, H. L., Sergeeva, O. A., 2007. Modafinil inhibits rat midbrain dopaminergic neurons through D2-like receptors. *Neuropharmacology* 52, 626-633.
- Kraft, G. H., Bowen, J., 2005. Modafinil for fatigue in MS: a randomized placebo-controlled double-blind study. *Neurology* 65, 1995-1997; author reply 1995-1997.
- Kruger, L., Saporta, S., Swanson, L. W., 1995. *Photographic Atlas of the Rat Brain: The Cell and Fiber Architecture Illustrated in Three Planes with Stereotaxic Coordinates*, 299 pp. Cambridge University Press, New York.
- Kulla, A., Manahan-Vaughan, D., 2000. Depotentiation in the dentate gyrus of freely moving rats is modulated by D1/D5 dopamine receptors. *Cereb Cortex* 10, 614-620.
- Larson, J., Lynch, G., 1988. Role of N-methyl-D-aspartate receptors in the induction of synaptic potentiation by burst stimulation patterned after the hippocampal theta-rhythm. *Brain Res* 441, 111-118.
- Lemon, N., Manahan-Vaughan, D., 2006. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *J Neurosci* 26, 7723-7729.
- Lin, J. S., Roussel, B., Akaoka, H., Fort, P., Debilly, G., Jouvet, M., 1992. Role of catecholamines in the modafinil and amphetamine induced wakefulness, a comparative pharmacological study in the cat. *Brain Res* 591, 319-326.
- Madras, B. K., Xie, Z., Lin, Z., Jassen, A., Panas, H., Lynch, L., Johnson, R., Livni, E., Spencer, T. J., Bonab, A. A., Miller, G. M., Fischman, A. J., 2006. Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. *J Pharmacol Exp Ther* 319, 561-569.
- Manns, I. D., Alonso, A., Jones, B. E., 2003. Rhythmically discharging basal forebrain units comprise cholinergic, GABAergic, and putative glutamatergic cells. *J Neurophysiol* 89, 1057-1066.
- Martin, S. J., Grimwood, P. D., Morris, R. G. M., 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci* 23, 649-711.
- McNaughton, B. L., Morris, R. G. M., 1987. Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends Neurosci* 10, 408-415.
- Mignot, E., Nishino, S., 2005. Emerging therapies in narcolepsy-cataplexy. *Sleep* 28, 754-763.
- Mignot, E., Taheri, S., Nishino, S., 2002. Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. *Nat Neurosci* 5 Suppl, 1071-1075.
- Minzenberg, M. J., Carter, C. S., 2007. Modafinil: A Review of Neurochemical Actions and Effects on Cognition. *Neuropsychopharmacology*.
- Moldofsky, H., Broughton, R. J., Hill, J. D., 2000. A randomized trial of the long-term, continued efficacy and safety of modafinil in narcolepsy. *Sleep Med* 1, 109-116.
- Morgan, R. E., Crowley, J. M., Smith, R. H., LaRoche, R. B., Dopheide, M. M., 2007. Modafinil improves attention, inhibitory control, and reaction time in healthy, middle-aged rats. *Pharmacol Biochem Behav* 86, 531-541.
- Morris, R., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11, 47-60.
- Morris, R. G. M., Frey, U., 1997. Hippocampal Synaptic Plasticity: Role in Spatial Learning or the Automatic Recording of Attended Experience? *Philos Trans R Soc Lond B Biol Sci* 352, 1489-1503.
- Morris, R. G. M., Moser, E. I., Riedel, G., Martin, S. J., Sandin, J., Day, M., O'Carroll, C., 2003. Elements of a neurobiological theory of the hippocampus: the role of activity-

- dependent synaptic plasticity in memory. *Philos Trans R Soc, London B Biol Sci* 358, 773–786.
- Moser, E. I., 1995. Learning-related changes in hippocampal field potentials. *Behav Brain Res* 71, 11–18.
- Moser, E. I., Moser, M. B., Andersen, P., 1994. Potentiation of dentate synapses initiated by exploratory learning in rats: dissociation from brain temperature, motor activity, and arousal. *Learn Mem* 1, 55-73.
- Nieves, A. V., Lang, A. E., 2002. Treatment of excessive daytime sleepiness in patients with Parkinson's disease with modafinil. *Clin Neuropharmacol* 25, 111-114.
- Packard, M. G., White, N. M., 1991. Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. *Behav Neurosci* 105, 295-306.
- Pavlidis, C., Greenstein, Y. J., Grudman, M., Winson, J., 1988. Long-term potentiation in the dentate gyrus is induced preferentially on the positive phase of theta-rhythm. *Brain Res.* 439, 383-387.
- Pierard, C., Liscia, P., Valleau, M., Drouet, I., Chauveau, F., Huart, B., Bonneau, D., Jouanin, J. C., Beaumont, M., Beracochea, D., 2006. Modafinil-induced modulation of working memory and plasma corticosterone in chronically-stressed mice. *Pharmacol Biochem Behav* 83, 1-8.
- Scammell, T. E., Matheson, J., 1998. Modafinil: a novel stimulant for the treatment of narcolepsy. *Expert Opin Investig Drugs* 7, 99-112.
- Schwartz, J. R., Nelson, M. T., Schwartz, E. R., Hughes, R. J., 2004. Effects of modafinil on wakefulness and executive function in patients with narcolepsy experiencing late-day sleepiness. *Clin Neuropharmacol* 27, 74-79.
- Shuman, T., Wood, S. C., Anagnostaras, S. G., 2009. Modafinil and memory: Effects of modafinil on Morris water maze learning and Pavlovian fear conditioning. *Behav Neurosci* 123, 257-266.
- Simon, P., Panissaud, C., Costentin, J., 1994. The stimulant effect of modafinil on wakefulness is not associated with an increase in anxiety in mice. A comparison with dexamphetamine. *Psychopharmacology (Berl)* 114, 597-600.
- Swanson-Park, J. L., Coussens, C. M., Mason-Parker, S. E., Raymond, C. R., Hargreaves, E. L., Dragunow, M., Cohen, A. S., Abraham, W. C., 1999. A double dissociation within the hippocampus of dopamine D1/D5 receptor and beta-adrenergic receptor contributions to the persistence of long-term potentiation. *Neuroscience* 92, 485-497.
- Tanganelli, S., Ferraro, L., Bianchi, C., Fuxe, K., 1994. 6-hydroxy-dopamine treatment counteracts the reduction of cortical GABA release produced by the vigilance promoting drug modafinil in the awake freely moving guinea-pig. *Neurosci Lett* 171, 201-204.
- Tanganelli, S., Perez de la Mora, M., Ferraro, L., Mendez-Franco, J., Beani, L., Rambert, F. A., Fuxe, K., 1995. Modafinil and cortical gamma-aminobutyric acid outflow. Modulation by 5-hydroxytryptamine neurotoxins. *Eur J Pharmacol* 273, 63-71.
- Tonner, P. H., Poppers, D. M., Miller, K. W., 1992. The general anesthetic potency of propofol and its dependence on hydrostatic pressure. *Anesthesiology* 77, 926-931.
- Turner, D. C., Robbins, T. W., Clark, L., Aron, A. R., Dowson, J., Sahakian, B. J., 2003. Cognitive enhancing effects of modafinil in healthy volunteers. *Psychopharmacology (Berl)* 165, 260-269.
- Ward, C. P., Harsh, J. R., York, K. M., Stewart, K. L., McCoy, J. G., 2004. Modafinil facilitates performance on a delayed nonmatching to position swim task in rats. *Pharmacol Biochem Behav* 78, 735-741.

Waters, K. A., Burnham, K. E., O'Connor, D., Dawson, G. R., Dias, R., 2005. Assessment of modafinil on attentional processes in a five-choice serial reaction time test in the rat. *J Psychopharmacol* 19, 149-158.

Wesensten, N. J., 2006. Effects of modafinil on cognitive performance and alertness during sleep deprivation. *Curr Pharm Des* 12, 2457-2471.

Wong, Y. N., King, S. P., Simcoe, D., Gorman, S., Laughton, W., McCormick, G. C., Grebow, P., 1999a. Open-label, single-dose pharmacokinetic study of modafinil tablets: influence of age and gender in normal subjects. *J Clin Pharmacol* 39, 281-288.

Wong, Y. N., Simcoe, D., Hartman, L. N., Laughton, W. B., King, S. P., McCormick, G. C., Grebow, P. E., 1999b. A double-blind, placebo-controlled, ascending-dose evaluation of the pharmacokinetics and tolerability of modafinil tablets in healthy male volunteers. *J Clin Pharmacol* 39, 30-40.

Figure legends:

Figure 1. Spatial learning but not motor activity is increased in modafinil-treated rats. Mean distance travelled (A) and mean latency to find platform (B) and across all trials in the water-maze for control (white rhombs), 10 mg/kg modafinil (grey dots) and 64 mg/kg modafinil (black squares) group on each day of training. C. Mean swimming velocity during the water-maze performance. Black asterisks represent the significance level of 64 mg/kg modafinil group versus control group; grey asterisks represent the significance levels of 10 mg/kg modafinil group versus control group; * $P < 0.05$. D. Representative traces of the rats' trajectory in water-maze for day 3 (upper traces) and probe trial (lower traces). Control, 10 mg/kg modafinil and 64 mg/kg modafinil cases are positioned left, middle and right traces respectively.

Figure 2. Probe trial experiment reveals similar performance for modafinil-treated and control rats. A. Percentage of the total trial time spent by control (white bars), 10 mg/kg modafinil (grey bars) and 64 mg/kg modafinil (black bars) group in each of the quadrants of the water-maze arena in the absence of

platform. The platform had been situated in the centre of the north east quadrant; NE (Target) = North East, NW = North West, SE = South East and SW = South West). B. Mean swimming velocity of each treatment group during the probe trial. C. Number of entries made into each quadrant by each treatment group. D. Total distance traveled by the control and the modafinil-treated groups during the probe trial. Asterisk denotes difference between the 10 mg/kg modafinil treatment group (grey bar) and the control group (white bar). * $P < 0.05$.

Figure 3. Samples of synaptic plasticity in dentate gyrus evoked by high-frequency stimulation (HFS) composed by different amount of 200Hz bursts. fEPSP slope (A) and population spike (PS) amplitude (B) measured for 30 min baseline period and 120 min post-stimulation period. Dotted line – one burst, black line – two bursts, dark grey line – three bursts, light grey line – four bursts.

Figure 4. Modafinil increases LTP in dentate gyrus. A. fEPSP slope comparison between modafinil treated (black circles) and control animals (white circles) after 200Hz, 2 bursts stimulation protocol. B. PS amplitude for modafinil treated (black circles) and control animals (white circles) after 200Hz, 2 burst stimulation protocol. C. Original analog traces showing the field potentials evoked from the dentate gyrus pre-HFS, 5 min, 30 min and 120 min following HFS in the presence of vehicle (NaCl 0.9%) or modafinil (150 mg/kg). Vertical scale-bar corresponds to 5 mV, horizontal scale-bar to 5 ms.

Figure 5. Modafinil augments granular excitability. A. I.p. injection of modafinil (150 mg/kg) evokes delayed gradual increase of fEPSP slope (black circles), compared to controls (white circles). B. Modafinil application results in a robust augmentation of PS amplitude (black circles), compared to the control PS values (white circles). C. Original analog traces show field potentials evoked from the dentate gyrus pre-injection, 5 min, 30 min and 120 min following injection for modafinil-treated animals (upper traces) and for controls (lower traces). Vertical scale-bar corresponds to 5 mV, horizontal scale-bar to 5 ms.

Figure 6. Oscillatory changes in dentate gyrus after modafinil application.

A. Sample of EEG power spectrogram during baseline period (grey line) and 120 min after modafinil injection (black line) of rats under urethane anaesthesia. B. Sample EEG traces represent different modes of oscillatory activity. Slow (delta) **and very slow (0.1-1.5 Hz)** oscillations (upper trace) were more evident during the baseline recordings. Faster (theta) oscillations (bottom trace) occurred more frequently after the HFS protocol. Horizontal bar: 500 msec, vertical bar 0.5 mV. C. Theta ratio (slow theta over delta) represented for a periods of 30 min in modafinil-treated rats (white bars) and controls (black bars). The drug is applied after the baseline period. * $P < 0.05$, ** $P < 0.01$.

Figure 7. Modafinil increases selectively theta rhythm. A. Modafinil application results in a gradual augmentation of low theta (3.0 – 5.5Hz, black symbols) and parallel tendency for suppression of delta (1.5 – 3.0Hz, white symbols) in dentate

gyrus. B. Low gradual increase is present in the oscillatory activity of urethane-anaesthetized animals. Theta (black symbols) and delta (white symbols) spectral powers augment in a concurrent manner. C. No significant change was observed in high theta (5.5 – 11Hz, black symbols) or gamma (30 – 80Hz, white symbols) after Modafinil injection.

Figure 8. Spectral powers analysis reveals increase of the theta episodes after modafinil application.

A. Example of theta band modulation throughout modafinil application experiment for an individual animal. B. The parallel delta band dynamics from the same animal. C. Original traces of field potential recordings in the dentate gyrus from the dotted windows marked in (A). The upper trace represents baseline oscillatory shift (1) and bottom trace represents the same event after modafinil injection (2). Horizontal bar: 500 msec, vertical bar: 1 mV. Colour-coded power spectrograms demonstrate the occurrence pattern of theta activity in the frequency band of 4 Hz before (D) and after (E) the application of modafinil, extracted from the field potential tracings in (C). Note the theta power epochs are with longer duration and higher amplitude. Time is on the x-axis, frequency is on the y-axis, power is colour-coded on a log scale.

Figure 9. Synaptic plasticity correlates with theta increase. Significant positive correlation (Pearson, $n = 7$, $p < 0.0001$, $r = 0.8118$) between the fEPSP slope and the slow theta power, recorded in dentate gyrus.

