EFFECT OF A NOVEL HISTAMINE SUBTYPE-3 RECEPTOR INVERSE AGONIST AND MODAFINIL ON
EEG POWER SPECTRA DURING SLEEP DEPRIVATION AND RECOVERY SLEEP IN MALE
VOLUNTEERS

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To be submitted to
Psychopharmacology

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Abstract

Rationale: Histamine and dopamine contribute to the maintenance of wakefulness.

Objective: Exploratory analysis of the effects of 10 and 50 mg of MK-0249, a novel histamine subtype-3 receptor inverse agonist, and 200 mg of modafinil, a presumed dopaminergic compound, on EEG power spectra during sleep deprivation and subsequent recovery sleep. **Methods:** 25 healthy men were recruited to a double-blind placebo-controlled cross-over design. EEG power spectra, an electrophysiological marker of changes in sleepiness and vigilance, were obtained at the beginning of wake maintenance tests at 2 hourly intervals throughout a night and day of sleep deprivation, which is an established model of excessive sleepiness. **Results:** After placebo, sleep deprivation was associated with enhancements in delta, theta and reductions in alpha and beta activity. Following dosing at 02:00, MK-0249 and modafinil reduced delta and theta activity and enhanced alpha and beta activity, compared to placebo. During recovery sleep initiated at 21:00 h, latency to sleep onset, and number of awakenings were not different from placebo for any of the active treatments. Wake after sleep onset and stage 1 % was increased and total sleep time, SWS % and REM% were reduced after both doses of MK-0249. Compared to placebo, MK-0249 and the 50 mg dose in particular, reduced activity in some delta and theta/alpha frequencies and enhanced beta activity during NREM sleep and REM sleep. After modafinil, no changes were observed for power spectra during sleep. **Conclusion:** both MK-0249 and modafinil exert effects on the EEG which are consistent with wake promotion.

Key words: H3, EEG, alerting effects, excessive daytime sleepiness

Introduction

Wake and sleep propensity vary with time awake (sleep homeostasis) and time of day (circadian phase) and excessive sleepiness is present in conditions such as narcolepsy, untreated sleep apnea, shiftwork sleep disorder and hypersomnia (Leibowitz et al. 2006). Changes in sleep-wake propensity can be assessed objectively by testing the ability to initiate sleep in the multiple sleep latency test (MSLT) or the ability to maintain wakefulness in the maintenance of wakefulness test (MWT) (Littner et al. 2005). The spectral composition of the EEG is an electrophysiological correlate of changes in sleepiness and vigilance. It has been shown repeatedly that during sleep deprivation EEG power in theta and delta activity increases and alpha activity decreases (Aeschbach et al. 1999; Akerstedt and Gillberg 1990; Cajochen et al. 1995). Analysis of the contribution of circadian phase and time awake during forced desynchrony have confirmed that these changes in the EEG are related to an interaction of a circadian alerting signal, in part reflected in EEG alpha activity, and a signal associated with the increase in sleep pressure during sustained wakefulness, reflected in an increase in delta and theta activity (Cajochen, et al. 2002). During the day, wakefulness and vigilance is maintained through activating neurotransmitters such as noradrenaline, dopamine, orexin and histamine, originating from brainstem and hypothalamic nuclei (Saper et al. 2005). These neurotransmitter systems are in part under circadian control and as we enter the biological night the strengths of these activating signals dissipates and sleepiness ensues (Dijk and Czeisler 1995). The extended wakefulness associated with sleep deprivation activates sleep homeostatic processes and associated changes in a variety of sleep regulatory substances, such as adenosine and TNF alpha, which also contribute to increased sleepiness and changes in the EEG (Krueger et al. 2008).

These neurochemical mechanisms underlying variations in sleep-wake propensity offer potential targets for sleepiness reducing/wake promoting interventions. Classical sleepiness reducing/wake promoting interventions target the noradrenergic/and dopaminergic systems (amphetamine) and adenosine (caffeine). A more recently developed wakefulness promoting compound (modafinil) appears to target the dopaminergic system, although there remains some debate about the precise mechanisms of action and it may currently not be

possible to define modafinil by a single mechanism of action (Bonnet, et al. 2005;Qu et al. 2008).

The histaminergic system is well placed to modify sleepiness because it has wide spread projections to many brain areas, is under circadian control, and is at least part of the pathway by which orexin exerts its effects on sleep propensity (Haas et al. 2008). Histaminergic neurons in the tuberomammillary nuclei are silent during NREM and REM sleep and are active during wakefulness, particularly during attentive wakefulness but not during drowsiness (Takahashi et al. 2006). It therefore appears that activity of histaminergic neurons is an important contributor to maintenance of vigilance.

Histaminergic tone is inhibited through constitutive activity of the H3 receptor, i.e. a pre-synaptic autoreceptor (Haas et al. 2008;Leurs et al. 2005). Inverse agonists of this receptor will increase synthesis and release of histamine and can thereby potentially reduce sleepiness in humans. Preclinical studies have shown that antagonists of the H3 receptor can induce wakefulness and that these effects are, unlike the wake promoting effects of modafinil, abolished in H3 receptor KO mice, demonstrating the very different mechanisms of actions of the two compounds (Parmentier et al. 2007). In one clinical study, reduction of sleepiness in narcoleptic patients was observed after administration of a H3- inverse agonists (Lin et al. 2008). In that study, however, no objective EEG data during either wakefulness or sleep were reported and it is currently not known how H3 antagonists and H3 inverse agonists affect the EEG during wakefulness and sleep.

We previously reported that the novel H3 inverse agonist MK-0249, similar to modafinil, exerts significant wake promoting effects as assessed by the latency to sleep onset during MWTs in a sleep deprivation paradigm (Iannone, et al. 2010). In that report we did not present data on the quantitative analysis of the EEG during the sleep deprivation or during the recovery sleep. To further characterize the effects of MK-0249 and modafinil on sleep-wake propensity we analysed EEG power spectra during the MWT (Chapotot et al. 2003) and subsequent recovery sleep, as well as sleep structure. Assessment of sleep following administration of a wake promoting compound is important in order to assess any residual alerting effects that may interfere with sleep when it is desired. A primary question of interest in the present analysis was whether these two compounds, which both have wake

promoting effects but very different mechanism of actions, have similar effects on the spectral composition of the EEG during wakefulness and sleep, as well as sleep structure.

Methods:

This report is based on an exploratory analysis of a randomized, double blind, double dummy, placebo and modafinil controlled, 4 period crossover study (Merck and Co., Inc. Protocol MK-0249 007-01). The aim of the study was to assess the acute wake promoting effects of MK 0249 using a sleep deprivation model in healthy male volunteers. The primary outcome variable of this study was sleep latency on the MWT, and these data have been reported elsewhere (Iannone et al. 2010). Here we report data on the effects of MK-0249 and modafinil on the spectral composition of the EEG during the MWT, sleep structure during recovery sleep as well as effects on the spectral composition of NREM and REM sleep. Details of the protocol can be found in (Iannone et al. 2010).

The study was performed at the Clinical Research Centre of the University of Surrey between October 2006 and February 2007. The protocol was approved by the Medicines and Healthcare products Regulatory Authority and the Ravenscourt Ethics Committee.

Subjects

25 male volunteers were recruited, aged between 18 and 45yrs. Participants had a BMI of between 19 and 31kg/m² and were free from any pre-existing pathological conditions including neurological, psychiatric, cardiovascular, respiratory, endocrine, vascular, haematological, gastrointestinal, hepatic, renal and genitourinary and any evidence or history of cancer. Participants gave written informed consent prior to any study procedures being conducted. A physician judged each participant to be healthy based on their ECG, medical history, physical examination, haematology, blood chemistry and urinalysis tests. Participants discontinued any medications for 14 days prior to day 1 and throughout the study. Those that were unable to do this were excluded from the study, with the exception of treatment for minor ailments. Participants showed no evidence of epileptic EEG activity during screening. During an acclimatisation stay in the unit, participants who fell asleep within 15 minutes of a 20 minute Maintenance of Wakefulness Test (MWT) after approximately 25hrs of continuous wakefulness were eligible for inclusion. Participants

smoking more than 5 cigarettes per day were not eligible and smoking was not allowed while participants were in the laboratory. Participants agreed not to consume any caffeinated beverages for 24hrs prior to each admission into the Surrey CRC and for the duration of their stay. Participants had not travelled through any time zones for 14 days prior to their acclimatisation stay and throughout the study. Participants were not shift workers, did not suffer from any sleep disorders, were deemed to have normal sleep habits and typically commenced habitual sleep between 20:00 and 00:00h.

Protocol

During each of the 4 treatment periods of the study, participants had an 8hr baseline sleep opportunity from 23:00 to 07:00 and were then kept awake until 21:00 on day 2 resulting in 38hrs of continuous wakefulness, at which time they commenced a 12hr recovery sleep opportunity (Figure 1).

MWTs were performed in a sound attenuated, light controlled individual bedroom at 0800 on day 1 and at 2 hourly intervals from 0000 until 2000 on day 2, as illustrated in Figure 1. Participants also completed a series of psychometric tests as part of the protocol, the result of which are presented in (Iannone et al. 2010). During times when participants were not completing tests they were allowed to move around in the unit and carry out various activities such as watching TV, playing games and reading. These activities were strictly controlled and consistent throughout the study periods. To ensure that subjects were awake, they were under continuous surveillance and not allowed to sit in comfortable chairs.

Drug administration occurred at 0200 on day 2 and consisted of a single dose of either 200mg of modafinil, 10 or 50 mg of MK-0249 or placebo. 10 and 50 mg of MK-0249 are expected to result in 88 and 93% receptor occupancy respectively (Iannone et al. 2010). Administration was carried out in a double-blind, double-dummy fashion and there was a washout period of at least 7 days between each of the 4 treatment periods.

EEG recordings during MWTs and Sleep Episodes

All PSG recordings for both sleep and waking EEG were electronically captured through the Siesta system (Compumedics, Abbotsford, Victoria, Australia). The montage for this study was C3-A2, C4-A1, O1-A2 and O2-A1, in addition to two EMG and two EOG channels for both sleep and wake recordings. All EEG signals were low-pass filtered at 70Hz and high-pass filtered with a time constant of 0.33sec which corresponds to a cut off frequency of 0.53Hz. Sampling rate was 256Hz and the data were stored at 128Hz for EEG, EOG and EMG signals. Data collected on the Siesta system was transmitted via Bluetooth and recorded directly to a computer file server and later downloaded into EDF files.

Spectral analysis

All wake and sleep EEG recordings were visually inspected and 2 second (MWT) or 4 second epochs (Sleep EEG) containing artefacts were annotated manually using Vitascore software (Temec, The Netherlands). Epochs annotated as artefacts were excluded from the analysis of the power spectra. Spectral analysis was conducted using a fast Fourier

Transform after the data were weighted with a squared cosine window, implemented in the Vitascore software. Power spectra were computed per 2 seconds, resulting in a 0.5 Hz resolution for the MWT EEGs. For the sleep EEG spectra were computed per 4 seconds, resulting in a 0.25 Hz resolution.

Scoring and processing of MWT EEG data

The first 2 minutes of EEG which was free from any significant movement artefact within the first 5 minutes of each MWT were selected by the first author for analysis. Any further artefacts were then manually removed from these 2min periods of data. At least 20sec of data had to be available after artefact removal for data to be included in the final dataset of power density values. It was considered that any less than this may not have given an accurate representation of the EEG being sampled. Analysis of these two minute periods was carried out regardless of vigilance state, and for waking EEG only. In this report EEG data during the MWT will be presented independent of vigilance state.

Scoring and processing of Sleep EEG data

A 12hr recovery sleep episode commenced at 21:00h on day 2, after 38hrs of continuous wakefulness and 19hrs after drug administration (Figure 1). Sleep stages and power spectra data were analysed to assess the effects of both 10 and 50mg of MK-0249 and modafinil on recovery sleep, compared to placebo. Sleep records were scored by the first author on a 30 second epoch basis according to the established criteria of Rechtschaffen and Kales (Rechtschaffen and Kales 1968). From this standard sleep parameters were calculated. They include latencies to sleep onset, defined as the first occurrence of stage 2, 3, 4 or REM sleep, the duration of stages 1,2,3,4, SWS and REM sleep, number and duration of sleep cycles, total sleep time (TST), sleep efficiency, i.e. TST/Time between lights-off and lights-on, the number of sleep stage changes, the number of awakenings (NAW) and wake after sleep onset (WASO) EEG data were then subjected to FFT power density spectral analysis, as described above and sleep stage specific power spectra were computed. Data for the entire 12hr recovery sleep episode was analysed in addition to the first 8hrs of the sleep episode in order to assess the effects of an extended recovery sleep opportunity and a typical 8hr sleep opportunity, both of which may represent 'real-life' scenarios.

Manuscript EEG power spectra

Statistics

A statistical analysis plan for these exploratory analyses was established prior to review of the data. All statistical analyses were carried out using SAS®, version 9.1.

Analysis of MWT data

In order to assess the effect of sleep deprivation, spectral data of each MWT in each subject were expressed relative to the baseline of this subject, which was the average of MWTs 1,2, and 3. These analyses were conducted with a resolution of 1 Hz. Ratios were log transformed and difference from baseline were assessed by a students t-test.

To assess the effects of the various treatments, values in each MWT and each frequency bin were expressed relative to the corresponding placebo value. A paired student's t-test was used to assess significant differences from placebo at each frequency from 1 to 25Hz on log transformed data. The level of significance used throughout was 0.05 and no correction for multiplicity was made. This obviously increases the likelihood of false positives. We nevertheless feel that this approach is justified because a) this is an exploratory analyses, b) it will allow for a direct comparison to previous studies, e.g. (Tinguely, Finelli et al. 2006) c) spectral values of adjacent frequency bins are highly correlated which makes selecting the appropriate correction for multiplicity problematic d) there is prior knowledge on the effects of sleep deprivation and modafinil on EEG power spectra on the MWT.

Visual inspection of the data shows that the significant effects are clustered in specific frequency ranges in accordance with prior studies and are unlikely to reflect statistical artefact.

Analysis of sleep EEG data.

Sleep Stages

Comparisons between conditions were made using SAS PROC MIXED with treatment and period as fixed factors and subject as a random factor, followed by the LS MEANS statement to compute the p-value of the contrast between conditions. The significance level used throughout was 0.05.

EEG power spectra during sleep

Power density spectra were created and a paired Student's t-test was used to assess significant differences from placebo at each frequency from 1 to 25Hz on log transformed data for the sleep stage specific spectral data. The difference between treatment groups and placebo for PSG measures were also assessed using linear mixed effects model.

One of the 25 enrolled subjects dropped out after Period 1 and was subsequently replaced and not included in the EEG analysis. Thus 24 subjects were included in the analyses but please note that for some of the comparisons the number of subjects may be less than 24 due to missing data.

Results:

Effects of Sleep Deprivation on EEG power spectra at the beginning of MWT

To document the effects of sleep deprivation on the EEG during the MWT, the EEG spectral data following dosing with placebo were expressed relative to the data collected pre-dosing, i.e. at MWT1 at 08:00, MWT2 at 0:00 and MWT 3 02:00 (See Figure 1). Sleep deprivation led to a substantial increase in low frequency activity, from 1 to 7Hz, in both central and occipital derivations (Figure 2). The changes were observed from 2 to 6 hours post-placebo dose, 8 to 12 and 14 to 18 hours post-dose. Evidence for a progressive increase in EEG power density was only observed in the 1 and 2 Hz bins, in which values at the later sampling times of 8 to 12 and 14 to 18 hours post-dose, were greater compared to those of 2 to 6 hours post-dose. Sleep deprivation also led to a reduction in alpha activity (10-11 and 10-12 Hz for the central and occipital derivation respectively). A significant increase in EEG activity with the sigma band was only observed at 14 Hz in the central derivation. Beta activity was reduced between 18 and 22 Hz in the central derivation and 17-25 Hz in the occipital derivation.

Effects of MK-0249 and Modafinil on EEG Power Spectra at the Beginning of MWT

The effects of the two doses of MK-0249 and Modafinil on the EEG were analyzed by comparing EEG power spectra in these three active conditions to the placebo condition for both the central (Figure 3) and occipital derivation (Figure 4). Comparison of the EEG

power spectra pre-dosing, (i.e. baseline values) revealed very few differences across conditions. By contrast, post-dosing many differences emerged when the EEG power spectra at the beginning of the MWT were analyzed for the interval 2 to 6, 8 to 12 and 14 to 18 hours post-dose (Figure 3 and Figure 4). In the 2-6 h post dose interval, modafinil and both doses of MK-0249 reduced low frequency activity up to approximately 6-7 Hz. During this interval all three active treatments enhanced alpha and beta activity in both the central and occipital derivation. During the 8-12 and 14 to 18 hours post-dose intervals, differences with placebo became in general smaller. Whereas in the modafinil condition the reduction of delta and theta activity persisted in the 8-12 and 14-18 hours post-dose interval, the spectral values in this frequency range after both doses of MK-0249 were no longer significantly different from placebo during these time periods. By contrast, the effects on alpha and beta activity in particular, appeared to persist throughout the 18 hours post-dose interval for all three active treatments, most clearly so for the highest dose of MK-0249.

Effects of MK-0249 and Modafinil on Sleep Initiation, Sleep Maintenance and Sleep Structure Following Sleep Deprivation.

No significant differences between placebo and modafinil or placebo and either dose of MK-0249 were observed for measures of sleep initiation, i.e. latency to sleep onset and latency to persistent sleep (Table 1). The latency to SWS and REM sleep was also not significantly affected by the active treatments. The number and duration of sleep cycles was neither affected by modafinil nor any of the two doses of MK-0249.

Both 10 and 50mg of MK-0249 reduced total sleep time while modafinil did not. Less SWS and more stage 1 sleep were present after administration of 10mg of MK-0249. In addition to these changes, 50mg of MK-0249 also significantly reduced the percentage of stage 2 sleep, compared to placebo. REM sleep was also reduced by administration of both doses of MK-0249. Modafinil did not affect any sleep stage, either analyzed for the entire 12 h sleep episode or first 8 hours of the recovery sleep episode.

The number of sleep stage changes was lower after administration of 50mg of MK-0249. No significant differences in the number of awakenings were present after administration of

modafinil or either dose of MK-0249. However wake after sleep onset was increased by both doses of MK-0249.

These differences between placebo and active treatments for the entire recovery sleep episode were largely replicated when only the first 8hrs of sleep were analysed with the exceptions that the increase in stage 2 sleep after administration of 50mg of MK-0249 and the reduction in REM sleep after administration of 10mg of MK-0249 did not reach statistical significance when the analysis was limited to the first 8 hours.

Effect of Modafinil and MK-0249 on EEG power spectra in NREM and REM sleep during recovery sleep

Effects of the treatments on the spectral composition of the EEG were investigated by analyzing power spectra per third of the 12-h sleep episode, for the central and occipital derivation and separately for NREM (Figure 5) and REM sleep (Figure 6).

The analysis revealed that modafinil had no consistent effects on EEG power spectra during either NREM or REM sleep in any of the intervals and derivations analyzed. By contrast, both doses of MK-0249 reduced very low frequency (0.25-2.0 Hz) activity and increased high spindle and beta activity in NREM sleep in both the central and occipital derivation. The latter effects were most pronounced for the highest dose. The enhancement of beta activity and the reduction of low delta during NREM sleep persisted throughout the 12 hour sleep episode primarily after the higher dose of MK-0249 and in both central and occipital derivation. Furthermore, the higher dose of MK-0249 reduced theta activity at 7 and 8Hz during NREM sleep but this effect was observed only in the central derivation.

Dose-dependent effects of MK-0249 on EEG power spectra during REM sleep were observed. The highest dose of MK-0249 led to an enhancement of beta activity during REM sleep which was primarily observed in the central derivation. The highest dose reduced very low frequency and theta activity which was primarily observed in the occipital derivation. The analysis per third of the night did not suggest that these effects on EEG power spectra dissipated in the course of the sleep episode.

Discussion

The effects of sleep deprivation on EEG power spectra during the MWT as observed in the present study are in accordance with previous reports showing enhancement of low frequency EEG activity and reduction in alpha activity during the MWT (Chapotot et al. 2003) or during the Karolinska Drowsiness Test (KDT) with eyes open (Akerstedt and Gillberg 1990) (Aeschbach et al. 1999). These effects persisted throughout the entire sleep deprivation period. Whereas in some previous reports sleep deprivation was associated with an increase in beta activity (Dumont et al. 1999), a decrease in beta EEG activity was observed in the present study. In the present study we also observed an increase in sigma activity. These minor discrepancies may be related to the use of the MWT vs. the KDT. Overall, the robust effects of sleep deprivation on the EEG power spectra at the beginning of the MWT, which can be summarized as an increase in delta and theta activity and a reduction in alpha activity, demonstrate the sensitivity of this method for detecting changes in vigilance under placebo conditions.

The effects of both MK-0249 and modafinil on EEG power spectra at the beginning of the MWT can be summarized as ‘countering the effects of sleep deprivation’. Thus the “drug induced-wakefulness” or “pharmacological sleep deprivation” was characterized by effects which were to a large extent a mirror image of the effects of sleep deprivation under placebo. In the first 6 hours post-dose both compounds reduced delta and theta activity and enhanced alpha activity. In addition, the compounds enhanced beta activity. Modafinil induced reduction of delta and theta activity was previously reported in a helicopter simulator study (Caldwell, Jr. et al. 2000), although in that study no enhancement of alpha or beta activity was reported. In a previous sleep-deprivation-MWT study (Chapotot et al. 2003), modafinil was reported to enhance alpha activity but not beta activity. Modafinil also reduced theta activity in that study, although significantly so only at a few time points. In that study, the effects of the stimulant d-amphetamine were also assessed and it was found that d-amphetamine reduced theta activity throughout the sleep deprivation, but did not significantly enhance alpha activity. Based on these previous studies with modafinil and d-amphetamine, as well as the effects of the stimulant caffeine (Landolt et al. 2004), and the effects of sleep deprivation under placebo it can be concluded that a reduction of delta and theta and enhancement of alpha activity are part of an alerting EEG signature. The

effects of MK-0249 on the EEG to a large extent encompass this alerting EEG signature. Not much evidence for a dose effect was found and this may indicate that the lower dose of MK-0249 has already near maximal effects. In the first six hours post-dose, the effects on EEG power spectra were very similar for modafinil and MK02409, despite their different mechanism of action.

The observed differences between modafinil and MK-0249 with respect to the time course of the effects on EEG powers spectra appeared frequency specific. The enhancement of beta activity, and to some extent alpha activity, persisted throughout the 18 h post-dose period for all active treatments. The reduction in delta and theta activity remained significant for approximately 14 h post dose period after modafinil administration but only for the first 4 hours or so after MK-0249. These frequency specific differences may relate to the different mechanism of actions or different pharmacokinetics of the two drugs.

During recovery sleep, initiated 19 hours following dosing with MK-0249, EEG power spectra in both NREM and REM sleep were altered relative to baseline. The effects differed between REM and NREM sleep and between the doses; for REM sleep the effects appeared to depend on EEG derivation as well. Sleep stage and EEG derivation specific effects have previously been reported after other pharmacological interventions. The effects of MK-0249 on EEG power spectra during NREM sleep and REM sleep are very different from the effects of compounds such as benzodiazepine and non-benzodiazepine agonists of the synaptic and extra synaptic GABA receptor (Borbely et al. 1985;Brunner et al. 1991;Dijk, et al. 2009;Trachsel et al. 1990), 5HT₂ antagonists (Dijk et al. 1989;Landolt et al. 1999) and alcohol (Dijk et al. 1992). The effects of MK-0249 and of the higher dose in particular on EEG power spectra during NREM sleep, probably reflect residual alerting effects because the number of awakenings and wake after sleep onset were also increased relative to placebo. These effects were, however, relatively mild, because sleep onset and the number of awakenings were not affected, although sleep efficiency, SWS% an REM% were suppressed. Thus our data suggest that modulation of the histaminergic system by MK-0249 appears to affect cortical activation and vigilance state.

EEG power spectra during NREM sleep and REM sleep were not affected by modafinil, even though analysis of the MWT EEG data suggested some maintained efficacy up to 18

hours post dose, i.e. one hour prior to the start of recovery sleep. This may indicate that the effects of modafinil on sleep EEG power spectra differ from its effects on the EEG during wakefulness. In other words, the effects of modafinil on the EEG are vigilance state specific: modafinil affects the EEG during wakefulness but not during sleep. Alternatively, these observations may simply imply that 19 hours post-dose the wake promoting effects of modafinil, with a half-life of 10-12 hours, have dissipated too much to still affect the sleep EEG and the effects of MK-0249, with a half-life of approximately 14 hours, may still be present. The lack of an effect of modafinil compared to placebo, on the EEG during recovery sleep following sleep deprivation contrast the effects of, for example, caffeine administration during sleep deprivation. Caffeine, in contrast to modafinil, does not just inhibit the expression of a sleep debt but rather reduce the build up of sleep pressure during wakefulness (Landolt, et al. 2004). The lack of an effect of modafinil on EEG power spectra during NREM sleep is in accordance with the absence of an effect on sleep structure and sleep maintenance measures. These findings are in accordance with previous studies from which it was concluded that modafinil does not disrupt sleep if administration takes place 10 hours or more before the start of sleep (Bonnet et al. 2005). Please note that after neither MK-0249 nor modafinil the rebound of SWS or TST following sleep deprivation exceed the placebo values.

Overall the data show that the H3-inverse agonist MK 0249 has robust effects on the EEG during sleep deprivation, as well as effects on the EEG during sleep. The latter effects appeared dose dependent and may also depend on the mechanisms of action of the two compounds. Despite the very different mechanism of action of modafinil and MK-0249, the effects of the two compounds on the EEG during sleep deprivation were rather similar. These data support the notion that H3-inverse agonists, similar to modafinil, have robust wake promoting effects.

Acknowledgements:

We thank the clinical, research, data management and statistics teams of the Surrey Clinical Research Centre for the conduct of the study and help with the data analysis. This research was funded by Merck Inc and Drs Dijk, James and Boyle have received research support

from Merck Inc. Dr Dijk has also served as a consultant to Merck Inc. All other authors are employees of Merck Inc. The data presented in this manuscript represent an exploratory analysis of the EEG data collected in the protocol as described in Iannone et al. 2010. As far as the original study is concerned, the company was involved in original concepts and systematic review of existing trial evidence. The protocol was developed by Merck Inc with input from Surrey Clinical Research Centre (SCRC). All data were collected at SCRC and monitored by Merck Inc. The company was also involved in the control of the allocation schedule for the original study. The analysis plan for this exploratory analysis was developed by Drs Dijk, James and Boyle and reviewed by Merck Inc. All analyses were conducted at SCRC, primarily by Dr James, and we agree to allow the journal to review the data. The manuscript was drafted by Drs James and Dijk and finalised after review and input from the other authors.

Reference List

1. Aeschbach D, Matthews JR, Postolache TT, Jackson MA, Giesen HA, Wehr TA (1999) Two circadian rhythms in the human electroencephalogram during wakefulness. *Am.J.Physiol* 277: R1771-R1779
2. Akerstedt T, Gillberg M (1990) Subjective and objective sleepiness in the active individual. *Int.J.Neurosci.* 52: 29-37
3. Bonnet MH, Balkin TJ, Dinges DF, Roehrs T, Rogers NL, Wesensten NJ (2005) The use of stimulants to modify performance during sleep loss: a review by the sleep deprivation and Stimulant Task Force of the American Academy of Sleep Medicine. *Sleep* 28: 1163-1187
4. Borbely AA, Mattmann P, Loepfe M, Strauch I, Lehmann D (1985) Effect of benzodiazepine hypnotics on all-night sleep EEG spectra. *Hum.Neurobiol.* 4: 189-194
5. Brunner DP, Dijk DJ, Munch M, Borbely AA (1991) Effect of zolpidem on sleep and sleep EEG spectra in healthy young men. *Psychopharmacology (Berl)* 104: 1-5
6. Cajochen C, Brunner DP, Krauchi K, Graw P, Wirz-Justice A (1995) Power density in theta/alpha frequencies of the waking EEG progressively increases during sustained wakefulness. *Sleep* 18: 890-894
7. Cajochen C, Wyatt JK, Czeisler CA, Dijk DJ (2002) Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. *Neuroscience* 114: 1047-1060
8. Caldwell JA, Jr., Caldwell JL, Smythe NK, III, Hall KK (2000) A double-blind, placebo-controlled investigation of the efficacy of modafinil for sustaining the alertness and performance of aviators: a helicopter simulator study. *Psychopharmacology (Berl)* 150: 272-282
9. Chapotot F, Pigeau R, Canini F, Bourdon L, Buguet A (2003) Distinctive effects of modafinil and d-amphetamine on the homeostatic and circadian modulation of the human waking EEG. *Psychopharmacology (Berl)* 166: 127-138
10. Dijk D, James L, Peters S, Walsh J, Deacon S (2009) Sex differences and the effect of gaboxadol and zolpidem on EEG power spectra in NREM and REM sleep. *J.Psychopharmacol.*

11. Dijk DJ, Beersma DG, Daan S, van den Hoofdakker RH (1989) Effects of seganserin, a 5-HT₂ antagonist, and temazepam on human sleep stages and EEG power spectra. *Eur.J.Pharmacol.* 171: 207-218
12. Dijk DJ, Brunner DP, Aeschbach D, Tobler I, Borbely AA (1992) The effects of ethanol on human sleep EEG power spectra differ from those of benzodiazepine receptor agonists. *Neuropsychopharmacology* 7: 225-232
13. Dijk DJ, Czeisler CA (1995) Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. *J.Neurosci.* 15: 3526-3538
14. Dumont M, Macchi MM, Carrier J, Lafrance C, Hebert M (1999) Time course of narrow frequency bands in the waking EEG during sleep deprivation. *Neuroreport* 10: 403-407
15. Haas HL, Sergeeva OA, Selbach O (2008) Histamine in the nervous system. *Physiol Rev.* 88: 1183-1241
16. Iannone R, Palcza J, Renger J J, Calder N, Cerchio K, Gottesdiener K, Hargreaves R, Dijk DJ, Boyle J, and Murphy G (2010). Acute alerting effects of a novel histamine subtype-3 receptor inverse agonist in healthy sleep-deprived male volunteers. *Clinical Pharmacology and Therapeutics*, 88:831-839 .
17. Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J (2008) Sleep as a fundamental property of neuronal assemblies. *Nat.Rev.Neurosci.* 9: 910-919
18. Landolt HP, Meier V, Burgess HJ, Finelli LA, Cattelin F, Achermann P, Borbely AA (1999) Serotonin-2 receptors and human sleep: effect of a selective antagonist on EEG power spectra. *Neuropsychopharmacology* 21: 455-466
19. Landolt HP, Retey JV, Tonz K, Gottselig JM, Khatami R, Buckelmuller I, Achermann P (2004) Caffeine attenuates waking and sleep electroencephalographic markers of sleep homeostasis in humans. *Neuropsychopharmacology* 29: 1933-1939
20. Leibowitz SM, Brooks SN, Black JE (2006) Excessive daytime sleepiness: considerations for the psychiatrist. *Psychiatr.Clin.North Am.* 29: 921-945
21. Leurs R, Bakker RA, Timmerman H, de E, I (2005) The histamine H₃ receptor: from gene cloning to H₃ receptor drugs. *Nat.Rev.Drug Discov.* 4: 107-120
22. Lin JS, Dauvilliers Y, Arnulf I, Bastuji H, Anaclet C, Parmentier R, Kocher L, Yanagisawa M, Leher P, Ligneau X, Perrin D, Robert P, Roux M, Lecomte JM, Schwartz JC (2008) An inverse agonist of the histamine H₃ receptor

improves wakefulness in narcolepsy: studies in orexin-/- mice and patients. Neurobiol.Dis. 30: 74-83

- 23. Littner MR, Kushida C, Wise M, Davila DG, Morgenthaler T, Lee-Chiong T, Hirshkowitz M, Daniel LL, Bailey D, Berry RB, Kapen S, Kramer M (2005) Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. Sleep 28: 113-121**
- 24. Parmentier R, Anaclet C, Guhenec C, Brousseau E, Bricout D, Giboulot T, Bozyczko-Coyne D, Spiegel K, Ohtsu H, Williams M, Lin JS (2007) The brain H3-receptor as a novel therapeutic target for vigilance and sleep-wake disorders. Biochem.Pharmacol. 73: 1157-1171**
- 25. Qu WM, Huang ZL, Xu XH, Matsumoto N, Urade Y (2008) Dopaminergic D1 and D2 receptors are essential for the arousal effect of modafinil. J.Neurosci. 28: 8462-8469**
- 26. Rechtschaffen A, Kales A (1968) A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. U.S. Government Printing Office, Washington, D.C.**
- 27. Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature 437: 1257-1263**
- 28. Takahashi K, Lin JS, Sakai K (2006) Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. J.Neurosci. 26: 10292-10298**
- 29. Tinguely G, Finelli LA, Landolt HP, Borbely AA, Achermann P (2006) Functional EEG topography in sleep and waking: State-dependent and state-independent features. Neuroimage. 32: 283-292**
- 30. Trachsel L, Dijk DJ, Brunner DP, Klene C, Borbely AA (1990) Effect of zopiclone and midazolam on sleep and EEG spectra in a phase-advanced sleep schedule. Neuropsychopharmacology 3: 11-18**

Figure 1: Protocol used to assess the wake-promoting effects of MK-0249 and modafinil. Yellow represents periods of wakefulness and black represents scheduled sleep periods. Arrows indicate MWT time points and time of drug administration which occurred at 0200, just after the third MWT. 2 minutes of data from the beginning of each MWT was used to assess the effects of MK-0249 and modafinil, in comparison to placebo, on the EEG throughout the period of sleep deprivation.

Figure 2: Effects of sleep deprivation on EEG power spectra during MWTs in the placebo condition

Power spectra for 2 to 6 (MWTs 4 to 6 combined; n=24; black circles), 8 to 12 (MWTs 7 to 9 combined; n=24; red circles) and 14 to 18hrs (MWTs 10 to 12 combined; n=24; blue circles) post-dose, expressed as a percentage of baseline (spectra for MWTs 1 to 3, prior to drug administration), for central (left) and occipital (right) derivations, in the placebo condition. Black, blue and red circles along the x-axis indicate significant differences between baseline and 2 to 6hrs, 8 to 12hrs and 14 to 18hrs post-dose respectively.

Figure 3: Comparison of the effects of modafinil and MK-0249 with placebo on EEG power spectra during MWTs for 4hr time bins in the central derivation

Central derivation EEG power density spectra of wake and sleep EEG combined for MWTs binned each 4hrs for modafinil (red triangles, $n = 22$ in top two panels and 23 in bottom two panels), MK-0249 10mg (blue circles, $n = 24$) and MK-0249 50mg (green squares, $n = 24$) expressed as a percentage of placebo for each MWT time point. Red triangles, blue circles and green squares on the x-axis indicate significant differences between placebo and modafinil, MK-0249 10mg and MK-0249 50mg respectively, $p < 0.05$.

Figure 4: Comparison of the effects of modafinil and MK-0249 with placebo on EEG power spectra during MWTs for 4hr time bins in the occipital derivation

Occipital derivation EEG power density spectra of wake and sleep EEG combined for MWTs binned each 4hrs for modafinil (red triangles, $n = 23$) and MK-0249 10mg (blue circles, $n = 24$) and 50mg (green squares, $n = 24$) expressed as a percentage of placebo for each MWT time point. Red triangles, blue circles and green squares on the x-axis indicate significant differences between placebo and modafinil, MK-0249 10mg and MK-0249 50mg respectively, $p < 0.05$.

Figure 5: Effects of modafinil and MK-0249 on EEG NREM power density spectra per third of the night

Power density spectra expressed as a percentage of placebo for the first (left), second (middle) and third (right) thirds of the recovery night for central (top row) and occipital (bottom row) derivations after administration of modafinil (red lines and filled triangles, $n = 21$), MK-0249 10mg (blue lines and open circles, $n = 22$) and MK-0249 50mg (green lines and filled squares, $n = 22$ in A, B and C and 21 in D, E and F). Blue circles and green squares along the x-axis indicate a significant difference between placebo MK-0249 10mg and MK-0249 50mg respectively, $P < 0.05$.

Figure 6: Effects of modafinil and MK-0249 on EEG REM power density spectra per third of the night

Power density spectra expressed as a percentage of placebo for the first (left), second (middle) and third (right) thirds of the recovery night for central (top row) and occipital (bottom row) derivations after administration of modafinil (red lines and filled triangles, $n = 21$ in A to E and 20 in F), MK-0249 10mg (blue lines and open circles, $n = 22$ in A to E and 21 in F) and MK-0249 50mg (green lines and filled squares, $n = 22$ in B and C, 21 in A, E and F). Red triangles, blue circles and green squares along the x-axis indicate a significant difference between placebo and modafinil, MK-0249 10mg and MK-0249 50mg respectively, $P < 0.05$.

Table 1: PSG assessment of sleep after administration of modafinil and MK-0249 compared to placebo for the entire 12 hour recovery sleep episode

PSG variables (mean $\pm$ SE) after administration of placebo (n=22), modafinil (n=23), MK-0249 10mg (n=24) and MK-0249 50mg (n=24). Effect of treatment was assessed with repeated measures ANOVA and p values are listed. Abbreviations used are as follows: sleep onset latency (SOL), slow wave sleep (SWS), rapid eye movement (REM), latency to persistent sleep (LPS), total sleep time (TST), number of awakenings (NAW) wake after sleep onset (WASO). * indicates a significant difference from placebo; * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

Treatment	Placebo	Modafinil	MK0249 10mg	MK0249 50mg
SOL (min)	5.7 $\pm$ 0.87	13.3 $\pm$ 2.7	14.1 $\pm$ 2.7	8.4 $\pm$ 1.6
SWS latency (min)	23.4 $\pm$ 5.9	24.5 $\pm$ 3.2	32.1 $\pm$ 5.6	41.3 $\pm$ 11.6
REM latency (min)	65.9 $\pm$ 4.5	65.7 $\pm$ 4.7	63.5 $\pm$ 6.1	80.2 $\pm$ 11.6
LPS (min)	16.1 $\pm$ 6.5	16.5 $\pm$ 3.2	19.0 $\pm$ 4.2	31.0 $\pm$ 11.9
# of cycles	5.9 $\pm$ 0.2	5.8 $\pm$ 1.2	5.8 $\pm$ 0.2	5.3 $\pm$ 0.2
Cycle duration (min)	97.7 $\pm$ 3.2	95.5 $\pm$ 2.8	92.3 $\pm$ 2.8	93.0 $\pm$ 4.4
TST (min)	670.6 $\pm$ 10.2	661.5 $\pm$ 10.6	619.3 $\pm$ 11.8***	580.4 $\pm$ 14.7***
Stage 1 (%)	5.6 $\pm$ 0.7	6.5 $\pm$ 0.8	7.0 $\pm$ 0.6*	7.8 $\pm$ 0.6***
Stage 2 (%)	40.5 $\pm$ 1.4	39.0 $\pm$ 1.6	38.2 $\pm$ 1.3	37.0 $\pm$ 1.8*
SWS (%)	21.3 $\pm$ 1.3	21.2 $\pm$ 1.5	18.4 $\pm$ 1.3**	16.2 $\pm$ 1.3***
REM (%)	28.2 $\pm$ 1.3	27.5 $\pm$ 1.1	25.0 $\pm$ 1.1*	21.7 $\pm$ 1.2***
Sleep efficiency (%)	94.4 $\pm$ 1.2	92.2 $\pm$ 1.4	86.4 $\pm$ 1.6***	81.6 $\pm$ 2.1***
Stage changes	172.5 $\pm$ 7.0	185.5 $\pm$ 8.3	165.6 $\pm$ 8.5	152.5 $\pm$ 6.3*
NAW	17.0 $\pm$ 2.7	18.1 $\pm$ 2.6	22.5 $\pm$ 1.8	21.4 $\pm$ 1.6
WASO (min)	29.8 $\pm$ 9.0	37.7 $\pm$ 9.4	81.1 $\pm$ 10.7***	121.0 $\pm$ 15.9***

Table 2: PSG assessment of sleep after administration of modafinil and MK-0249 compared to placebo for the first 8 hours of the recovery sleep episode

PSG variables (mean $\pm$ SE) after administration of placebo (n=22), modafinil (n=23), MK-0249 10mg (n=24) and MK-0249 50mg (n=24). Effect of treatment was assessed with repeated measures ANOVA and p values are listed. * indicates a significant difference from placebo; * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

First 8hrs of recovery sleep episode				
Treatment	Placebo	Modafinil	MK0249 10mg	MK0249 50mg
# of cycles	3.5 $\pm$ 0.2	3.6 $\pm$ 0.2	3.4 $\pm$ 0.2	3.2 $\pm$ 0.2
Cycle duration (min)	97.4 $\pm$ 3.3	95.7 $\pm$ 3.5	95.7 $\pm$ 5.0	94.1 $\pm$ 4.5
TST (min)	448.7 $\pm$ 7.9	446.5 $\pm$ 5.3	421.9 $\pm$ 8.1*	395.4 $\pm$ 11.8***
Stage 1 (%)	4.3 $\pm$ 0.6	4.8 $\pm$ 0.7	5.4 $\pm$ 0.5*	5.9 $\pm$ 0.5**
Stage 2 (%)	38.9 $\pm$ 1.7	38.8 $\pm$ 1.8	38.2 $\pm$ 1.6	36.8 $\pm$ 1.9
SWS (%)	29.5 $\pm$ 1.7	29.2 $\pm$ 1.8	25.3 $\pm$ 1.6**	22.8 $\pm$ 1.9***
REM (%)	23.8 $\pm$ 1.1	23.7 $\pm$ 1.1	22.6 $\pm$ 1.1	19.3 $\pm$ 1.2***
Sleep efficiency (%)	95.3 $\pm$ 1.2	93.6 $\pm$ 1.1	88.0 $\pm$ 1.7***	83.1 $\pm$ 2.5***
Stage changes	115.3 $\pm$ 5.5	122 $\pm$ 4.9	110.8 $\pm$ 5.8	100.3 $\pm$ 4.4*
NAW	8.7 $\pm$ 1.7	9.0 $\pm$ 1.6	11.4 $\pm$ 1.3	10.6 $\pm$ 1.2
WASO (min)	14.5 $\pm$ 6.1	15.7 $\pm$ 4.5	42.1 $\pm$ 6.7**	70.6 $\pm$ 11.2***

Definitions:

ANOVA -	Analysis of variance between groups
BMI -	Body Mass Index
CRC	Clinical Research Centre
EEG -	Electroencephalograph
EMG -	Electromyography
EOG -	Electrooculography
KDT -	Karolinska Drowsiness Test
LPS -	Latency to persistent sleep
MSLT -	Multiple sleep latency test
MWT -	Maintenance of wakefulness test
NAW -	Number of awakenings
NREM -	Non-rapid eye movement
PSG	Polysomnogram
REM -	Rapid eye movement
SOL -	Sleep onset latency
SWS -	Slow-wave sleep
TST -	Total sleep time
WASO -	Wake after sleep onset