Acute sleep deprivation: The effects of CX717

Acute sleep deprivation: The effects of the AMPAKINE compound, CX717, on human cognitive performance, alertness and recovery sleep.

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Abstract

Introduction: AMPA receptor modulation is a potential novel approach to enhance cognitive performance. CX717 is a positive allosteric modulator of the AMPA receptor that has shown efficacy in rodent and primate cognition models.

Methods: CX717 (100mg, 300 mg and 1000 mg) and placebo were studied in 16 healthy male volunteers (18 – 45 yrs) in a randomized, crossover study. Cognitive function, arousal and recovery sleep (by polysomnography) were assessed during the extended wakefulness protocol.

Results: Placebo condition was associated with significant decrements in cognition, particularly at the circadian nadir (between 03:00 and 05:00 hours). Pre-specified primary and secondary analyses (general linear mixed modelling, GLMM) at each separate time point did not reveal consistent improvements in performance or objective alertness with any dose of CX717. Exploratory repeated measures analysis, a method used to take into account the influence of individual differences, demonstrated an improvement in attention-based task performance following the 1000 mg dose. Analysis of the recovery sleep showed that CX717 1000 mg significantly reduced stage 4 and slow-wave sleep ($P \leq 0.05$) with evidence of reduced slow-wave and spindle activity.

Conclusions: The study suggests that CX717, only at the 1000 mg dose may counteract effects of sleep deprivation on attention-based tasks and that it may interfere with subsequent recovery sleep.

Key words: AMPAKINE, sleep deprivation, cognitive enhancer

Introduction

AMPA (α -amino-3-hydroxy-5-methyl-4-isooxalopropionic acid) type glutamate receptors are predominantly responsible for mediating fast synaptic transmission throughout the central nervous system (CNS). Activation of AMPA receptors leads to generation of fast excitatory postsynaptic potentials (EPSP) and is linked to long-term potentiation, a form of synaptic plasticity (Staubli et al., 1994a). AMPA receptors are tetramers composed of four subunits with each subunit existing as two splice variants, flip and flop. Although the splice variants represent only small changes in amino acid sequence these variants modify calcium permeability and channel kinetics, determining the speed of desensitization and re-sensitization, and are differentially distributed throughout the brain (Foster and Kemp, 2006).

AMPAKINES are positive allosteric modulators of AMPA receptors, limiting receptor deactivation and desensitization (Lynch, 2002). They have been shown to enhance long-term and short-term forms of memory, including spatial and olfactory memory encoding in rats (Staubli et al., 1994a; Staubli et al., 1994b; Hampson et al., 1998), spatial (delayed-match-to-sample) memory in primates (Porrino et al., 2005) and there is evidence to suggest AMPAKINES may facilitate spatial, visual and olfactory memory in humans (Ingvar et al., 1997). In addition to a cognitive facilitatory effect, there is evidence suggesting that some AMPAKINES have a neurotrophic and / or neuroprotective role within the CNS, with increased brain-derived neurotrophic factor (BDNF) expression and cell proliferation (Lauterborn et al., 2000; Bai et al., 2003). These findings have led some to suggest that AMPAKINES may be of use in the treatment / management of neurodegenerative disorders such as Alzheimer's and Parkinson's disease and indeed in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rodent model of Parkinson's disease, the novel AMPAKINE, LY404187, reduced dopamine cell loss, indicative of a neuroprotective role (O'Neill et al., 2004).

Acute sleep deprivation: The effects of CX717

In order to assess the efficacy of AMPAKINES in circumstances where cognitive performance is compromised, Porrino et al., (2005) used a model of sleep deprivation to investigate the effects of CX717 in non-human primates. Using a delayed-match-to sample (DMTS) memory task they showed that CX717 improved total performance in both normal alert and sleep deprived (30-36 h sleep deprivation protocol) conditions. Surface electroencephalogram (EEG) over the parietal cortex, demonstrated marked changes in power spectral density in the sleep deprived compared with the control condition, with increased delta and beta power following sleep deprivation. In addition local cerebral metabolism was also affected with glucose metabolism reduced in brain areas associated with memory processes. CX717 appeared to normalise the EEG and glucose metabolism profile, shifting values back to the normal, alert condition (Porrino et al., 2005).

From behavioural, electrophysiological and brain imaging research in humans, the prefrontal cortex (PFC) has been identified as one of the main areas affected by short-term sleep deprivation (Harrison et al., 2000; Thomas et al., 2000; Chee and Chuah, 2008). As pressure for sleep increases, the waking state becomes increasingly unstable (Doran et al., 2001). This has a direct negative impact upon working memory, attention and other executive functions such as verbal fluency and flexible thinking (Wimmer et al., 1992; Harrison and Horne, 1998).

Interestingly, healthy aging is also reported to be attributed to functional and structural changes including the prefrontal cortex (Moscovitch and Winocur, 1995; Fjell and Walhovd, 2010) this observed decrement in cognition is not dissimilar to that observed in sleep deprived individuals and as such sleep deprivation may provide a model of cognitive decline (Harrison et al., 2000).

Here we present the results of a 27 h extended wakefulness protocol in healthy male volunteers with cognitive testing overnight at the circadian nadir to assess the efficacy of CX717 on reversing cognitive deficits associated with sleep deprivation in human subjects.

Methods

The study was a single-centre, placebo controlled, randomised, double-blind, 4-way crossover study of 3 doses of the compound CX717 (100mg, 300mg, 1000mg) and placebo on cognitive performance and recovery sleep using an extended wakefulness protocol. The study was conducted at the Surrey Clinical Research Centre, Faculty of Health and Medical Sciences, University of Surrey, United Kingdom. The study was approved by Quorn Research Review Ethics Committee, UK and by the University of Surrey Ethics Committee. The study was carried out in accordance with principles based on the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Subjects: Sixteen healthy male volunteers, aged between 18-45 (mean 30) years with a body mass index between 18-30 kg/m² (mean 24.8 kg/m²) were recruited onto the study. They were in good health without a clinical history of mental or physical illness. Volunteers were excluded if they had participated in another study or had donated blood within 3 months prior to the study. In addition, those with a history of drug abuse, including alcohol, a history of primary insomnia (DSM IV criteria) or with a sleep disorder were excluded.

Volunteers underwent a medical examination that included a 12-lead electrocardiogram (ECG), electroencephalogram (EEG) screen with photic stimuli, estimates of blood constituents (haematology and biochemistry) and urinalysis, as well as testing for breath alcohol and drugs of abuse. The physical examination, ECG and laboratory tests were repeated at the end of the study

Acute sleep deprivation: The effects of CX717

within 9 days of the last dosing period. Adverse events were recorded throughout the study using established techniques.

Participants were required to avoid working shifts at night for 2 weeks prior to, and for the duration of, the study. Unusual levels of physical activity had to be avoided, and alcohol, nicotine and products containing caffeine were forbidden from 24 hours before treatment up to the end of the evaluation (24 hours after administration of the compound). Tests for breath alcohol and for drugs of abuse were performed each day before the ingestion of medication and the subjects were supervised throughout.

Study design: A single-centre, placebo controlled, randomised, double-blind, 4-way crossover study with placebo and 3 doses (100, 300 and 1000 mg) of CX717 compound was carried out to assess aspects of alertness and cognitive function during a 27 hour period of extended wakefulness. Recovery sleep was also assessed immediately following the extended wake period. Subjects were admitted to the Surrey CRC where they underwent training on psychometric tests prior to night 1 during the first study period.

The four assessments each involved two successive nights (baseline and medication), and these assessments were separated by approximately 14 days (allowed range 10-21 days). On the baseline night (night of Day-1) subjects were allowed to sleep from 23:00 until 0700 hours after which volunteers were kept awake for a period of 27 hours. Baseline psychometric assessments were conducted at 0900 hours (rested) and 2100 (pre-dose) hours on Day 1. The medication (placebo, 100, 300 or 1000 mg) was given in identical capsules at 2300 hours (Day 1), and the psychometric tests were carried out at 0000, 0100, 0300, 0500, 0700, 0900 and 1630 hours (1, 2, 4, 6, 8, 10 and 17.5 hours post dose) the next day (Day 2). Alertness was objectively measured at 0400 hours (5 hours post dose) by the modified Maintenance of Wakefulness Test (mMWT). On day 2, from 1000 through 1600 hours subjects underwent a recovery sleep during which polysomnography measures were recorded for manual sleep scoring and spectral analysis.

Modified Maintenance of Wakefulness Test (mMWT): The modified Maintenance of Wakefulness Test observes the capacity to remain fully awake when placed in an environment with minimal stimuli and as such it identifies objective alertness (Frey et al., 2004). Subjects were requested to sit semi-supine in a darkened, sound attenuated, temperature controlled (16 ± 2 °C) sleep laboratory, close their eyes and stay awake. The time taken to reach 30 s of stage 2 sleep was recorded. The test was terminated when 30 s of stage 2 sleep had been observed or at 15 minutes if the subject had not fallen asleep.

Cognition: A psychometric battery was administered at 1, 2, 4, 6, 8, 10 and 17.5 hours post dose. Due to the length of the complete test battery not all tests were included at each time point. Please refer to Table 1 for test battery details. [Need to insert Table 1 here]

Critical Flicker Fusion (CFF). This task provides an index of cortical arousal and assesses the ability to distinguish between discrete sensory information (Curran, 1990) and has been shown to be sensitive to a variety of psychoactive compounds (Hindmarch, 1975; 1982 and 1994; Smith and Misiak, 1976). Subjects were required to discriminate between flicker from fusion, and vice versa. There was a set of four light emitting diodes arranged in a one centimetre square. The diodes were held in foveal fixation when viewed at a distance of one metre. Response measures included the mean ascending threshold (discriminating fusion from flicker), mean descending threshold (discriminating flicker from fusion) and mean threshold frequency.

Continuous Tracking Task (CTT). This is a task of psychomotor function and bilateral attention (Parkin et al., 1998). During the task subjects were required to keep a cursor in alignment with a moving target on a visual display unit screen whilst simultaneously responding to peripheral stimuli that appeared at random. The response measures were the mean deviation from the moving target and cursor (CTT MD, pixels) and the response time (CTT RT, ms) to the peripheral stimuli.

Sustained Attention to Response Task (SART). The SART assesses sustained attentional performance and has been shown to be a sensitive measure of attentional failures and deficits (Roberston, 1997; Manly, 1999; Manly, 2002). Subjects were required to respond to every stimulus presented, but withhold any response if the stimulus was presented with a “target” stimulus. The measure was the number of slips of attention.

Rapid Visual Information Processing Task (RVIP). This task assesses the performance of visual attentional mechanisms in remaining vigilant to periodically occurring events (Coull et al., 1996; Wesnes and Warburton, 1984). The test required subjects to monitor a series of single numbers (1-9) appearing on a screen and to respond to three consecutive odd or three consecutive even numbers by pressing a button as quickly as possible. The measure was the number of valid responses.

Sternberg Short Term Memory Scanning Task (STM). Working memory as assessed in this test (Sternberg, 1966, 1969 and 1975) was measured by the ability to memorise a random series of 3, 5 or 7 letters (stimulus set) presented sequentially. A single probe letter was presented after the stimulus set and the subject was required to indicate whether the probe had been present in the original stimulus set or not. The response measure was the mean reaction time (ms).

Immediate and delayed Word Recall (IWR, DWR). This task assesses both working and explicit memory by scoring immediate and delayed recall respectively (Baddeley and Hitch, 1974; Baddeley, 1986). Subjects were given 2 minutes to learn a word list of 20 nouns with a recall period of 2 minutes immediately after. After 30 minutes subjects were given 2 minutes to recall as many of the learned words as possible.

Digit Symbol Substitution Test (DSST). This pen and paper task assesses various measures including information processing (Parrott, 1991), psychomotor performance (Lezak, 1995) and it also assesses visuomotor coordination, sustained attention and concentration. Subjects were

Acute sleep deprivation: The effects of CX717

given 120 seconds to substitute as many digits for nonsense symbols according to a key provided. The response measure was the number of symbol substitutions completed by the subject in the given time.

Hick's Choice Reaction Time (H-CRT). The H-CRT test is an indicator of sensorimotor performance, assessing the ability to attend and respond to a critical stimulus (Hindmarch et al., 2002; Sherwood and Kerr, 1993). Subjects were required to extinguish one of six equidistant red lights by pressing the associated response button as quickly as possible. Behind each red light was a corresponding green light, which was illuminated to indicate whether 1, 3, 4 or all 6 of the red lights were potential stimuli. The response measures were recognition reaction time (RRT), time taken to recognise the red light stimulus, motor reaction (MRT), time between the subject lifting his/her finger from the start button and touching the response button and the total reaction time (TRT), sum of both in milliseconds (ms).

Trails A and B (TAB). The TAB is a pen and paper test of psychomotor speed and mental set shifting (Spren and Strauss, 1991). During Trails A subjects were requested to connect (consecutively) circles numbered 1 to 25. Trails B required subjects to connect circles by alternating between ascending numbers and letters in alphabetical order. The time taken to complete Trails B after controlling for Trail A was analysed.

Subjective Assessments: Subjective feelings of sedation, mood and co-ordination during the extended wakefulness period and the effect of treatment on these were assessed by using linear analogue scales (LARS). A score of 50 was used to indicate that no change in subjective feelings had been perceived from their usual state for that particular factor.

Sleep: The electroencephalographic (EEG) correlates of sleep were recorded using the Vitaport 3 System (TEMEC Instruments B.V., Kerkrade, The Netherlands). During the recovery sleep, four channels were recorded (C3-A2, C4-A1, O1-A2, O2-A1) and digitised at a sample rate of 256Hz.

Acute sleep deprivation: The effects of CX717

Records were manually scored according to standard criteria (Rechtschaffen and Kales, 1968). The variables scored were the sleep period time (min), sleep efficiency (SE, %), total sleep time (TST, min), sleep onset latency (SOL, based on 3 epochs of stage 2 sleep), latency to sleep stages (1, 2, 3, 4) and rapid eye movement (REM) sleep (min), numbers of awakenings (NAW) and duration (min) and percentage of time awake throughout the night. Duration and percentage of the sleep stages (1, 2, 3, 4), slow-wave sleep (SWS, stages 3 and 4 combined) and REM sleep, and latency to persistent sleep (LPS, time to 10 mins of consolidated sleep) were also calculated.

Where records allowed spectral analysis was carried out. Vitaport data files were imported into Vitabase/Vitascore software (TEMEC Instruments B.V., Kerkrade, The Netherlands), all EEGs were visually inspected and artefacts were manually removed. Artefact free 4sec sub-epochs of EEG were subjected to offline spectral analysis using Fast Fourier Transform (FFT). Changes in EEG power spectra in NREM sleep and REM sleep, across sequence bands: slow-wave (0.25 - 0.70 Hz and 0.75 - 4.5 Hz), theta (4.75-7.75 Hz), alpha (8.0-12.0 Hz), sigma (12.0 – 15.0 Hz) and beta (15.0 – 20.0 Hz) were assessed.

In order to represent data in such a fashion as to clearly indicate differences between the different treatment conditions power density values were expressed as a percentage of placebo, a method which has previously been used to illustrate the effects of various sleep deprivation paradigms (Dijk et al., 1987; Brunner et al., 1990; Aeschbach et al., 1997) and pharmacological (Brunner et al., 1991; Aeschbach et al., 1994) intervention studies. In order to achieve this, geometric means of the data were calculated as percentages of placebo, the placebo condition being assigned a value of 100%.

Statistical Analysis

The analyses of sleep and psychometric measures were carried out using the Full Analysis Data Set (FADS), and included all randomised subjects who received at least one dose of study medication and gave rise to at least one post dosing efficacy measurement. Cognitive and psychomotor data sets were analysed by analysis of variance at each separate time point using SAS® PROC MIXED with fixed effect factors for subject, treatment and period with subtraction of the individual period rested baseline value. 'Subject' was used as a fixed effect in all statistical analyses, instead of as a random effect, because the pattern of missing values necessitated this choice. Sleep, spectral analysis, MWT and LARS data sets were similarly analysed but with no baseline applied. Treatment effects were evaluated on a two sided significance test with a significance level of 0.05.

Where possible a repeated measures, restricted estimation maximum likelihood (REML) option, general linear mixed modelling (GLMM) was also performed on cognitive and psychomotor data (change from baseline), for all time points up to and including 17.5 hours post dose. Repeated measures GLMM was used in order to assess more fully whether a treatment effect was evident by controlling for inter-individual differences. In each of these analyses, the relevant performance data formed the dependent variable in a GLMM with subject and treatment as fixed effects independent variables, with a categorical independent variable having a separate level for each combination of period and time of measurement, with time of measurement as a repeated measure independent variable using the anisotropic spatial power variance-covariance matrix option, and with a treatment by time of measurement interaction.

Results

Following placebo there was a significant deterioration in performance with extended wakefulness compared with the rested baseline for CFF ($F_{(3,129)} = 20.5$, $P \leq 0.001$), CTT MD ($F_{(5,248)} = 20.5$, $P \leq 0.001$), CTT RT ($F_{(5,234)} = 19.8$, $P \leq 0.001$), SART ($F_{(3,174)} = 11.3$, $P \leq 0.001$), RVIP ($F_{(1,53)} = 46.8$, $P \leq 0.001$), STM ($F_{(6,297)} = 7.9$, $P \leq 0.001$, $P \leq 0.001$), DWR ($F_{(2,144)} = 13.2$, $P \leq 0.001$), CRT RRT ($F_{(3,164)} = 8.5$, $P \leq 0.001$), CRT MRT ($F_{(3,152)} = 5.3$, $P \leq 0.01$), CRT TRT ($F_{(3,163)} = 9.1$, $P \leq 0.001$) and DSST ($F_{(3,146)} = 3.5$, $P \leq 0.05$) with the nadir in performance between 05:00 and 07:00 hours. There was no statistically significant deterioration in performance on IWR ($F_{(2,144)} = 2.7$) and Trails B ($F_{(3,194)} = 2.4$) [Need to insert Figure 1 and Table 2 here].

1. General linear mixed modelling (GLMM) at each individual time point

GLMM at each individual time point was the primary analysis of interest. This analysis did not reveal a significant effect of CX717 (100 to 1000 mg) on levels of alertness as assessed by the MWT or on any of the cognitive measures (CTT, SART, RVIP and CFF) during the extended wakefulness period. There was, however, a significant improvement in SART performance (fewer slips of attention) ($F_{(3,35)} = 3.5$, $P \leq 0.05$) after the recovery sleep when comparing CX717 300mg and 1000 mg with placebo (LSMn Diff = 2.6 and 3.8, CI = 0.2: 5 and 1.4: 6.3, $P \leq 0.05$ and 0.01, respectively) (Figure 2d).

GLMM of the LARS data at individual time points showed that for subjective ratings there was a statistically significant worsening of mood ($F_{(3,38)} = 3.8$, $P \leq 0.05$) and co-ordination ($F_{(3,38)} = 3.4$, $P \leq 0.05$) 4 hours post dose when comparing CX717 1000 mg with placebo (LSMn Diff = -5.5, CI = -10.2: -0.8, $P \leq 0.05$ and LSMn Diff = -9.1, CI = -15.3: -2.8, $P \leq 0.01$, respectively).

There was no significant treatment effect on objective alertness as assessed by the MWT.

[Need to insert figure 2 here]

2. Repeated Measures GLMM

A repeated measures GLMM demonstrated a significant treatment effect for the SART ($F_{(3,152.8)} = 4.5$, $P \leq 0.01$), TAB ($F_{(3,149.1)} = 3.9$, $P \leq 0.05$) and CFF ($F_{(3,164.2)} = 3.1$, $P \leq 0.05$) tests. CX717 300 and 1000 mg reduced the number of slips of attention omission in the SART (LSMn Diff = 1.2, CI 0.0: 2.5, $P \leq 0.05$ and LSMn Diff = 1.9, CI 0.7: 3.1, $P \leq 0.01$) and CX717 300 mg improved performance on the trails task (LSMn Diff = 2.9, CI 0.3: 5.4, $P \leq 0.05$). There was an indication of improved performance on the CFF test with CX717 1000 mg although it did not reach statistical significance (LSMn Diff = -0.5, CI -1.1: 0.1, $P = 0.09$).

There were no significant treatment effects for any of the other performance measures. Treatment effects were limited to the assessments listed above.

Recovery Sleep: Manual staging and spectral analysis

There was a significant reduction in stage 4 and SWS following CX717 1000 mg (LSMn Diff = -19.27, CI = -32.95: -5.59, $P \leq 0.01$ and LSMn Diff = -21.75, CI = -38.43: -5.08, $P \leq 0.05$, respectively). No other statistically significant changes in sleep architecture were reported.

[Need to insert Table 3 here]

Spectral Analysis:

There was no significant effect of treatment on individual 1Hz width frequency bins (1 – 32 Hz) during NREM (Figure 3a) or REM sleep (Figure 3b), although power density in the delta and theta frequencies as well as in the spindle frequencies for the active treatments were consistently below those for placebo. When looking at certain pre-specified, frequency bands a significant reduction was seen in slow-wave activity (SWA) (0.25 – 0.5 Hz and 0.7 – 4.5 Hz) during NREM sleep following 1000 mg CX717 compared with placebo (ratio estimate 1.28, CI = 1.02: 1.60,

Acute sleep deprivation: The effects of CX717

$P \leq 0.05$ and ratio estimate 1.27, CI = 1.03: 1.57, $P \leq 0.05$). There was no significant reduction in power density during REM sleep with CX717 100, 300 or 1000 mg.

[Need to insert figure 3 here]

Safety

There were no serious adverse events or discontinuations due to adverse events during the study. Fourteen subjects (87.5%) reported a total of 14 clinical adverse experiences (AEs). Of these five subjects (31.25%) experienced 5 AEs following placebo, seven subjects (43.75%) experienced 7 AEs following the 100 mg dose and one subject (6.25%) experienced 1 AE after 300 and 1000 mg. The most common AEs were nervous system (headache, paraesthesias and dizziness) and gastrointestinal (diarrhoea, nausea and vomiting). The incidence of AEs did not increase with dose. [Need to insert table 4 here]

Discussion

Performance deficits during sleep deprivation have been well documented. Extended wakefulness into the biological night is associated with deterioration in cognitive performance and subjective alertness particularly around the circadian nadir (Horner and Pettitt, 1985; Dijk et al., 1992; Lorenzo et al., 1995; Rogers et al., 2003; Viola et al 2007). The study reported here assessed the efficacy of CX717 on reversing cognitive deficits during a 27 hour extended wakefulness period. Performance was assessed using attention-based tasks known to be particularly sensitive to sleep deprivation (Dinges and Kribbs, 1991). In this study, cognitive testing was performed throughout the night and the combined circadian and sleep deprivation effects would be expected to lead to decrements in performance on vigilance and reaction time tasks with a performance trough between 0300 and 0700 hours (Van Dongen and Dinges, 2005; Schweitzer et al., 2006; Viola et al 2007). As expected this study showed that for the group as a whole performance on tests of attention, information processing, sensorimotor performance and memory deteriorated over a period of 27 hours extended wakefulness with the lowest performance evident during the early morning hours.

In a primate sleep deprivation model the AMPAKINE CX717 was able to reverse performance deficits (Porrino et al., 2005) and thus it was hypothesised that CX717 may act to attenuate decrements in performance in human subjects during a period of extended wakefulness. Analysis of variance of the individual time points revealed a significant improvement of sustained attention following the recovery sleep but no consistent improvement on other performance or alertness measures during the extended wakefulness period. Closer inspection of the individual data however, suggested that there was a relatively high 'between subject' variability in response to sleep loss. Recent research has highlighted substantial inter-individual differences between a subject's vulnerability to sleep loss and consequent performance deficits (Drummond et al., 2001; Drummond et al., 2004; Frey et al., 2004; Van Dongen et al., 2004b).

Acute sleep deprivation: The effects of CX717

Studies have consistently shown that some subjects are relatively resilient to extended periods of sleep loss whilst others display marked decrements in performance, and it has been suggested that there is a trait-like differential vulnerability to impairment from sleep loss (Van Dongen et al., 2004a; Viola et al., 2007) which has correlates at the level of brain imaging (Chee and Chuah, 2008; Vandewalle et al., 2009).

The most appropriate way to quantify inter-individual variability in neurobehavioural responses to sleep deprivation is via the intraclass correlation coefficient (ICC) (Van Dongen et al., 2004b). This however was not assessed in this current study as no two sleep deprivation periods were identical and instead each period was distinct, with a unique treatment under investigation.

Exploratory analysis of the data, ranking subjects according to their z score for each test around the point of circadian nadir (03:00 to 05:00 hours), suggested that subjects who showed greater cognitive deficits during a sleep deprivation paradigm demonstrated significant cognitive improvement following CX717 1000 mg (Boyle et al., 2006). This analysis however can be criticized for possible 'regression to the mean' (Wesensten et al., 2007) and is not appropriate to assess whether a treatment effect is present.

We therefore used repeated measures (REML option) GLMM in order to assess whether a consistent treatment effect was evident (Van Dongen et al., 2004b). This analysis suggested that CX717 did counteract cognitive decline on attention based tasks, such as CFF and SART, following sleep deprivation but only at the higher, 1000 mg dose. There was however no evidence of cognitive enhancement on other performance measures including memory and sensorimotor tasks and no improvement in levels of alertness as assessed by the MWT. Lack of positive effects of CX717 on performance has previously been noted in a simulated night shift work study (Wesensten et al., 2007). In the study by Wesensten et al. (2007) however, no improvement was seen in any of the performance measures including attention based tasks. The

reason for the difference in findings is unclear. In the Wesensten study attentional performance was assessed using the psychomotor vigilance task (PVT) rather than the SART and CFF and there were distinct differences in the methodology with the Wesensten study a parallel design, shift work paradigm compared with the current study which was an extended wakefulness study.

Manual staging and spectral analysis of the recovery sleep data demonstrated that CX717 at the 1000 mg dose significantly reduced stage 4 and SWS. This finding is consistent with the Wesensten et al. study (2007) which showed that CX717 1000mg significantly reduced SWS during a daytime sleep opportunity. The slow-waves that dominate the EEG during SWS can be quantified as SWA and we found that SWA was reduced after CX717-1000 mg. SWA is an important aspect of sleep and is thought to correlate with cognitive performance, learning and memory and subjective ratings of sleep quality (Jewett, et al., 1999; Walker, 2009). The recovery sleep data from both the Wesensten and current study therefore suggest that CX717 may impair sleep and its restorative abilities.

The effect of CX717 1000mg on SWA during the recovery sleep however may account for the improvement in performance on the sustained attention task upon awakening. Similar improvements in sustained attention have been observed for caffeine and may to be linked to a reduction in sleep inertia upon awakening possibly associated with a reduction in SWA during the sleep period (Landolt et al., 1995; Van Dongen et al., 2001).

From a safety perspective CX717 was well tolerated with no significant dose related adverse events and no clinical changes in safety parameters. Following CX717 1000 mg subjects reported a worsening in mood and 4 hours post dose. It is unclear whether this is a direct effect of the drug or whether an improvement in cognitive functioning following CX717 enables the subjects to rate their feelings more accurately. It is known that sleep deprivation compromises a

subject's ability to rate subjective feelings of fatigue (Van Dongen et al., 2003) and this may account for the anomaly in subjective ratings following placebo and CX717 treatments.

Overall, the data suggest that CX717 at the 1000 mg dose may improve performance on some attention based tasks, in particular information processing and sustained attention tasks, during a period of extended wakefulness. There is evidence that the effects of CX717 continue up to 17.5 hours post dose with reductions in SWS, delta EEG power and a possible reduction in sleep homeostatic pressure. It is clear from the study that subjects vary in their response to extended wakefulness. These data could therefore be supported by further studies where the between subject variability is either more carefully controlled, for example by selecting only those subjects vulnerable to performance deficits during sleep loss, or where the protocol design allows for the variability to be estimated.

In conclusion, the AMPAKINE CX717 may be of use in overcoming cognitive deficits associated with extended wakefulness and may be of particular benefit for those subjects who are more vulnerable to decrements in performance during sleep loss.

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Acute sleep deprivation: The effects of CX717

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Acute sleep deprivation: The effects of CX717

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Figure legends and Figures:

Figure 1: Overnight performance during placebo condition

- a) Critical flicker fusion mean threshold (CFF, Hz), ($F_{(3,129)} = 20.5$, $P \leq 0.001$). A lower CFF threshold indicates poorer performance.
- b) Continuous tracking task mean deviation (CTT MD, pixels), ($F_{(5,248)} = 20.5$, $P \leq 0.001$). A higher deviation indicates poorer tracking ability.
- c) Continuous tracking task reaction time (CTT RT, seconds), ($F_{(5,234)} = 19.8$, $P \leq 0.001$). A higher reaction time indicates poorer performance.
- d) Sustained attention to response task (SART, number of attentional slips), ($F_{(3,174)} = 11.3$, $P \leq 0.001$). A higher score indicates a greater number of slips in attention.

Symbols show mean and error bars represent standard error of the mean (SEM).

* indicates significant change from rested (09:00) baseline at <0.05 ; ** indicates significant change from rested baseline (09:00) placebo at <0.01 ; *** indicates significant change from rested baseline (09:00) at <0.001 . Bottom x-axis indicates hours post-dose.

Figure 2: Effects of CX717 on wake performance measures

- a) Critical flicker fusion mean threshold (CFF, Hz). Change from placebo following CX717 300 (■) and 1000 (□) mg, ($F = 3.1_{(3,164)}$, $P \leq 0.05$).
- b) Continuous tracking task mean deviation (CTT MD, pixels). Change from placebo following CX717 300 (■) and 1000 (□) mg. ($F = 2.6_{(3,232)}$, $P = 0.0521$).
- c) Modified maintenance of wakefulness test (MWT, seconds). Bars show change from placebo following CX717 100, 300 and 1000 mg. ($F = 1.1_{(3,27)}$, $P = \text{not significant}$).
- d) Sustained attention to response task (SART, number of attentional slips). Change from placebo following CX717 100 (▲), 300 (■) and 1000 (□) mg. ($F = 4.5_{(3,152)}$, $P \leq 0.01$).

Acute sleep deprivation: The effects of CX717

Symbols or bars show mean and error bars represent standard error of the mean (SEM). †

indicates significant improvement from placebo at $P \leq 0.05$ following CX717 300mg and **

indicates significant improvement from placebo at $P \leq 0.001$ following CX717 1000mg. Upper

x-axis indicates clock time as a categorical value. Bottom x-axis indicates hours post-dose.

Figure 3: Effects of CX717 on NREM sleep EEG power density spectra

Absolute NREM and REM sleep EEG power density spectra (left panel) and power spectra of treatment conditions expressed as a percentage of placebo (right panel). Placebo (black line at 100%), CX717 100mg (▲), 300mg (■) and 1000mg (●).

3ANREM **3B**Relative power density (%)
(Placebo = 100%) **3C**REM **3D**Relative power density (%)
(Placebo = 100%)

Table 1: Schedule of psychometric assessments
Schedule of assessment of cognitive, psychomotor, subjective and clinical alertness measures

Clock Time (hh:mm)	Time Post Dose	Psychometric Tests
00:00	1 hr	STM; SART; CTT; CFF
01:00	2 hr	STM; CTT; HCRT; TAB; DSST
03:00	4 hr	STM; SART; CTT; CFF; WRi; WRd; LARS
05:00	6 hr	STM; CTT; HCRT; RVIP; TAB; DSST
07:00	8 hr	STM; SART; CFF; WRi; WRd
09:00	10 hr	STM; CTT; HCRT; TAB; DSST; LARS
16:30	17.5 hr	STM; SART; CTT; CFF; HCRT; TAB; DSST; WRi; WRd; LARS

Table 2.

Cognitive Test	Pre-dose mean (sem)	Time Point Post Dose mean (sem)						
		1 hr	2 hr	4 hr	6 hr	8 hr	10 hr	17.5 hr
	Rested Baseline							
CFF Mean (Hz)	31.2 (0.9)	30.8 (0.8)		30.3 (0.8)		29.7 (0.7)		32.5 (0.8)
CTT MD (pixels)	8.4 (0.6)	8.1 (0.7)	8.4 (0.8)	10.1 (2.0)	12.9 (1.9)		10.5 (0.9)	8.3 (0.8)
CTT RT (ms)	652.5 (22.5)	683.2 (19.5)	694.6 (23.6)	714.0 (22.3)	726.9 (22.8)		697.9 (24.1)	666.2 (25.4)
SART (NAS)	3.1 (0.6)	4.5 (0.9)		5.1 (0.8)		5.6 (0.6)		5.9 (1.1)
RVIP (VR)	73.1 (7.1)				55.1 (5.7)			
STM RT (ms)	701.9 (42.5)	695.2 (46.0)	731.1 (45.5)	716.9 (45.1)	738.7 (36.5)	760.6 (40.0)	768.6 (43.3)	680.1 (43.3)
IWR (number correct)	11.1 (1.0)			11.9 (1.0)		10.9 (1.0)		12.1 (0.9)
DWR (number correct)	7.1 (1.1)			7.4 (1.2)		5.2 (1.0)		7.8 (1.3)
H-CRT TRT (ms)	573.0 (22.9)		559.7 (21.4)		627.5 (38.1)		609.4 (32.2)	552.2 (17.7)
DSST (number responses)	54.6 (3.2)		52.8 (3.8)		53.4 (2.9)		52.1 (2.7)	57.1 (3.2)
Trails B (ms)	11.1 (0.8)		9.9 (0.8)		10.4 (0.8)		10.9 (0.9)	9.8 (0.6)

Table 3: Treatment Related Adverse Events

Body System	Placebo		CX717 100 mg		CX717 300 mg		CX717 1000 mg	
	NAE	NS	NAE	NS	NAE	NS	NAE	NS
Total	5	5	7	7	1	1	1	1
Back Pain	1	1	1	1	0	0	0	0
Head Discomfort	1	1	0	0	0	0	0	0
Headache	1	1	1	1	0	0	0	0
Paraesthesia	1	1	1	1	0	0	0	0
Depression	1	1	0	0	0	0	0	0
Depressed Mood	0	0	0	0	1	1	0	0
Atrioventricular First Degree Block	0	0	2	2	0	0	0	0
Nausea	0	0	1	1	0	0	1	1
Dry Throat	0	0	1	1	0	0	0	0