Differential effects of a dual orexin receptor antagonist (SB-649868) and zolpidem on sleep initiation and consolidation, SWS, REM sleep, and EEG power spectra in a model of situational insomnia

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Abstract

Orexins play a role in sleep regulation and orexin receptor antagonists are under development for the treatment of insomnia. We conducted a randomised, double-blind, placebo-controlled, four-period crossover study to investigate the effect of single doses of the dual orexin receptor antagonist SB-649868 (10 or 30 mg) and a positive control zolpidem (10 mg), an allosteric modulator of $GABA_A$ receptors. Objective and subjective sleep parameters and next day performance were assessed in 51 healthy male volunteers in a traffic noise model of situational insomnia. Compared to placebo, SB-649868 10 and 30 mg increased Total Sleep Time (TST) by 17 and 31 minutes ($p<0.001$), whereas after zolpidem TST was increased by 11.0 min ($p=0.012$). Wake after sleep onset was reduced significantly by 14.7 min for the SB-6489698 30 mg dose ($p<0.001$). Latency to persistent sleep was significantly reduced after both doses of SB-6489698 ($p=0.003$) but not after zolpidem. Slow Wave Sleep (SWS) and EEG power spectra in nonREM sleep were not affected by either dose of SB-640868, whereas SWS ($p< 0.001$) and low delta activity (<=1.0Hz) were increased, and 2.25-11.0Hz activity decreased after zolpidem. REM sleep duration was increased after SB-649868 30 mg ($p=0.002$) and reduced after zolpidem ($p=0.049$). Latency to REM sleep was reduced by -20.1 ($p=0.034$) and -34.0 min ($p<0.001$) after 10 and 30 mg of SB-649868. Sleep onset REM episodes were observed. SB-649868 was well tolerated. This dual orexin receptor antagonist exerts hypnotic activity, with effects on sleep structure and the EEG that are different from those of zolpidem.

Keywords: insomnia, orexin, hypocretin, hypnotics, circadian, GABA, SB-649868.

Introduction

Insomnia is a serious health problem with an estimated prevalence ranging from approximately 4-22 % (Roth et al. 2011) and associated costs which are significant (Sarsour et al. 2011). Current pharmacological treatments of insomnia are based primarily on positive allosteric modulators of several $GABA_A$ receptor subtypes, i.e., benzodiazepines and non-benzodiazepines such as zolpidem (Nutt and Stahl 2010; Winsky-Sommerer 2009). Several novel pharmacological treatments for insomnia are under development including antagonists of the orexin 1 and orexin 2 receptors (OX1R, OX2R) (Scammell and Winrow 2011; Sullivan and Guilleminault 2009). The development of OXR antagonists for the treatment of insomnia is based on the large body of evidence supporting a role for orexin neuropeptides (also known as hypocretins) in the control of arousal and sleep/wake states (Brown *et al.* 2001; Eriksson *et al.* 2001; Eriksson *et al.* 2004; Eriksson *et al.* 2010; Hagan *et al.* 1999; Piper *et al.* 2000; Sakurai and Mieda 2011). In particular, (1) Orexin-producing neurons in the lateral hypothalamus exhibit high firing rates during wakefulness which dissipate during nonREM and REM sleep (Lee et al. 2005) and orexins activate the key neurotransmitter systems implicated in maintaining wakefulness, such as histamine, serotonin and noradrenaline, as well as cholinergic neurons (Sakurai and Mieda 2011); (2) Deficiency in the orexinergic system is associated with disruption of the sleep-wake cycle. This is observed in human narcolepsy-cataplexy, in canine and rodent animal models of human narcolepsy (i.e., knock-out (KO), in mice devoid of either the prepro-orexin precursor or the orexin receptors and in transgenic mice and rats with selective postnatal degeneration of orexin-expressing neurons) (Chemelli *et al.* 1999; Mignot 2004; niz Behn *et al.* 2010; Sakurai and Mieda 2011); (3) Orexin concentrations in CSF fluctuate with a circadian rhythm which is synchronized to the wake-sleep cycle with peaks during the day in the day-active squirrel monkey (Zeitzer et al. 2003) and during the night in the nocturnal rat (Deboer et al. 2004); (4) The circadian

variation in orexins is abolished by lesioning of the suprachiasmatic nuclei, the locus of the circadian pacemaker in mammals, which drives the circadian variation in sleep propensity and structure (Deboer et al. 2004).

To our knowledge, until recently the only data available on effects of dual orexin receptor antagonists on sleep in humans are those recently published by Brisbare-Roch and colleagues (Brisbare-Roch et al. 2007). The authors reported on the effect of almorexant on sleep propensity and the electroencephalogram (EEG) in sleep recorded during two 25 min sessions during the daytime in healthy volunteers. Thus, there are currently only very limited data available on the effects of dual orexin receptor antagonists on sleep propensity and structure, as well as on the sleep EEG in humans. Furthermore, it is unknown how such effects compare to the effects of allosteric modulators of $GABA_A$ receptors, which are currently the most commonly prescribed hypnotics for the treatment of insomnia.

SB-649868 is a potent, orally acting, selective OX1/OX2 receptor antagonist under investigation for the treatment of insomnia. Studies in rodent and primate models have demonstrated sleep-promoting effects and lack of motor impairment following administration of SB-649868 (Gerrard et al. 2009). In healthy volunteers, SB-649868 was shown to be safe and well-tolerated at doses up to 80 mg, with mechanism-related adverse events (e.g., somnolence and fatigue) observed in a majority of subjects after 60 and 80 mg single doses. Evening administration of doses up to 60 mg to healthy volunteers without sleep complaint and under normal sleeping conditions resulted in significant dose-dependent decrease in latency to persistent sleep, and wake after sleep onset, and increase in total sleep time as measured by polysomnography (Bettica *et al.* 2011).

We here assess and compare the effect of SB-649868 on sleep structure and the spectral composition of the sleep EEG and those of zolpidem, a non-benzodiazepine

allosteric modulator of $GABA_A$ receptor subtypes, relative to placebo, in a validated model of situational insomnia.

Materials and Methods

This was a randomised, double-blind, double dummy, placebo-controlled, four-period crossover study (Study code: GSK104094; ID# NCT00440323 on http://ClinicalTrials.gov) to investigate the effect of single oral doses of SB-649868 (10 or 30 mg) and of a positive control (zolpidem, 10 mg) in a model of noise induced situational insomnia in healthy male volunteers (Cluydts et al. 1995). In this model disruption of sleep maintenance and initiation is induced by standardized traffic noise and efficacy of hypnotics can be assessed reliably (Cluydts *et al.* 1995; Dijk *et al.* 2007; Dijk *et al.* 2011);

The study was conducted at a single site (Surrey Clinical Research Centre, University of Surrey) and the protocol was approved by Ravencourt Ethics Committee, which is a Phase 1 Research Ethics committee. The primary objective of the study was to determine the effect of SB-649868 on total sleep time, a measure of hypnotic efficacy comprising both sleep initiation and sleep maintenance elements, in healthy volunteers undergoing a noise-disturbed sleep model. Secondary objectives were to study: a) the changes induced by SB-649868 and zolpidem on other polysomnography (PSG) sleep parameters such as the nonREM sleep stages 1,2, 3, 4, REM sleep, the number of awakenings as well as the spectral composition of the sleep EEG; b) to investigate the effects of SB-649868 and zolpidem on subjective sleep quality, on the morning after dosing and on the following 3 days; c) to investigate the effects of SB-649868 and zolpidem on daytime cognitive functioning on the morning following dosing, and d) to investigate the safety and the pharmacokinetic profile of SB-649868 and zolpidem in healthy volunteers. Under the conditions and dose range used in the present study SB-649868 has been reported to have a half-life in the range of 3.4 h to 3.9 h (Bettica *et*

al. 2011). In this report we will focus on the primary outcome measures, as a prime indicator of hypnotic efficacy, as well as on the sleep related secondary outcome variables.

Population: All subjects provided written informed consent to participate in the study and were screened approximately 28 days prior to treatment. Fifty-one subjects entered the study; 44 subjects completed the study while 7 subjects prematurely discontinued. Subjects were healthy adult males aged 18 – 55 years inclusive with a body weight ≥50 kg and a body mass index within the range 18.5 – 29.9 kg/m^2. Subjects were to have a history of going to bed from 22:00 to 00:00 on at least 5 –7 nights per week with a reported nightly sleep duration of 6.5 – 8.5 h over the previous 3 months or more at Screening. Subjects who had consumed beverages and medications that could interfere with the effects of treatments or study assessments were excluded. Other key exclusion criteria were sleep apnoea and periodic limb movements' disorders and other sleep disturbances, as assessed during a clinical polysomnographic screening, sleep complaints, and a recent history of or current shift work. Subjects who were not prepared to use protocol-specified methods of contraception or sexual abstinence, as appropriate, could also not be included. All subjects had to have laboratory values, vital signs and electrocardiographic (ECG) values within the normal reference range.

Study Design and Treatments: The study consisted of four treatment sessions. Each treatment session consisted of 2 nights (Fig.1). Subjects were admitted to the unit on Day 1 at approximately 16:00 in the afternoon, prior to the first night (Night 1), and underwent admission procedures. At approximately 18:00, subjects were served a standard light snack, and at approximately 21:00 a standardised dinner was served. At approximately 22:30 they retired to bed with lights off at 23:00 and lights on at 07:00. During Night 1, study medication was not administered and subjects underwent PSG monitoring only (no noise disturbance).

During the second night (Night 2) for all sessions, after a standard light snack at approximately 18:00 and a standardised dinner at approximately 21:00, randomised subjects were administered two double-blinded doses of study medication. Thirty minutes after a standard dinner, at approximately 21:30, subjects received either placebo or SB-649868 (10 mg or 30 mg). This timing of administration was chosen because within the used dose range and administered in the fed state average t_{max} is approximately in the 2.5-2.75 h range (Bettica *et al.* 2011). At approximately 22:30 subjects received either placebo or zolpidem (10 mg). Subjects retired to bed at 22:30 with lights off at 23:00 (Fig. 1).

During Night 2, subjects underwent PSG monitoring, blood sampling for pharmacokinetic analysis (before lights off) and were exposed to the pre-recorded traffic noise. Calibrated noise lasted from lights off (23:00) continuously until lights on (07:00).

All subjects remained in the unit during each of the treatment sessions. Subjects were discharged at the end of each treatment session when all study procedures had been completed at the discretion of the Investigator depending on the nature of any ongoing adverse events. During the study, each single-dose treatment was separated by a 7-day washout period (±1 day) and preferably occurred on the same day of the week, or were consistently held on either workdays or days of rest, for any given subject. Subjects were instructed to maintain a normal pattern of sleep between 23:00 and 07:00 when outside the unit between treatment sessions. Compliance with the maintenance of a regular sleep-wake cycle instruction was monitored by actigraphy recordings for the duration of the study. Subjects were instructed to return to the unit within 7 – 14 days of the last dose of study treatment for the follow up visit.

EEG/PSG recordings: Nine EEG channels (C4-A1, C3-A2, O2-A1; O1-A2, Fpz-A1,Fz-A1,Cz-A1, Oz-A1, Pz-A1), two electrooculogram (EOG) channels and one submental electromyogram (EMG) channel, using gold electrodes were recorded onto a Compumedics

Siesta digital EEG machine. Signals were digitized at 256 (EEG, EMG) or 128 Hz (EOG/ECG) and stored at 128 Hz for the EEG and EOG/EMG. The low and high frequency filters were set at 0.3 and 70 Hz for the EEG/EOG and at 10 and 70 Hz for the EMG. A single channel ECG was also recorded. For the PSG screening night, this montage was complemented by recordings from the left and right Anterior Tibialis muscle, recording of nasal/oral airflow, thoracic and abdominal effort, body position, tracheal microphone and oximetry. All PSG were scored according to the standard criteria of Rechtschaffen and Kales (Rechtschaffen and Kales 1968). Epochs annotated as artefacts were excluded from the analyses of the EEG power spectra by visual inspection of the records while blind to treatment. Nights were only included in the spectral analysis if at least 5 hours of artefact free data were available. EEG spectral analysis was performed on the C3-A2 and C4-A1 channel using a fast Fourier transform, as previously described (Dijk *et al.* 2010). Briefly, EEG power spectra were computed per 4 s epochs and the 0.25 Hz spectra were collapsed into 1Hz bins. Average spectra were computed separately for nonREM sleep (encompassing stages 1-4) and REM sleep for C3-A2 or, if this channel was not available, C4-A1.

Assessments: Treatment effects on sleep duration, maintenance and initiation were assessed by quantifying Total Sleep Time (TST), Wake After Sleep Onset (WASO), Number of Awakenings (NAW), Sleep Efficiency (SE), Latency to Persistent Sleep (LPS) and Latency to REM sleep as measured with PSG. In addition we assessed effects on sleep structure, i.e., REM sleep and nonREM sleep and its sub-stages 1,2,3,4 as well as Slow Wave Sleep (SWS) which is the sum of stage 3 and 4. Sleep questionnaires were used to assess subjective TST, subjective WASO, subjective NAW, subjective sleep latency, and sleep quality. A computerised battery of standard tests was used to assess treatment effects on subjective alertness, mood, working/short-term memory, long-term memory, attention/executive functions, and motor control. The battery, comprising three blocks of

tests, was used on the morning after dosing, beginning approximately 30 minutes after waking (i.e.,10 h post-dose for SB-649868 and 9 h post-dose for zolpidem), and ending approximately 90 minutes later. While the battery enables assessment of a wide range of functions, here we report the effects on two widely used tests of residual effects: Psychomotor Vigilance Task (PVT), which lasted for 10 minutes and Digit-Symbol Substitution (DSST), which was administered in each of the three test blocks.

Treatments safety was assessed by collecting adverse events, vital signs, 12-lead ECG and clinical laboratory assessments. Plasma concentrations were assessed at different time-points after the administration of both SB-649868 and zolpidem.

Statistical analysis: A sample size of 46 subjects completing the study was planned as it would have provided 80% power to detect a difference between SB-649868 and placebo of at least 22 minutes in total sleep time, assuming a within-subject standard deviation of about 36.8 minutes. A pre-specified interim analysis was performed when approximately half of the subjects had completed the study and showed that no sample size adjustment was required, because the variability of the data collected was in line with that expected.

Available data from withdrawals were used for all statistical analyses, as planned in the protocol and as properly managed by the mixed models applied. For the final analysis, a mixed-effects model was applied on PSG data of the 2nd nights, with period and treatment as fixed effects and subject as random effect. Estimates for mean treatment differences of SB-649868 compared with placebo and with zolpidem were derived. Similar estimates were provided for zolpidem compared with placebo. Tests of significance were performed at the 5% level.

Subjective Sleep Questionnaire data were also analysed in this mixed model with the pre-dose' values included as a 'baseline' covariate.

Measures of DSST and PVT underwent an exploratory analysis of covariance (ANCOVA). Cognitive performance after the night with No-Noise Exposure (baseline) was used as a covariate, period and treatment as fixed effects and subject as random effect. Estimates for mean treatment differences of SB-649868 compared with placebo were derived.

In an exploratory analysis of the effects of treatment on EEG power spectra, nonREM and REM sleep spectra in each of the three treatments were expressed as a percentage of the placebo condition for each individual and geometric means and 95% confidence intervals were computed for each of the conditions.

Safety data were summarised by means of descriptive statistics.

Results

Subject disposition

Fifty-one healthy men were recruited, and received at least one treatment. Forty four subjects completed the study as planned. Baseline characteristics of the subjects are reported in Table 1.

Effects on sleep maintenance and initiation

A summary of the effects of placebo, of SB-649868 (10 and 30 mg) and of zolpidem (10 mg) is presented in Table 2.

Although a baseline no-noise condition was not recorded in this study, sleep in the placebo condition showed the disruptive effects of the model on sleep, i.e., a long LPS and relatively low sleep efficiency (87%), reflecting a TST of only 419 min. When compared to placebo both doses of SB-649868 significantly increased TST by 17 and 31 min for the 10 mg and 30 mg dose respectively. A significant increase in TST was also observed after zolpidem, although to a lower extent (+11 min). The increase in TST after SB-649868 10 mg was not significantly different from zolpidem, but the increase after SB-649868 30 mg was

statistically significantly greater than after zolpidem. The significant changes in TST were accompanied by significant changes in SE (%). WASO was significantly reduced for the SB-648969 30 mg dose, but not so for the other active treatments, although the improvement of around 7 min obtained with the 10 mg dose was already of the same extent of that estimated for zolpidem. The number of awakenings was not significantly different from placebo for any of the treatments. LPS was significantly reduced after both doses of SB-648969 (of -8.5 and -17.4 min at 10 mg and 30 mg respectively) while the effect of zolpidem on LPS was not statistically significant.

Effects on Sleep Structure and the EEG

All-Night Measures: SB-649868 and zolpidem had different effects on sleep architecture. When analysed as % of sleep period time, SB-649868 did not affect nonREM sleep with the exception of a significant prolongation of Stage 1 observed for SB-649868 10 mg. By contrast, zolpidem significantly increased the % of sleep period time spent in Stage 3 and 4 and SWS. Furthermore, with regard to REM sleep SB-640868 30 mg significantly increased the % of sleep period time spent in REM sleep, while a significant reduction in % sleep period time spent in REM sleep was observed with zolpidem 10 mg. Latency to REM sleep was reduced after both doses of SB-649868. Analysis of the distribution of REM latencies is presented in Figure 2. Sleep onset REM latencies, i.e., intervals between sleep onset and the first occurrence of REM sleep less than or equal to 15 minutes were observed for SB-640868 10 mg (n=1) and 30 mg (n=2), but not for placebo (n=0) or zolpidem (n=0).

Analyses per Third of Sleep Episode. The effects of the treatment on REM sleep and SWS were also analyzed per third of the sleep episode. REM sleep increased from the first to the final third whereas SWS decreased in all conditions (Fig. 3). In the first third REM sleep was increased compared to placebo for both SB-649868 10 and 30 mg. In the second third a

significant increase compared to placebo was only observed after SB-649868 30 mg and no significant differences from placebo were observed for REM sleep in the final third.

In the first third of the sleep episode, SWS was enhanced compared to placebo after both SB-649868 30 mg and zolpidem. In the second third a significant increase of SWS was only observed after zolpidem and in the final third no statistically significant differences from placebo were present.

SB-649868 did not alter the EEG power density in either nonREM sleep (Fig. 4) or REM sleep (data not shown), i.e., all values were close to placebo. In contrast, during nonREM sleep zolpidem increased EEG power density in the 0.25-1.0 Hz bin while a decrease was observed for frequencies between 2.25 and 11Hz (Fig. 4).

Subjective sleep measures

Subjective sleep measure assessed in the morning following baseline sleep (i.e., no noise) and the morning following noise exposure are presented separately for placebo, SB-649868 (10 and 30 mg) and of zolpidem (10 mg) in Table 3. Exposure to the noise model was associated with an increase in subjective sleep latency, and the number and duration of night awakenings and a reduction in subjective total sleep time in particular in the placebo condition. The effects of the treatments on subjective sleep assessments were quite variable although all treatments appeared to counter the negative effects of the noise model to some extent. Significant improvements compared to placebo were only observed for subjective sleep latency ($p=0.029$) and subjective total sleep time ($p=0.008$) after treatment with the SB-649868 30 mg dose.

Performance

Performance across all tests was consistently high, indicating that participants were well motivated.

Digit Symbol Substitution Test.

Performance on the DSST is summarized by presenting the number of correct responses during the three assessments at baseline and following noise exposure, separately for the three treatments (Table 4). Mean and median numbers of correct responses were consistently high. Median correct responses varied from 14-15 whereas the median number of attempted response varied from 15-16. ANCOVA did not detect any significant differences from placebo.

Psychomotor Vigilance Task

Performance on the PVT was summarized by presenting the number of lapses (response time > 500 ms) and the median response times (Table 5). For lapses some indication of an effect of treatment was present in the SB-649868 30 mg condition in which according to the ANCOVA the mean number of lapses was significantly greater than in the placebo condition ($p=0.012$). The median number of lapses was 1 in all conditions. The average median response time was short in all conditions, with some indication of an increase in the average median response time in the SB-649868 30 mg condition ($p=0.013$ *vs.* placebo). However, also in the SB-649868 30 mg condition the median of the median response time was identical to placebo.

Adverse Events

Overall, the frequency of adverse events was similar for the active treatment groups and placebo. Table 6 reports the most frequent adverse events, i.e., those reported by at least 5% of the subjects. More than twice as many subjects reported somnolence and disturbance of attention after SB-649868 30 mg compared to placebo. On the other hand, headache was reported in more than twice as many subjects after placebo compared to the other treatments and insomnia was reported in more than twice as many subjects after placebo compared to SB-649868 30 mg. Most of the adverse events were mild or moderate in intensity. Only one subject experienced an adverse event of severe intensity (severe somnolence after receiving

SB-649868 30 mg that was judged by the Investigator to be related to investigational product). One subject reported a mild hallucination which started 90 minutes after the administration of SB-649868 30 mg and lasted 2 hours; the adverse event recovered and the subject continued the study. There were no clinically relevant abnormalities in urinalysis, 12-lead ECG and vital signs parameters.

Discussion

This is a first report of the effects of the orexin receptor 1 and 2 antagonist SB-649868 on sleep and the EEG in a traffic noise model of situational insomnia. Sleep under placebo conditions showed the characteristic disruptive effect of this validated model (Cluydts et al. 1995) on PSG assessed sleep initiation and total sleep time as well as subjective measures of sleep initiation. These disruptive effects were countered to some extent by the active treatments. Both doses (10 and 30 mg) of SB-649868 showed significant hypnotic efficacy as indexed by a significant increase in total sleep time and a reduction in the latency to persistent sleep. The effects on total sleep time are not only due to the reduction in the latency to sleep onset because wakefulness after sleep onset was reduced after SB-649868 30 mg. Thus these data indicate that SB-649868 has positive effects on both sleep initiation as well as sleep maintenance, as indexed by wake after sleep onset. Number of awakenings, on the other hand, was not affected by SB-649868. The effects observed in this study are in line with those observed in healthy volunteers in normal sleeping conditions (Bettica *et al.* 2011). Also in that case SB-649868 did not affect the number of awakenings. As we cannot say whether noise disturbance significantly disrupted number of awakenings, we cannot conclude an SB-649868 effect on number of awakenings. The positive control (zolpidem 10 mg) showed its well established hypnotic efficacy as indexed by an increase in total sleep time. Interestingly, the effect on total sleep time was greater for SB-649868 30 mg than zolpidem. Zolpidem did

not improve sleep initiation in this study, but, compared to SB-649868 30 mg, led to a greater reduction in the number of awakenings. The effects of dual orexin receptor antagonists on sleep initiation and total sleep time are likely to be mediated by a reduced orexinergic drive on several neuronal populations known to play a key role in the transitions between wakefulness and sleep and to be densely innervated by the orexin-producing neurons. These include the monoaminergic noradrenergic neurons of the locus coeruleus and serotonergic neurons of the raphe nuclei, expressing respectively OX1R and OX1R/OX2R, as well as the histaminergic neurons located in the tuberomammillary nucleus via OX2R (Fort *et al.* 2009; Mieda *et al.* 2011). Thus, dual orexin receptor antagonists may exert their effects by reducing the excitatory action of orexins on these wake-promoting neurostransmitter systems, resulting in a decreased monoaminergic tone, which in turn will reduce cortical activation and an increased sleep propensity. In addition, dual orexin receptor antagonists may exert their effects on cholinergic neurons of the basal forebrain and of the latero-dorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei that are part of the arousal-promoting circuits (Fort *et al.* 2009). Zolpidem acts through a positive allosteric modulation of $GABA_A$ receptors, showing a preferential affinity for the α1-$GABA_A$ receptor subtype and lower affinity to α2- and α3-$GABA_A$ receptor subtypes (Mohler 2006; Winsky-Sommerer 2009). Whether and how these differential effects on total sleep time and number of awakening relate to the different roles of these specific $GABA_A$ subtypes and the orexinergic system in 'sleep state switching' (Fort *et al.* 2009; Saper *et al.* 2010) cannot be determined from our data.

The effects on sleep structure differed between the two compounds such that the percentage of sleep period spent in the SWS stage was enhanced after zolpidem and not affected by SB-649868, whereas the percentage of sleep period spent in REM sleep was enhanced after SB-649868 and reduced after zolpidem. SB-649868 enhanced REM sleep

propensity as evidenced by both an increase in REM sleep duration and a reduction in REM latency. Enhancement of visually scored SWS by zolpidem has been reported previously (Kanno et al. 2000). In this study, zolpidem led to an increase in EEG activity in the 0.25-1.0 Hz range and a reduction in higher delta and theta frequencies, consistent with previous reports (Brunner *et al.* 1991; Dijk *et al.* 2010). The lack of an effect of SB-649868 on EEG power spectra in nonREM sleep in the present study can therefore not be attributed to a lack of sensitivity of spectral measures in this model of situational insomnia. Suppression of REM sleep by benzodiazepines is also well documented although for zolpidem and other non-benzodiazepine allosteric modulators of the $GABA_A$ receptor this effect is not always observed (Lancel 1999;Mohler 2006). Effects of dual orexin receptor antagonists on REM sleep have been previously shown in animals (e.g., Brisbare-Roch *et al.* 2007; Gerrard *et al.* 2009) and humans possibly mediated at least in part by their effects on LDT/PPT cholinergic neurons implicated in REM sleep regulation (Sakurai and Mieda 2011). This would be in accordance with previous studies in cats showing that microinjection of orexin-A in the LDT significantly suppressed REM sleep (Xi *et al.* 2001). In addition, REM-off noradrenergic and serotonergic neurons, located respectively in the locus coeruleus and the raphe nuclei, are known to be implicated in the control of the gating to REM sleep (Luppi *et al.* 2011), and to display high expression levels of OX1R and OX1R/OX2R respectively (Mieda *et al.* 2011). Thus, SB-649868 is likely to suppress serotonergic and noradrenergic transmission that would counteract REM sleep suppression by, in part, reducing the noradrenergic excitation of REM-off neurons in the dorsal deep mesencephalic reticular nucleus (Crochet *et al.* 2006). Furthermore, a recent study suggested that suppression of REM sleep by orexin-A involves both O1X and OX2 receptors (Mieda *et al.* 2011). Thus the effects of the dual orexin receptor antagonist on sleep structure as assessed by visual scoring of polysomnographic records, is very different from the effects of zolpidem even though both compounds have hypnotic

effects. Quantitative EEG analysis of the nonREM EEG further differentiated the effects of these compounds. While zolpidem altered the nonREM sleep EEG in accordance with the extensively described benzodiazepine EEG fingerprint shared by non-benzodiazepine compounds, SB-649868 did not induce noticeable changes in the EEG power density.

Overall, our results suggest that the dual orexin antagonist receptor SB-649868 exerts direct or indirect effects on several transmitter systems involved in the transitions between vigilance states, while it does not -at least at the 10 and 30 mg doses- alter neuronal networks involved in the generation of EEG rhythms.

The characteristic dynamics of sleep structure across the sleep episode when sleep occurs during the night, i.e., a decline of SWS and an increase in REM sleep (Dijk and Czeisler 1995) was preserved in all conditions indicating that major regulatory process underlying sleep are not significantly disrupted by either the traffic noise model or the pharmacological treatments.

The distribution of REM sleep latencies as observed after SB-649868 is reminiscent of the bimodal distribution which has been observed in disorders such as depression and has been successfully simulated based on mathematical models of REM sleep regulation (Beersma et al. 1984).

Some neuronal pathways involved in the control of autonomic function, as well as several regions of the limbic system, associated with stress, emotions and anxiety, provide neuronatomical and physiological input onto orexinergic neurons (Yoshida *et al.* 2006) and may contribute to hyper-arousal associated with insomnia and orexin antagonists may reduce this arousal. We note that a limitation of the traffic noise insomnia model is that some of the arousal circuits activated may be different from those activated in insomnia.

The Digit Symbol Substitution Task is a sensitive task to assess residual effects (e.g., Boyle *et al.* 2009; Stone *et al.* 2002). In the present study neither of the two doses of SB-

649868, nor 10 mg of zolpidem induced residual effects on the DSST. The absence of an effect of zolpidem is in accordance with previous studies (Hindmarch et al. 2001). Performance on the psychomotor vigilance task (PVT), a task used extensively to document the negative effects of sleep loss (e.g., Lim and Dinges 2008) did also not indicate major residual effects, although for the SB-649868 30 mg dose some indication for reduced performance on this task was observed.

Both SB-649868 and zolpidem were well tolerated. Adverse events which were most frequent and which occurred, more often after SB-649868, were related to its pharmacological effect. This result is in line with what previously reported in healthy volunteers who received SB-649868 in the morning, after lunch or after dinner (Bettica *et al.* 2011). Somnolence was reported by 29% of subjects treated with SB-649868 30 mg. This is not unexpected considering the potent sleep-inducing effect of SB-649868, the fact that dosing was timed to maximize the hypnotic effect, i.e., subjects were dosed 90 minutes before bedtime, and that circulating levels of SB-649868 were still relatively high at lights on (data not shown). Future studies should explore different doses and dosing regimens of the drug both in healthy subjects and in the target patient population.

Narcolepsy/cataplexy could be a possible consequence of orexin antagonism based on preclinical and human data suggesting a deficiency of the orexinergic system in narcolepsy (Chen et al. 2005). Symptoms common in narcolepsy/cataplexy are hallucinations, sleep paralysis, cataplexy and sleep onset REM episodes (SOREM) (Narcolepsy fact sheet, National Institute of Neurological Disorders and Stroke. http://www.ninds.nih.gov/disorders). One mild hallucination was reported by a subject treated with SB-649868 30 mg and SOREM episodes were reported only after the administration of SB-649868. It should be noted that

also in this case the timing of drug administration two hours before lights out, may have artificially increased the risk of hallucinations and SOREM episodes. Future studies in patients and with different timing of dosing will be needed to assess the risk of SB-649868 to induce narcolepsy/cataplexy.

Overall, these data indicate that the dual orexin receptor antagonist SB-649868 has significant hypnotic effects in a model of situational insomnia, with effects on REM sleep, SWS and the EEG that are different from those of zolpidem. These differences may be related to the different mechanisms of action of these two compounds.

Disclosure of Interest

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Table 1. Baseline Characteristics of recruited subjects

Demographic parameter	N = 51
Age in Years, Mean (standard deviation)	28.9 (8.77)
Sex, n (%) Male:	51 (100)
White, n (%)	39 (76)
Body mass index in kg/m^2, Mean (standard deviation)	24.08 (2.371)
Height in cm, Mean (standard deviation)	177.0 (6.856)
Weight in kg, Mean (standard deviation)	75.63 (10.177)

Table 2. PSG Sleep Parameters

PSG Parameter	Mean (SD)				Estimated difference *vs* Placebo (p-value)			Estimated difference *vs*Zolpidem (p-value)	
	Placebo	SB-649868 10 mg	SB-649868 30 mg	Zolpidem	SB-649868 10 mg	SB-649868 30 mg	Zolpidem	SB-649868 10 mg	SB-649868 30mg
	n=45	n=45	n=45	n=43					
LPS (min)	27.2	18.8	9.0	22.3	-8.5	-17.4	-3.8	-4.7	-13.6
	(23.1)	(19.2)	(10.0)	(10.9)	(0.003)	(<0.001)	(0.180)	(0.099)	(<0.001)
NAW[1] (#)	21.2	20.2	22.0	18.2	+0.1	+1.4	-2.5	+2.6	+3.9
	(11.5)	(9.2)	(14.4)	(12.1)	(0.956)	(0.294)	(0.069)	(0.064)	(0.005)
REM (%)	18.3	19.2	20.8	17.1	+0.8	+2.3	-1.4	+2.3	+3.7
	(4.0)	(4.6)	(4.5)	(5.0)	(0.242)	(0.002)	(0.049)	(0.002)	(<0.001)
SE (%)	87.2	90.7	93.9	89.9	+3.5	+6.4	+2.3	+1.2	+4.1
	(7.9)	(7.1)	(4.4)	(6.5)	(<0.001)	(<0.001)	(0.012)	(0.180)	(<0.001)
ST1 (%)	10.4	10.9	10.7	10.0	+1.1	+0.6	+0.0	+1.1	+0.5
	(4.7)	(6.0)	(6.7)	(6.2)	(0.039)	(0.290)	(0.969)	(0.046)	(0.316)
ST2 (%)	50.7	50.6	51.1	49.6	-0.9	0.1	-1.4	+0.6	+1.5
	(10.5)	(8.8)	(10.6)	(8.6)	(0.396)	(0.951)	(0.161)	(0.578)	(0.144)
ST3 (%)	5.6	5.9	5.9	6.4	+0.2	+0.2	+0.7	-0.6	-0.5
	(2.4)	(2.3)	(2.4)	(2.9)	(0.637)	(0.585)	(0.046)	(0.128)	(0.142)
ST4 (%)	7.0	7.1	7.1	11.5	+0.3	+0.2	+4.5	-4.2	-4.3
	(6.6)	(5.8)	(5.4)	(6.7)	(0.554)	(0.738)	(<0.001)	(<0.001)	(<0.001)
SWS (%)	12.5	12.9	13.0	17.9	+0.5	+0.4	+5.2	-4.7	-4.8
	(7.7)	(6.8)	(6.5)	(7.1)	(0.428)	(0.539)	(<0.001)	(<0.001)	(<0.001)
TST (min)	418.9	435.9	451.2	431.9	+16.8	+30.7	+11.0	+5.8	+19.7
	(38.0)	(34.0)	(21.2)	(31.3)	(<0.001)	(<0.001)	(0.012)	(0.185)	(<0.001)
WASO (min)	37.3	30.2	21.6	29.5	-6.5	-14.7	-6.8	+0.4	-7.9
	(27.0)	(26.5)	(19.8)	(29.5)	(0.102)	(<0.001)	(0.088)	(0.931)	(0.050)
REM latency (min)	142.9	121.6	106.9	139.6	-20.1	-34.0	-0.6	-19.5	-33.4
	(57.3)	(54.6)	(48.6)	(58.5)	(0.034)	(<0.001)	(0.949)	(0.043)	(<0.001)

(#): NAW[1]= Number of awakenings after sleep onset

Table 3. Subjective Sleep Parameters

	[n]; Mean (SD)				
Variable	Night	Placebo	SB-649868 10 mg	SB-649868 30 mg	Zolpidem 10 mg
S-Sleep Latency (min)	Baseline	[38] 17.3 (12.3)	[39] 18.7 (20.6)	[39] 18.2 (12.6)	[36] 17.3 (12.0)
	Noise Exposure	[39] 39.9 (35.2)	[40] 50.0 (84.3)	[38] 20.7 (13.3)	[34] 38.4 (36.6)
S-Total Sleep Time (min)	Baseline	[46] 453.6 (21.7)	[47] 446.1 (48.4)	[46] 449.2 (29.7)	[45] 452.9 (22.4)
	Noise Exposure	[45] 380.1 (94.2)	[48] 395.5 (97.6)	[46] 413.3 (79.0)	[45] 394.5 (93.0)
S-Number of Awakenings	Baseline	[46] 1.5 (1.3)	[47] 1.7 (2.1)	[46] 1.5 (1.4)	[45] 1.4 (1.3)
	Noise Exposure	[46] 3.6 (4.4)	[48] 3.2 (3.0)	[47] 3.4 (3.2)	[44] 2.8 (2.1)
S-Wake After Sleep Onset (min)	Baseline	[42] 11.5 (13.6)	[42] 12.0 (20.7)	[41] 14.6 (19.4)	[39] 10.0 (11.8)
	Noise Exposure	[38] 54.8 (59.2)	[42] 37.6 (41.5)	[38] 44.0 (56.4)	[35] 35.3 (42.4)

Table 4. Digit Symbol Substitution Test; Number Correct: [n]; Mean/Median (SD)

Night/Measurement	Placebo	SB-649868 10 mg	SB-649868 30 mg	Zolpidem 10 mg
Baseline (1)	[46] 14.8/15 (1.4)	[48] 14.3/14 (1.7)	[48] 14.5/14 (1.8)	[45] 14.5/14 (1.7)
Baseline (2)	[46] 14.3/14 (1.5)	[48] 14.0/14 (1.4)	[48] 14.1/14 (2.2)	[45] 14.3/14 (1.8)
Baseline (3)	[45] 14.2/14 (1.5)	[48] 14.2/14 (1.6)	[48] 13.9/14 (1.9)	[45] 14.3/14 (1.6)
Noise Exposure (1)	[46] 14.5/14.5 (1.7)	[48] 14.5/14 (2.0)	[48] 14.3/14 (1.7)	[45] 14.8/15 (1.5)
Noise Exposure (2)	[46] 14.5/14 (1.5)	[48] 14.5/14 (1.6)	[48] 14.4/14 (1.5)	[45] 14.4/14 (1.8)
Noise Exposure (3)	[46] 14.5/14 (1.6)	[48] 14.7/15 (1.4)	[47] 14.3/15 (1.8)	[45] 14.4/15 (1.9)

Table 5. Psychomotor Vigilance Task: [n]; Mean/Median (SD)

Variable	Night	Placebo	SB-649868 10 mg	SB-649868 30 mg	Zolpidem 10 mg
Lapses (number)	Baseline	[45] 2.0/1 (3.1)	[48] 2.3/1 (3.3)	[48] 2.8/1 (3.8)	[45] 2.3/1 (3.4)
	Noise Exposure	[46] 2.2/1 (3.2)	[48] 1.9/1 (2.9)	[47] 4.7/1 (8.4)	[45] 2.1/1 (2.9)
Median Reaction Time (ms)	Baseline	[45] 268/266 (37)	[48] 267/264 (30)	[48] 276/266 (39)	[45] 270/262 (40)
	Noise Exposure	[46] 268/263 (37)	[48] 269/263 (35)	[47] 286/263 (59)	[45] 267/258 39

Table 6. Summary of Adverse Events occurring in at least 5% of subjects

Preferred Term	Placebo (N=46)	10 mg SB649868 (N=48)	30 mg SB649868 (N=48)	10 mg Zolpidem (N=45)
Fatigue	9 (20%)	6 (13%)	10 (21%)	11 (24%)
Vessel puncture site haematoma	3 (7%)	2 (4%)	4 (8%)	4 (9%)
Somnolence	2 (4%)	4 (8%)	14 (29%)	3 (7%)
Headache	4 (9%)	1 (2%)	2 (4%)	1 (2%)
Disturbance in attention	1 (2%)	0 (0%)	3 (6%)	1 (2%)
Insomnia	4 (9%)	6 (13%)	1 (2%)	3 (7%)
Hypoglycaemia	3 (7%)	2 (4%)	5 (10%)	4 (9%)

Fig1. Schematic representation of the protocol. This was a four-period crossover study, each period consisting of two consecutive nights (Night 1: adaptation; Night 2: treatment). During Night 2 for all sessions, randomised subjects were administered two double-blinded doses of study medication (i.e., either placebo or SB-649868 (10 mg or 30 mg); or either placebo or zolpidem 10 mg), while exposed to the traffic noise model of situational insomnia

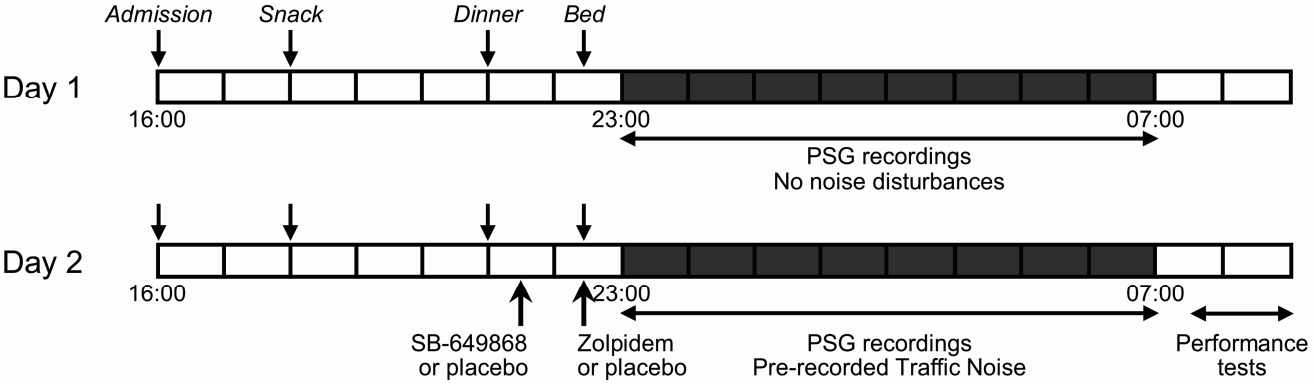

Fig2. Distribution of latency to REM sleep during the four conditions.

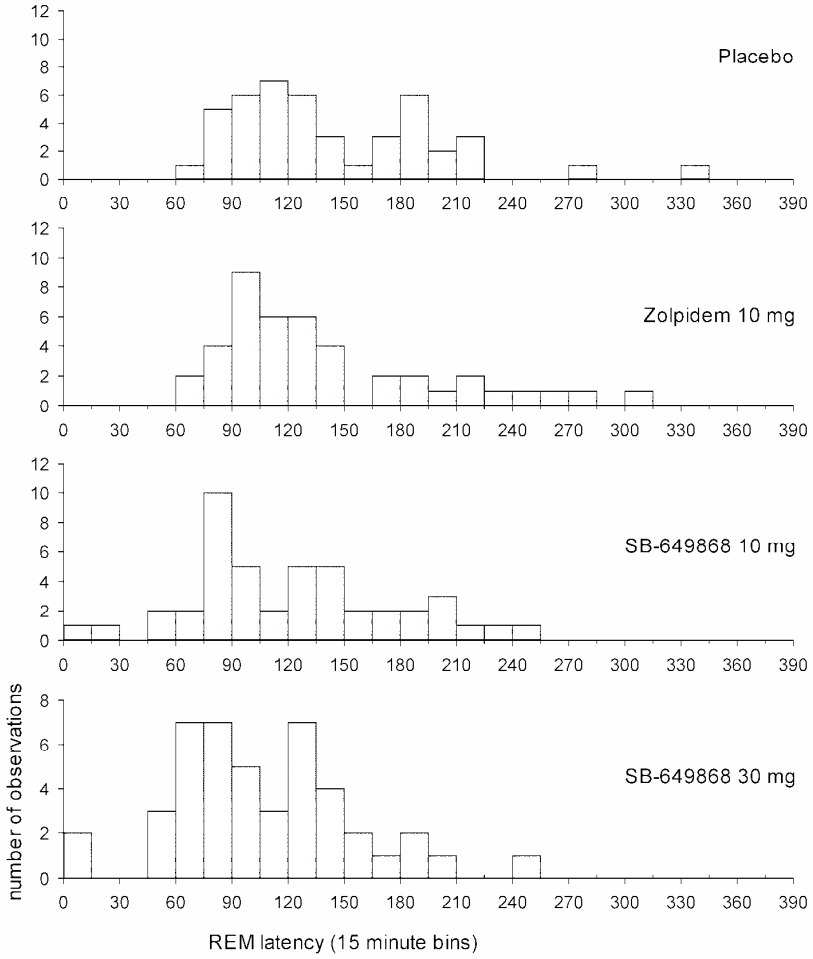

Fig 3. REM sleep and SWS per third of sleep episode during noise exposure after treatment with placebo, 10 mg SB-649868, 30 mg SB-649868 and 10 mg zolpidem. Error bars indicate 1 SEM. * P<0.05; § P<0.01; # P<0.001, Compared to placebo

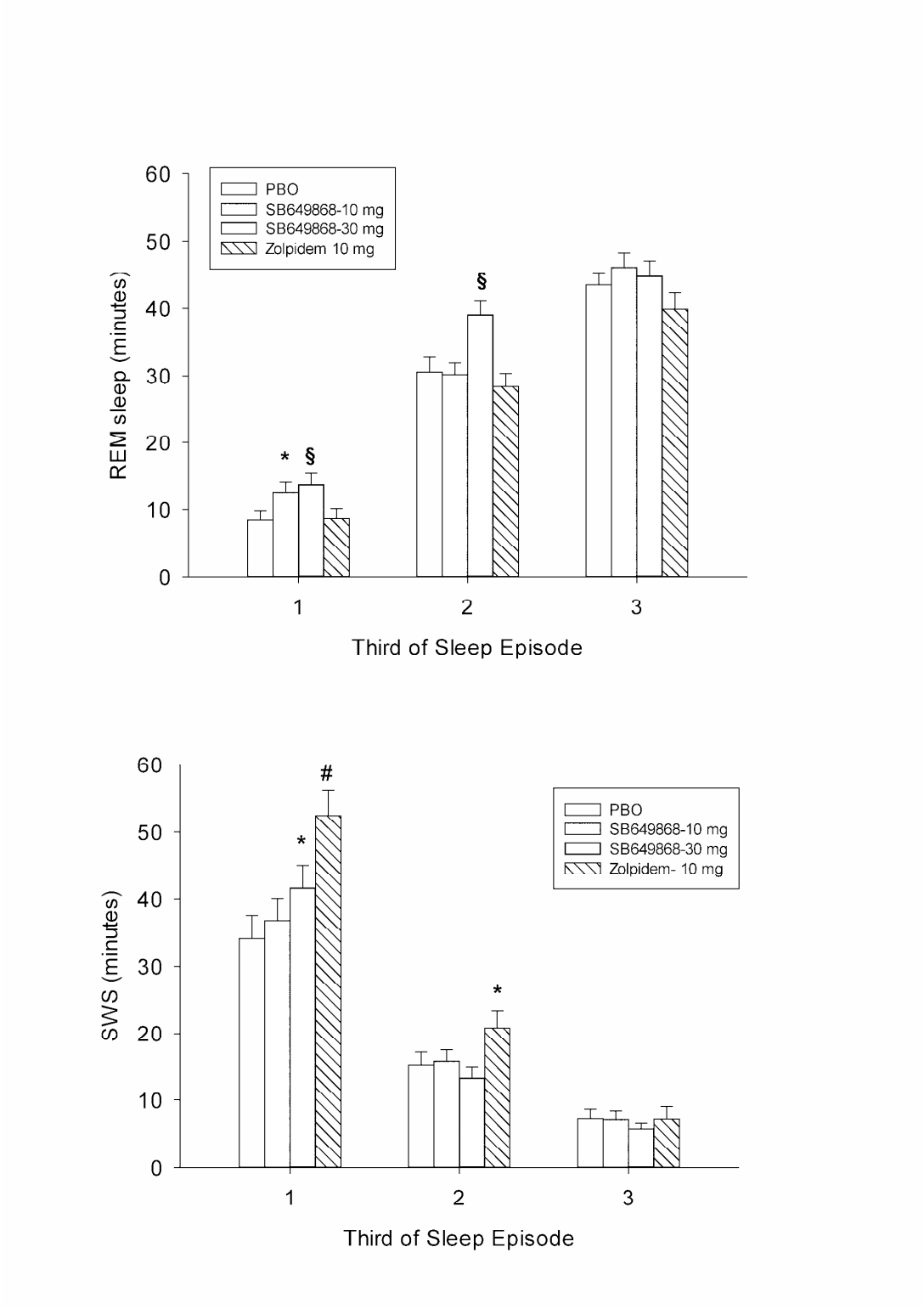

Fig 4. Effect of SB-649868 (10 and 30 mg) and zolpidem (10 mg) on EEG power density spectra during non REM sleep. All data are geometric means expressed relative to placebo (=100%). Vertical bars indicate 95% confidence intervals. 36-38 subjects contributed to each of the comparisons for frequencies up to 15 Hz. Data are plotted at the upper limits of the frequency bins. Thus, values plotted at 1 Hz represent the 0.25-1.0 Hz bin.