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# Randomised clinical trial of the effects of prolonged-release melatonin, temazepam and zolpidem on slow-wave activity during sleep in healthy people

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## Abstract

Current pharmacological treatments for insomnia include benzodiazepine and non-benzodiazepine hypnotics targeting  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> receptors, as well as agonists of the melatonin receptors MT1 and MT2. Melatonin, temazepam and zolpidem are thought to exert their effect through different mechanisms of action, but whether this leads to differential effects on electroencephalogram (EEG) power spectra during sleep in middle-aged people is currently not known. To establish whether the effects of prolonged-release melatonin (2 mg) on the nocturnal sleep EEG are different to those of temazepam (20 mg) and zolpidem (10 mg). Sixteen healthy men and women aged 55–64 years participated in a double-blind, placebo-controlled, four-way cross-over trial. Nocturnal sleep was assessed with polysomnography and spectral analysis of the EEG. The effects of single oral doses of prolonged-release melatonin, temazepam and zolpidem on EEG slow-wave activity (SWA, 0.75–4.5 Hz) and other frequencies during nocturnal non-rapid eye movement (NREM) sleep were compared. In an entire night analysis prolonged-release melatonin did not affect SWA, whereas temazepam and zolpidem significantly reduced SWA compared with placebo. Temazepam significantly reduced SWA compared with prolonged-release melatonin. Prolonged-release melatonin only reduced SWA during the first third of the night compared with placebo. These data show that the effects of prolonged-release melatonin on the nocturnal sleep EEG are minor and are different from those of temazepam and zolpidem; this is likely due to the different mechanisms of action of the medications.

## Keywords

Prolonged-release melatonin, temazepam, zolpidem, EEG, slow-wave activity

## Introduction

Insomnia is common in many psychiatric disorders and also in older people (Riemann et al., 2011). Pharmacological treatment options for insomnia include benzodiazepine and non-benzodiazepine hypnotics that target  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> receptors and other hypnotics including melatonin receptor agonists (Ebert et al., 2006; Rudolph and Knoflach, 2011), the latter of which are sometimes referred to as chronobiotics. Current understanding of the neurochemical basis of sleep regulation provides a rational basis for pursuing both mechanisms of action (Saper et al., 2005). There is considerable evidence for a role of the wide range of GABA<sub>A</sub> receptor subtypes in sleep regulation (Lancel, 1999; Winsky-Sommerer, 2009). The classical benzodiazepine temazepam targets the majority of GABA<sub>A</sub> receptor subtypes in an unspecific manner, whereas the widely prescribed imidazopyridine zolpidem displays some selectivity for GABA<sub>A</sub> receptors containing the alpha-1 subunit.

Evidence for a role of melatonin in sleep regulation consists of the close association between the endogenous circadian rhythm of melatonin secretion and the sleep propensity rhythm, as well as specific electroencephalogram (EEG) characteristics such as sleep spindle activity (Cajochen et al., 2003; Dijk et al., 1997). Sleep scheduled outside the phase of nocturnal melatonin secretion is disrupted and characterised by delayed sleep initiation and reduced sleep consolidation, and this can be alleviated by exogenous melatonin administration, and several studies have

demonstrated this in healthy individuals (Rajaratnam et al., 2004; Stone et al., 2000; Wyatt et al., 2006). The effects of melatonin are thought to be mediated primarily by MT1 and MT2 receptors, which have a high density in the suprachiasmatic nucleus of the hypothalamus, amongst other areas (Arendt and Rajaratnam, 2008; Srinivasan et al., 2009). The different mechanisms of action of temazepam, zolpidem and prolonged-release melatonin could lead to differential effects on sleep structure and the sleep EEG, some of which may be undesirable. Effects on sleep structure may include reduced time spent in slow-wave sleep (SWS), which has been reported following benzodiazepines but not after zolpidem (Lancel, 1999; Winsky-Sommerer, 2009), or melatonin (Srinivasan et al., 2009). SWS is defined on the basis of the presence of slow waves (<2 Hz) with an amplitude exceeding 75  $\mu$ V.

Spectral analysis by fast Fourier transform (FFT) provides a description of the sleep EEG by computing power density (square of the amplitude, with no minimum amplitude criterion) within

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defined frequency ranges. Changes in the slow frequency range of the EEG after non-pharmacological (Aeschbach et al., 1997; Dijk et al., 1990a, 1990b) and pharmacological manipulations (Aeschbach et al., 1994) have been quantified by computing slow wave activity (SWA), defined as power density in the 0.75–4.5 Hz range. Spectral analysis of the EEG often confirms results of visual scoring of SWS, but may be more sensitive in assessing acute drug effects (Luthringer et al., 1995) as well as their chronic effects (Bastien et al., 2003) and also provides a description of changes in other frequencies. Sometimes dissociations between visual scoring and spectral analyses have been observed (Dijk et al., 1990a). In healthy individuals temazepam reduces visually scored SWS, SWA and theta activity, whilst enhancing spindle activity (Dijk et al., 1989). Zolpidem on the other hand, leads to no change, or an increase in SWS, while at the same time it reduces SWA and theta/alpha activity, and enhances spindle activity (Bettica et al., 2012; Brunner et al., 1991; Walsh et al., 2007). The effects of melatonin on the sleep EEG are equivocal. Administration of melatonin prior to daytime sleep causes no change in SWS, enhances spindle activity, but sometimes some reduction of SWA is observed in healthy individuals (Aeschbach et al., 2009; Dijk et al., 1995). Melatonin administration to healthy individuals prior to nocturnal sleep did not lead to significant changes in either visually scored SWS or in the spectral composition of the sleep EEG (Cajochen et al., 1997), and an absence of changes in the sleep EEG was observed also following a prolonged-release formulation in elderly insomnia patients (Luthringer et al., 2009).

The efficacy of prolonged-release melatonin in the treatment of the subjective complaint of insomnia has been reported (Lemoine and Zisapel, 2012; Lemoine et al., 2007, 2011; Wade et al., 2007, 2010, 2011). However, we are not aware of any published studies that have directly compared the effects of a benzodiazepine, a non-benzodiazepine and prolonged-release melatonin on the sleep EEG. A prolonged-release formulation of melatonin (Circadin, RAD Neurim Pharmaceuticals EEC Ltd, Reading, UK) has been approved for the treatment of insomnia in people aged  $\geq 55$  years. Very few data on the effects of hypnotics on the spectral composition of the EEG are available for this age group and we are not aware of any studies of the effects of melatonin on the spectral composition of the sleep EEG in this age group. We therefore investigated the acute effects of prolonged-release melatonin, temazepam and zolpidem on the spectral composition of the EEG during nocturnal sleep in healthy individuals aged 55–64 years. In view of the continuing interest in the functional significance of SWS (Dijk, 2010), the primary objective was to compare the EEG power spectra during non-rapid eye movement (NREM) sleep in the slow-wave frequencies following a single dose of prolonged-release melatonin to a single dose of temazepam, and secondary objectives were to compare to a single dose of zolpidem or placebo. Other secondary objectives were to compare the effects of treatment on EEG power spectra during NREM and rapid eye movement (REM) sleep in all EEG frequencies up to 32 Hz. The EEG power density spectra during NREM sleep following prolonged-release melatonin was compared to temazepam, zolpidem and placebo in other traditional EEG frequency bands as an exploratory measure. Other exploratory objectives were to compare polysomnography (PSG) and subjective sleep quality measures following prolonged-release melatonin to temazepam, zolpidem and placebo.

## Methods

### Ethics

The protocol was reviewed by an independent ethics committee (Brent Medical Ethics Committee/National Health Service (NHS) National Research Ethics Service) and was approved on the 20<sup>th</sup> March 2009 and a Clinical Trials Authorisation was provided by the Medicines and Healthcare products Regulatory Agency (MHRA). The study was conducted at the Surrey Clinical Research Centre (Surrey CRC) of the University of Surrey in accordance with principles based upon the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all subjects prior to any study specific procedures. This study was sponsored by the University of Surrey and registered at Clinicaltrials.gov: NCT00940550.

### Subjects

Subjects were recruited via the Surrey CRC database and through advertisements in the media. Study recruitment commenced in July 2009 and the last subject last visit was in August 2009. Fifty-two healthy men and women aged 55–64 years were medically screened for eligibility, and 36 were excluded due to screening failure. Sixteen healthy men and women (12 men; mean age 58.8 years, standard deviation (SD) 2.9), were enrolled in the study and were paid for their participation. Subjects had no sleep complaints and were judged healthy by physical examination, 12 lead electrocardiogram (ECG), medical and psychiatric history, haematology and clinical chemistry. Other key inclusion criteria included a regular sleep-wake cycle with a bedtime between 22:00 and 00:00 on at least five nights per week, reported typical sleep duration of 6.5–8.5 h and being a non-smoker. Exclusion criteria included being of childbearing potential; a known sensitivity to temazepam, zolpidem or melatonin; body mass index (BMI)  $<19$  or  $>33$  kg/m<sup>2</sup>; a score  $>5$  on the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989); a history of clinically significant sleep pathology according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) or an apnoea-hypopnoea index (AHI)  $>10$  or a periodic limb movements arousal index (PLMAI)  $>10$  as assessed at the clinical PSG screening visit. Other exclusion criteria included being a shift worker; having recently travelled through more than two time zones; drinking more than five caffeinated beverages per day or consuming more than three (men) or two (women) units of alcohol per day; use of psychotropic medications and other medication that may interfere with of the study medications.

### Study design

This was a single centre, randomised, double-blind, placebo-controlled, four-way crossover trial to study the effects of prolonged-release melatonin, temazepam and zolpidem on the spectral composition of the EEG during nocturnal sleep in healthy middle-aged men and women.

*Visit 1.* A general medical screening visit occurred up to 28 d prior to the first treatment visit and written informed consent was obtained. The visit included assessment of general health by history and examination, bloods for haematology, biochemistry and serology. Urinary screening for drugs of abuse and an alcohol

breath test were performed. For females a pregnancy test was also required. Subjects received actiwatches (Actiwatch, Cambridge Neurotechnology Ltd) to document the regularity of their sleep-wake schedules for the duration of the study, ensuring subjects remained compliant with the inclusion criteria (bedtime between 22:00 and 00:00 on at least five out of seven nights per week, including the night before a study visit and a sleep duration of 6.5–8.5 h).

**Visit 2.** The adaptation and clinical PSG screening visit occurred at least two days prior to the first treatment visit. Subjects arrived at the Centre at ~17:00. Medical status, concomitant medications and actigraphic recordings of sleep-wake schedules were reviewed and drug screening and alcohol breath tests were performed. Lights out was from 23:00–07:00 and PSG was recorded during this time. Blood samples (20:00, 21:00, 22:00, 23:00, 02:00, 05:00 and 08:00) and pooled urine (19:00–21:00, 21:00–23:00, and 23:00–07:00, with scheduled bladder voids at 20:30, 22:30 and 07:00) were collected to assess subjects' plasma melatonin profile and urinary 6-sulphatoxymelatonin excretion. The Karolinska Sleepiness Scale (KSS) a nine-point Likert scale used to assess subjective sleepiness in the immediately preceding period (Akerstedt and Gillberg, 1990), was completed at 20:00, 22:00 and at 07:15. The Sleep Quality Questionnaire (SQQ) comprising 10 questions regarding the previous nights' sleep was completed shortly after awakening to assess subjective sleep quality.

**Visits 3-6: Treatment periods (TPs) 1-4.** Subjects arrived at the Centre at ~17:00. Procedures were conducted as in visit 2 with the exception of collection of blood samples and with the addition of dosing with the study medications occurring at appropriate times before lights off. In order to allow for an adequate washout period between treatments, the TPs were separated by a minimum of five days. Lights out remained from 23:00–07:00 throughout the study. A post-study telephone contact occurred after visit 6. Evening meals were served at 19:00 on all study visits and after that further food intake was not allowed.

**Safety.** Adverse events were recorded and monitored from the time of the first dose of study medication until the follow up telephone contact.

### *Study medications, doses and timing of administration*

Temazepam and zolpidem represent common choices in clinical practice for insomnia treatment. Doses of 20 mg of temazepam and 10 mg of zolpidem are indicated for insomnia in adults younger than 65 years. Prolonged-release melatonin 2 mg is indicated for individuals  $\geq 55$  years who suffer from insomnia. Therefore, the study population consisted of individuals aged 55–64 years to assess and compare the influence of temazepam 20 mg, zolpidem 10 mg and prolonged-release melatonin 2 mg on nocturnal sleep measured by EEG. Prolonged-release melatonin or matching placebo tablets were administered at 21:00. Temazepam or zolpidem or matching placebo capsules were administered at 22:45. The difference in timing of administration of study drugs was to account for the differences in time to reach peak serum concentration (C<sub>MAX</sub>), and therefore ensured that C<sub>MAX</sub> occurred at approximately the same time. The summary of product characteristics for

prolonged-release melatonin indicates that C<sub>MAX</sub> is reached after approximately three hours in the fed state, whereas, temazepam and zolpidem reach C<sub>MAX</sub> quicker, after approximately 1 h and 0.5–3 h respectively. All study medications and placebos were supplied by clinical supply management vendor Bilcare GCS (Europe) Ltd (Powys, UK). Prolonged-release melatonin 2 mg tablets were sourced from H Lundbeck A/S (Valby, Denmark) and matching placebo tablets were sourced from Penn Pharmaceutical Services Ltd (Gwent, Wales). Temazepam 10 mg tablets and zolpidem 10 mg tablets were sourced from Teva Pharmaceutical Industries Ltd (Eastbourne, UK) and were over-encapsulated by Bilcare GCS to produce temazepam 20 mg and zolpidem 10 mg capsules that were identical in appearance and matching placebo capsules were also supplied by Bilcare GCS. Tests were carried out by Bilcare GCS to confirm that over-encapsulated tablets complied with the pharmacopoeia standard for in vitro tablet disintegration. Any small delay in tablet disintegration caused by the over-encapsulation is not considered to have a significant effect on dissolution. We therefore can be confident that the pharmacodynamics and pharmacokinetics of study medications were not markedly affected by over-encapsulation. All study medications were administered at the recommended doses and frequency in accordance with applicable Summaries of Product Characteristics. Four treatment sequences were generated in a Latin square design. Bilcare GCS generated a randomisation list to ensure that subjects were randomised in balanced order to a treatment sequence (1:1:1:1 ratio) and packaged medications into treatment cartons according to the sequences and applied a randomised label to each. Subjects were assigned a randomisation number at visit 3 (TP1) in sequential order. Both staff and subjects were blind to the study treatments until after data review and after the database was locked.

### *PSG recordings and scoring of sleep stages*

Subjects slept in individual, sound attenuated, temperature controlled, windowless bedrooms. All PSGs were recorded on Siesta digital recorders (Compumedics, Abbotsford, Victoria, Australia) and the same recorder was used for each subject throughout the study. EEGs were recorded from C3–A2, C4–A1, O1–A2 and O2–A1 derivations, in addition to two submental electromyogram (EMG) and two electrooculogram (EOG) derivations. EEG signals were high-pass filtered at 0.3 Hz and low-pass filtered at 70 Hz and these settings were appropriate for both visual scoring and spectral analysis of the EEG. Sampling and storage rate was 256 Hz for EEG, EOG and EMG signals. All PSG recordings were scored by registered polysomnographic technologists (RPSGTs) who were blinded to treatment. PSG recordings were scored per 30 s epochs, using ProFusion PSG software application within neXus Control (Compumedics Limited, Melbourne, Victoria, Australia). We used the standardised criteria of Rechtschaffen and Kales, to ensure compatibility with previous studies, sleep staged hypnograms were then exported to the data management database.

### *EEG spectral analysis*

Spectral analysis of EEG data recorded from 23:00–07:00 on each treatment night comprised the primary and secondary outcome measures of the study. PSG recordings were exported from the Compumedics system in EDF format and, together with the sleep staged hypnograms, were imported in Vitabase-Vitascore

software, version 1.40 (RC.1) (Temec Instruments B.V., Kerkrade, The Netherlands). All sleep EEG data were visually inspected per 30 s epoch and artefacts were annotated manually by the first author (EA), within Vitascore software. Sub-epochs annotated as artefacts were excluded from the analysis of the power spectra. Spectral analysis was conducted using a fast Fourier transform (FFT). The data were weighted with a squared cosine window, implemented in the Vitascore software. Spectra were computed per four-second sub-epoch, resulting in a 0.25 Hz resolution up to 32 Hz. In addition, power was computed in eight traditional EEG frequency bands 0.25–0.5 Hz (low-delta); 0.75–4.5 Hz (delta (SWA)); 4.75–7.75 Hz (theta); 8.0–11.75 Hz (alpha); 12.0–14.75 Hz (sigma); 15.0–19.75 Hz; 20.0–24.75 Hz; 25.0–32.0 Hz (beta-1, 2, 3 respectively). Sleep stage specific power spectra (stages 1, 2, 3, 4, NREM and REM) were computed for the entire night as well as per thirds of the night. All these computations were conducted in Vitascore software. One central derivation (C3–A2 in preference to C4–A1) was selected per subject, per visit, for analysis. We used the central derivation to ensure compatibility with previous studies. The criteria set for the amount of sub-epochs of data remaining in the entire night for each derivation following artefact removal was 62.5%. Data reduction was achieved by computing power per 1 Hz bin through addition of consecutive 0.25 Hz values and then dividing by the number of contributing 0.25 Hz values. These data were then exported to the data management database.

### *Statistical approaches for primary, secondary and exploratory objectives*

A sample size of 15 was needed to detect, with a two-sided statistical test, at the 5% level of significance, with 80% power, a difference of 28% in suppression between prolonged-release melatonin 2 mg and temazepam 20 mg, assuming a between-subject standard deviation of 35%. Hence it was decided to recruit 16 subjects for this study. All statistical analyses were performed by Surrey CRC statisticians using SAS, version 9.1 or later (SAS Institute Inc., Cary, North Carolina, USA) and all EEG power density values were  $\log_{10}$  transformed prior to statistical analysis. The primary study objective was to compare EEG power spectra during NREM sleep in the slow-wave frequencies following administration of prolonged-release melatonin 2 mg to temazepam 20 mg, and secondary objectives were also to compare prolonged-release melatonin to zolpidem 10 mg and placebo in the slow-wave frequencies. We conducted contrast analyses for four adjacent 1 Hz bins (0.25–1.0, 1.25–2.0, 2.25–3.0, 3.25–4.0). To assess the robustness of the results we also investigated the effects on EEG activity in the slow-wave range by excluding the 0.25–1.0 Hz range and including the 4.25–5.0 Hz range (1.25–5.0 Hz) The slow-wave frequency ranges were assessed by implementing SAS PROC MIXED with the REML option, with subject as random effect, with factors treatment (prolonged-release melatonin, temazepam, zolpidem and placebo), frequency bin (four 1 Hz bins) and period (TP number), and including the condition by frequency bin interaction. This latter interaction allows for the assessment of any differential effects of treatment on particular frequencies within the slow-wave range. Frequency bin was used as a repeated measure with an unstructured variance-covariance matrix option. Using the LSMEANS statement of SAS PROC MIXED, the levels of treatment were statistically compared; estimates, unadjusted 95% confidence limits, and

unadjusted *p* values were calculated and reported. In addition, Cohen's *d* effect sizes were calculated from the mixed model results by taking the *t* value for the comparison of treatments, multiplying by two, then dividing by the square root of the number of degrees of freedom in the *t*-test. Other secondary objectives included a comparison across conditions of EEG power spectra during NREM and REM sleep in all EEG frequencies between 0.25 and 32 Hz, and due to limitations of mixed model estimation, geometric means of power densities within each treatment conditions were expressed relative to placebo (100%) and 95% confidence limits were used to compare the effects of treatment to placebo across the entire frequency spectrum. The eight traditional EEG frequency bands, including the SWA range (0.75–4.5 Hz), during NREM sleep were assessed as an exploratory objective across the entire night and per thirds of the night. The contrast analyses for the eight traditional bands were calculated using PROC MIXED as described above for the slow-wave frequencies, however, frequency bin was not used as a factor in the model and therefore no repeated statement was implemented in the model either. Exploratory analysis of PSG sleep measures and subjective sleep quality measures were conducted by calculating means and standard errors and implementing SAS PROC TTEST to calculate paired *t*-tests to compare treatment means. No multiplicity significance level adjustment was applied to the analyses concerning the secondary and exploratory endpoints.

## **Results**

### *Subject disposition*

Sixteen subjects (14 White, 2 Black; 12 men; mean age 58.8 years, SD 2.9; mean BMI 25.6) were enrolled in the study and comprised the All Subjects Treated Set (ASTS). One subject did not attend one visit (TP3, treatment due to be administered at TP3 was administered at TP4, and the subject was subsequently withdrawn and therefore did not receive their final scheduled treatment) and was excluded from the Per Protocol Set (PPS) (mean age and range as above). In three subjects some EEG recordings contained too many artefacts for a reliable assessment i.e. less than 62.5% of data remained following artefact removal; therefore six EEG recordings were also excluded from the PPS (2×placebo and zolpidem nights and 1×temazepam and prolonged-release melatonin nights). Data review and assignment to the PPS were completed prior to unblinding. Here we report data on the PPS.

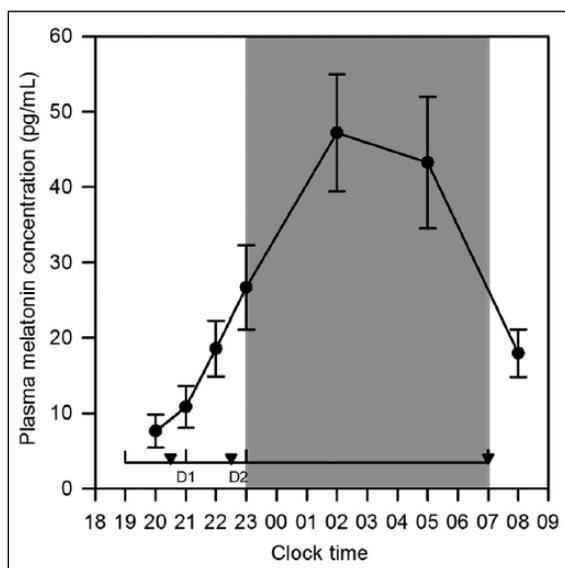
### *Plasma melatonin and 6-sulphatoxymelatonin*

Average plasma melatonin assessed at adaptation visit 2 rose early in the evening peaking at around 02:00 (see Figure 1). Assessment of subjects' plasma melatonin profiles confirmed that no subjects were melatonin deficient. During treatment periods urinary excretion of 6-sulphatoxymelatonin increased with each pooled urine sample (19:00–21:00, 21:00–23:00, 23:00–07:00) (see Table 1). Compared to placebo, treatment with temazepam and zolpidem did not lead to differences in urinary 6-sulphatoxymelatonin, whereas treatment with prolonged-release melatonin caused a large increase in excretion of 6-sulphatoxymelatonin in all subjects, which was evident in both the 21:00–23:00 and the 23:00–07:00 pooled urine samples.

### Slow-wave frequencies

The primary and secondary study objectives were to compare the EEG power spectra during NREM sleep in the slow-wave frequencies following prolonged-release melatonin compared with temazepam, zolpidem and placebo. General linear mixed model analyses (see methods) with factors treatment (prolonged-release melatonin, temazepam, zolpidem, placebo), frequency bin (four consecutive 1 Hz bins) and period (TP number), were performed over three NREM sleep frequency ranges to assess the robustness of the effects on the slow-wave frequencies. The slow-wave ranges analysed included 0.25–4.0 Hz and 1.25–5.0 Hz, each comprising four 1 Hz bins. Additionally, mixed model analysis was performed with factors treatment and period for the traditional SWA band (0.75–4.5 Hz).

We observed a significant effect of treatment and frequency bin in both the 0.25–4.0 Hz and the 1.25–5.0 Hz ranges, however, no significant interactions between treatment and frequency bin were observed (see Table 2). Following prolonged-release melatonin, power density was significantly higher in all three slow-wave ranges assessed compared with temazepam, but not compared with zolpidem or placebo (see Table 2 and Table 3).



**Figure 1.** Study protocol and baseline average plasma melatonin.  
 • Mean ( $\pm$ standard error(SE)) plasma melatonin (pg/mL) collected on adaptation visit 2 ( $n=12-15$ ). D1 and D2 (dosing 1 and 2) represents dosing on visits 3–6 with prolonged-release melatonin or matching placebo at 21:00 and with temazepam or zolpidem or matching placebo at 22:45.  
 □ Scheduled bladder voids and horizontal lines represent pooled urine collection periods. Grey shaded area represents lights off period.

**Table 1.** Urinary 6-sulphatoxymelatonin excretion rate.

Pooled urine sample time	Placebo ( $n=15$ ) mean pg/h (SE)	PR melatonin ( $n=15$ ) mean pg/h (SE)	Temazepam ( $n=15$ ) mean pg/h (SE)	Zolpidem ( $n=15$ ) mean pg/h (SE)
19:00–21:00	170.75 (20.95)	141.86 (14.67)	139.77 (23.18)	153.80 (21.55)
21:00–23:00	344.18 (75.62)	66590.69 (12769.22)	320.46 (63.29)	329.33 (72.61)
23:00–07:00	1597.83 (340.87)	139880.67 (12131.39)	1198.02 (242.91)	1448.83 (320.38)

h: hour; pg: picograms; PR melatonin: prolonged-release melatonin; SE: standard error.

Temazepam suppressed activity in all three slow-wave ranges compared with placebo. Zolpidem suppressed activity in the 1.25–5.0 Hz and the traditional SWA ranges; but not the 0.25–4.0 Hz range compared with placebo (see Table 2 and Table 3). Compared with zolpidem, temazepam also significantly reduced activity in all three slow-wave ranges (see Table 2 and Table 3). Figure 2(a) displays power in the traditional SWA range following prolonged-release melatonin, temazepam and zolpidem administration relative to placebo for the entire night (see Figure 2). Effect sizes calculated based on Cohen's  $d$  were all large except for the comparisons of prolonged-release melatonin with zolpidem in the 0.25–4.0 Hz range and with placebo in the 1.25–5.0 Hz range which produced small and medium effect sizes respectively, these differences are likely due to the effect of zolpidem on the lowest 1 Hz bin. The effect sizes demonstrated that temazepam had the largest effect on the EEG compared with placebo and prolonged-release melatonin had the smallest.

### Traditional EEG frequency ranges

As an exploratory measure the effect of treatment on NREM EEG power density within other traditional EEG ranges: 0.25–0.5 Hz (low-delta); 4.75–7.75 Hz (theta); 8.0–11.75 Hz (alpha); 12.0–14.75 Hz (sigma); 15.0–19.75 Hz; 20.0–24.75 Hz; 25.0–32.0 Hz (beta-1, 2, 3 respectively), was analysed in the same way as for the traditional SWA range (0.75–4.5 Hz (delta)) (see Table 3). Power density within the low-delta range was significantly lower following prolonged-release melatonin compared with zolpidem ( $p=0.0007$ ) and approached significance compared with temazepam ( $p=0.0520$ ). Please note that in this frequency range, values following zolpidem tended to be above placebo values ( $p=0.0634$ ), whereas for prolonged-release melatonin the values tended to be below placebo ( $p=0.0779$ ). Temazepam significantly decreased power within the SWA, theta and alpha ranges compared with prolonged-release melatonin and placebo. Zolpidem significantly decreased power within the theta and alpha ranges compared with prolonged-release melatonin and the SWA, theta and alpha ranges compared with placebo administration. Temazepam also significantly decreased activity in the SWA range when compared with zolpidem.

The traditional EEG frequency ranges were also analysed per thirds of the night. Given that effects of temazepam and zolpidem on the EEG are typically reported for NREM sleep in the SWA and spindle frequency ranges, only these ranges are discussed here. Figures 2(b), 2(c) and 2(d) display power in the traditional SWA range following prolonged-release melatonin, temazepam and zolpidem administration relative to placebo for the first, second and last third of the night respectively (see Figure 2). NREM SWA significantly decreased following prolonged-release melatonin

**Table 2.** Effect of treatment on the slow-wave frequencies during non-rapid eye movement (NREM) sleep: mixed model factors and contrasts.

Factor	Frequency range, Hz	DF	F	p		
Treatment	0.25–4.0	(3,6)	6.30	0.0241		
	1.25–5.0	(3,12)	9.53	0.0018		
Frequency bin	0.25–4.0	(3,48)	1110.76	<0.0001		
	1.25–5.0	(3,48)	1439.89	<0.0001		
Treatment*frequency bin	0.25–4.0	(9,72)	0.89	0.5376		
	1.25–5.0	(9,72)	1.02	0.4306		

Contrast	Frequency range, Hz	Ratio estimate	95% CL		p	Cohen's d
			Upper	Lower		
PR melatonin vs temazepam	0.25–4.0	1.19	1.38	1.03	0.0243	2.29
	1.25–5.0	1.28	1.47	1.12	0.0016	2.36
PR melatonin vs zolpidem	0.25–4.0	1.03	1.19	0.89	0.6680	0.35
	1.25–5.0	1.10	1.27	0.96	0.1435	0.90
PR melatonin vs placebo	0.25–4.0	0.92	1.06	0.79	0.2026	1.12
	1.25–5.0	0.94	1.07	0.82	0.3063	0.62
Temazepam vs placebo	0.25–4.0	0.77	0.89	0.67	0.0044	3.31
	1.25–5.0	0.73	0.84	0.64	0.0003	2.90
Zolpidem vs placebo	0.25–4.0	0.89	1.04	0.77	0.1139	1.43
	1.25–5.0	0.85	0.97	0.74	0.0238	1.50
Temazepam vs zolpidem	0.25–4.0	0.86	1.00	0.75	0.0469	1.96
	1.25–5.0	0.86	0.98	0.75	0.0313	1.45

CL: confidence limits; DF: degrees of freedom; PR melatonin: prolonged-release melatonin.

compared with placebo during the first third of the night ( $p=0.0093$ ). Following temazepam SWA significantly decreased compared with placebo during all thirds of the night ( $p=0.0237$ ,  $<0.0001$ ,  $<0.0001$  respectively), and compared with prolonged-release melatonin and zolpidem during the second ( $p=0.0001$ ,  $0.0021$  respectively) and last ( $p=0.0001$ ,  $0.0025$  respectively) thirds of the night. Following zolpidem SWA significantly decreased compared with placebo during the first and second thirds of the night ( $p=0.0268$ ,  $0.0162$  respectively), and compared with prolonged-release melatonin during the last third of the night ( $p=0.0158$ ). Following temazepam and zolpidem there was a significant increase in sigma activity (spindle frequency range) compared with prolonged-release melatonin ( $P=0.0050$ ,  $0.0325$  respectively) during the last third of the night.

### Power density spectrum

Other secondary objectives were to assess the effects of treatment on the EEG power density up to 32 Hz during NREM and REM sleep. Power spectra following treatment were expressed relative to placebo for the entire night (see Figure 3) and per thirds of the night during NREM sleep for prolonged-release melatonin treatment (see Figure 4), and 95% confidence limits were used to indicate significant differences between treatment and placebo. Even though the interaction between treatment and frequency bin was not significant in either the 0.25–4 Hz or 1.25–5.0 Hz range analyses, visual inspection of the EEG frequency spectrum (see Figure 3) suggests that the response to the various treatments was not uniform across the slow-wave frequencies during NREM sleep.

Prolonged-release melatonin did not cause any marked changes to EEG power spectra during NREM or REM sleep

compared with placebo. However, a small decrease in activity in the low-delta and SWA ranges was observed during the first third of the night (see Figure 4), as confirmed by analyses of traditional SWA range per thirds of the night (see above). Temazepam had the greatest effect on the lower frequencies during NREM sleep, and caused a substantial decrease in power up to approximately 10 Hz compared with placebo, encompassing slow-wave, theta and low-alpha frequency ranges. Temazepam also marginally increased activity in the spindle frequency range at 12 Hz compared with placebo. Zolpidem exerted similar effects to temazepam on NREM sleep; however, the decrease in activity in the slow-wave frequencies was less pronounced, and in the lowest 1 Hz bin zolpidem increased power compared with placebo. The increase in power in the lowest bin following zolpidem reflects the significant increase in power following zolpidem compared with prolonged release melatonin observed in the low-delta band, which also approached significance compared with placebo. Compared with placebo, temazepam and zolpidem did not exert distinctive effects on the EEG power density spectra during REM sleep.

### Visually scored PSG data

Exploratory analyses included investigation of the effects of treatment on PSG measures by calculating paired  $t$ -tests (see Table 4). No significant effects of prolonged-release melatonin were observed for any of the visually scored PSG sleep variables when compared with placebo. Total sleep time (TST), duration of stage 2, duration of NREM sleep and sleep efficiency increased following temazepam and zolpidem compared with placebo and wake after sleep onset (WASO) decreased. Additionally, temazepam significantly decreased number of awakenings (NAW),

**Table 3.** Mixed model analysis of traditional electroencephalogram (EEG) frequency bands during non-rapid eye movement (NREM) sleep.

Frequency band	PR melatonin vs temazepam				PR melatonin vs zolpidem				Temazepam vs zolpidem			
	Ratio estimate	95% CL		<i>p</i>	Ratio estimate	95% CL		<i>p</i>	Ratio estimate	95% CL		<i>p</i>
		Upper	Lower			Upper	Lower			Upper	Lower	
Low-delta (0.25–0.5 Hz)	0.86	1.00	0.74	0.0520	0.75	0.88	0.65	0.0007	0.87	1.02	0.75	0.0782
Delta (0.75–4.5 Hz)	1.21	1.34	1.09	0.0009	1.06	1.18	0.95	0.3070	0.88	0.97	0.79	0.0156
Theta (4.75–7.75 Hz)	1.40	1.57	1.24	<.0001	1.27	1.43	1.13	0.0003	0.91	1.02	0.81	0.0990
Alpha (8.0–11.75 Hz)	1.12	1.24	1.01	0.0359	1.13	1.26	1.01	0.0301	1.01	1.12	0.91	0.8894
Sigma (12.0–14.75 Hz)	0.90	1.01	0.81	0.0731	0.91	1.02	0.81	0.1069	1.01	1.13	0.90	0.8835
Beta-1 (15.0–19.75 Hz)	1.04	1.15	0.94	0.4057	1.02	1.13	0.92	0.6783	0.98	1.08	0.89	0.6845
Beta-2 (20.0–24.75 Hz)	1.04	1.15	0.93	0.5241	1.00	1.12	0.89	0.9795	0.96	1.08	0.86	0.5098
Beta-3 (25.0–32.0 Hz)	1.00	1.15	0.88	0.9515	0.99	1.14	0.86	0.9034	0.99	1.13	0.86	0.8539

Frequency band	PR melatonin vs placebo				Temazepam vs placebo				Zolpidem vs placebo			
	Ratio estimate	95% CL		<i>p</i>	Ratio estimate	95% CL		<i>p</i>	Ratio estimate	95% CL		<i>p</i>
		Upper	Lower			Upper	Lower			Upper	Lower	
Low-delta (0.25–0.5 Hz)	0.87	1.02	0.75	0.0779	1.01	1.18	0.87	0.8706	1.16	1.35	0.99	0.0634
Delta (0.75–4.5 Hz)	0.91	1.01	0.82	0.0875	0.76	0.84	0.68	<.0001	0.86	0.96	0.77	0.0097
Theta (4.75–7.75 Hz)	0.98	1.10	0.87	0.6958	0.70	0.79	0.62	<.0001	0.77	0.87	0.68	0.0001
Alpha (8.0–11.75 Hz)	0.95	1.06	0.85	0.3461	0.85	0.95	0.76	0.0041	0.84	0.94	0.76	0.0033
Sigma (12.0–14.75 Hz)	0.95	1.06	0.85	0.3589	1.05	1.18	0.94	0.3822	1.04	1.17	0.93	0.4711
Beta-1 (15.0–19.75 Hz)	0.99	1.09	0.89	0.7620	0.95	1.05	0.86	0.2685	0.96	1.07	0.87	0.4819
Beta-2 (20.0–24.75 Hz)	1.01	1.13	0.91	0.8333	0.98	1.09	0.87	0.6781	1.01	1.13	0.90	0.8175
Beta-3 (25.0–32.0 Hz)	1.01	1.17	0.88	0.8387	1.01	1.16	0.88	0.8866	1.02	1.18	0.89	0.7502

CL: confidence limits; PR melatonin: prolonged-release melatonin.

whilst zolpidem significantly increased REM latency compared with placebo. TST, duration of stage 2, duration of NREM sleep and sleep efficiency were significantly higher and WASO was significantly lower following treatment with both temazepam and zolpidem, compared with prolonged-release melatonin. Duration of stage 1 and NAW were significantly lower following temazepam compared with prolonged-release melatonin. Duration of stage 4 and stages 3+4 (SWS) were significantly higher and duration of REM was significantly lower following zolpidem compared with prolonged-release melatonin. Stage 4 and NAW were significantly higher following zolpidem compared with temazepam.

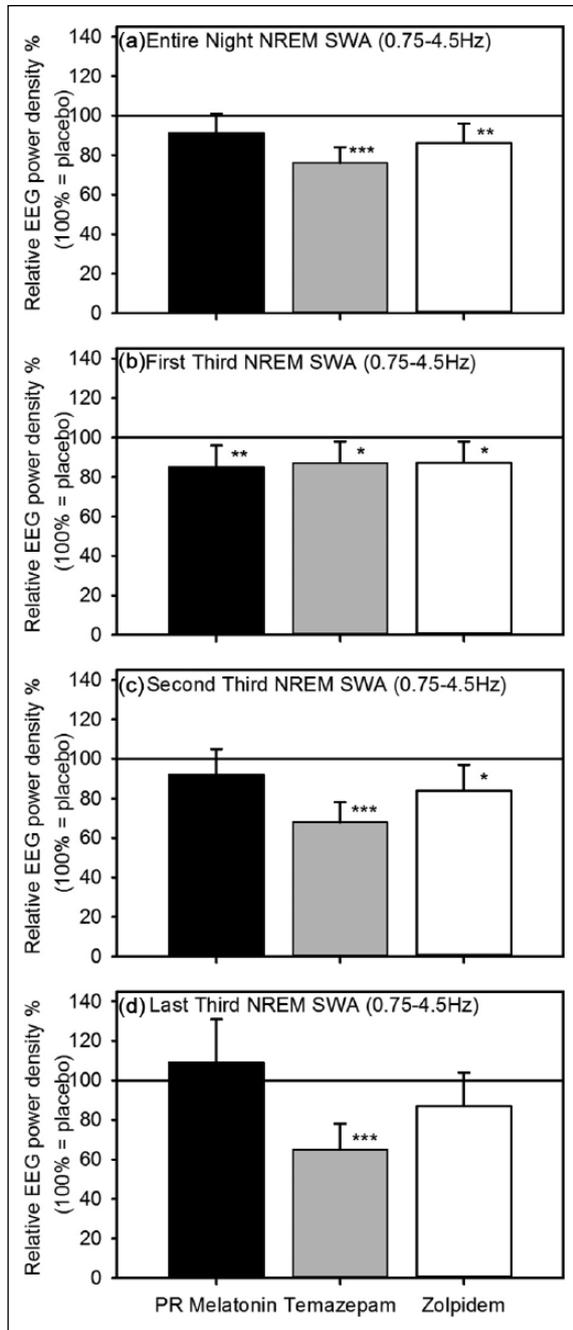
### Subjective sleep quality

Other exploratory analyses included comparisons of the effect of treatment on subjective sleep quality as assessed by the SQQ and the KSS, using paired *t*-tests. The question 'How was the quality of your sleep last night?' scored significantly better following temazepam and zolpidem compared with placebo ( $p=0.0091$ ,  $0.0132$  respectively) and prolonged-release melatonin ( $p=0.0410$ ,  $0.0379$  respectively). Subjects answered the question 'How long did you sleep altogether?' with significantly longer following temazepam compared with placebo and prolonged-release melatonin ( $p=0.0173$ ,  $0.0308$  respectively). The question 'How many times did you wake up?' was answered with significantly fewer awakenings following temazepam compared with prolonged-release melatonin ( $p=0.0281$ ), and following zolpidem compared

with placebo and prolonged-release melatonin ( $p=0.0358$ ,  $0.0018$  respectively). When asked 'What was the total duration of those night awakenings?' subjects scored awakenings as significantly shorter following zolpidem compared with prolonged-release melatonin ( $p=0.0446$ ). Assessment of sleepiness using the KSS scale did not reveal any significantly different scores between treatments at any time.

### Safety

During the study conduct no deaths, serious adverse events or serious drug adverse reactions were reported and no subjects were withdrawn from the study due to an adverse event. In total 48 adverse events were reported and the majority of the reports were mild in intensity. Out of 48 events, 39 adverse events were deemed by the investigator to be drug-related (placebo, eight; prolonged-release melatonin, 10; temazepam, eight; zolpidem, 13 events). The most widely reported adverse drug reactions (ADRs) under treatment with prolonged-release melatonin were gastro-intestinal disorder related which were reported for four subjects (constipation, dry mouth, flatulence and nausea). Additionally, three subjects reported nervous system disorder ADRs (balance disorder, somnolence and headache) and two subjects reported general disorders and administration site condition ADRs (fatigue and feeling hot) under treatment with prolonged-release melatonin. Under treatment with temazepam five subjects reported nervous system related ADRs (balance disorder, headache and somnolence). Additionally, gastro-intestinal disorder related ADRs (dry mouth),



**Figure 2.** Electroencephalogram (EEG) slow-wave activity (SWA) power density during non-rapid eye movement (NREM) sleep. Mean SWA (0.75–4.5 Hz) ( $\pm$  95% confidence limits) following prolonged release (PR) melatonin, temazepam and zolpidem for (a) entire night, and (b) first, (c) second and (d) last third of night expressed relative to placebo (100%). \* indicates a significant difference from placebo at  $\leq 0.05$ ; \*\* indicates significant difference from placebo at  $\leq 0.01$ ; \*\*\* indicates significant difference from placebo at  $\leq 0.001$ .

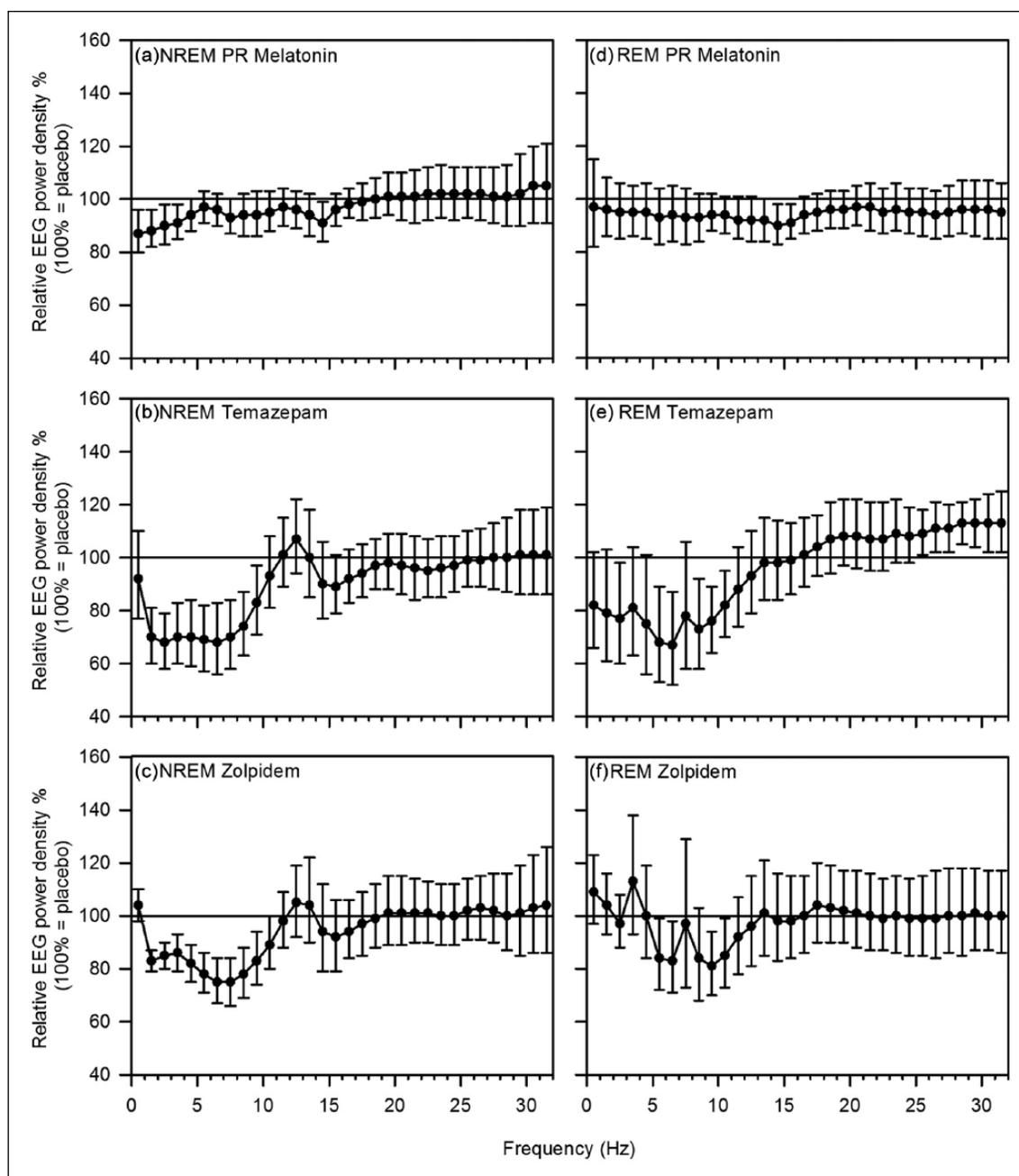
and psychiatric disorder related ADRs (nightmare) were reported by one subject each, under treatment with temazepam. Two subjects reported nervous system related ADRs (balance disorder and headache) and two subjects reported ADRs relating to general disorders and administration site condition (asthenia and thirst)

under treatment with zolpidem. Other ADRs reported under treatment with zolpidem included gastro-intestinal disorders (nausea), and psychiatric disorders (anxiety and disorientation), each reported by one subject. It should also be noted that six subjects in total reported at least one ADR under the placebo condition. Two subjects reported renal and urinary disorder ADRs and two subjects reported nervous system disorder ADRs (dizziness and headache) under the placebo condition. In addition, gastro-intestinal disorder (dry mouth), psychiatric disorder (dissociation) and general disorder and administration site condition (fatigue) ADRs were reported by one subject each, under the placebo condition. No clinically significant influence of treatment was noted on vital signs and clinical laboratory values. Overall all study medications were well tolerated.

## Discussion

The data demonstrate that single doses of temazepam, zolpidem and prolonged release melatonin exert differential effects on sleep and the EEG. The data show that evening administration of prolonged-release melatonin to older subjects, who are not melatonin deficient, has relatively minor effects on NREM slow-wave frequency ranges of the nocturnal EEG in comparison to those of temazepam and zolpidem. These findings are in accordance with previous studies which found no major effects of prolonged-release melatonin on the spectral composition of the nocturnal EEG in both healthy individuals (Cajochen et al., 1997) and insomnia patients (Luthringer et al., 2009). Other researchers have found that daytime administration of melatonin in healthy individuals induces effects on the EEG reminiscent of those induced by benzodiazepines and non-benzodiazepine z-drugs including a reduction in SWA and an increase in spindle activity (Aeschbach et al., 2009; Dijk et al., 1995), whereas in our study we observed that prolonged-release melatonin only suppressed SWA during the first third of the night. If we accept that the current and published data show that during the night melatonin has at best minor effects on the EEG and that during the day melatonin affects the EEG then it may be hypothesised that effects of exogenous melatonin on the EEG are only observed when endogenous melatonin levels are low. The minor reduction in SWA in the first third of the night may be indicative of effects on core body temperature or other effects related to the pharmacokinetic properties of the drug. Temazepam and zolpidem on the other hand significantly reduced SWA as was expected, and this was observed in all three slow-wave ranges analysed for temazepam and in the 1.25–5.0 Hz and the traditional slow-wave range for zolpidem when compared with placebo. The effects of temazepam on the EEG were more pronounced than for the effects of zolpidem, this is likely due to the selectivity of zolpidem for GABA<sub>A</sub> receptors containing the alpha-1 subunit.

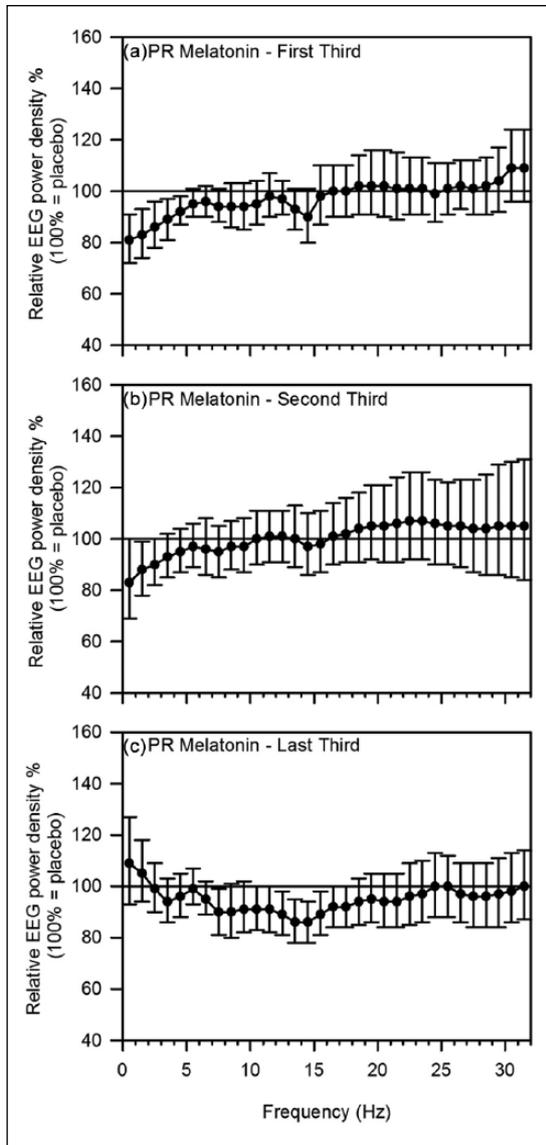
Spectral analysis of the eight traditional EEG frequency bands and 1 Hz bins up to 32 Hz revealed that both temazepam and zolpidem induced the well-characterised EEG signature that is observed following administration of benzodiazepines and non-benzodiazepine z-drugs. Most notably, in accordance with previous research in healthy individuals (Bettica et al., 2012; Brunner et al., 1991; Dijk et al., 1989; Walsh et al., 2007) treatment with temazepam and zolpidem reduced power in the slow-wave, theta and alpha ranges compared to placebo, whereas prolonged-release melatonin did not significantly differ in any of the traditional EEG frequency



**Figure 3.** Entire night electroencephalogram (EEG) power density spectra. • Mean EEG power ( $\pm 95\%$  confidence limits) following prolonged-release (PR) melatonin ((a), (d);  $n=13$ ), temazepam ((b), (e);  $n=12$ ) and zolpidem ((c), (f);  $n=12$ ) expressed relative to placebo (100%) for non-rapid eye movement (NREM) (left panel) and rapid eye movement (REM) (right panel) sleep.

bands compared to placebo. It has previously been observed by several groups that activity in the lowest slow-wave frequencies is enhanced or at least not reduced after zolpidem administration (Bettica et al., 2012; Brunner et al., 1991; Walsh et al., 2007), and such findings were also observed in the current study in the lowest 1 Hz frequency bin during NREM sleep. This increase in activity in the 1 Hz range may be related to the observed increase in visually scored SWS. It should be noted that this increase in SWS after zolpidem is accompanied by a very different spectral profile than the increase in SWS after sleep deprivation. After the latter

physiological manipulation, enhancement of SWS and SWA is accompanied by enhancements of spectral power in a broad frequency range, up to 10 Hz (Dijk et al., 1990b, 1993); whereas after pharmacological intervention with zolpidem, activity in the range of approximately 2–11 Hz is reduced (Bettica et al., 2012; Brunner et al., 1991; Walsh et al., 2007). The behaviour of very low frequency components of the EEG in response to hypnotics is also of some interest because reductions of these EEG frequencies or of delta activity encompassing these slow components have been observed in chronic fatigue syndrome (Le Bon et al., 2012)



**Figure 4.** Non-rapid eye movement (NREM) sleep electroencephalogram (EEG) power density spectra per thirds of the night following prolonged-release (PR) melatonin. • Mean EEG power ( $\pm 95\%$  confidence limits) for the first (a), second (b) and last (c) third of the night expressed relative to the corresponding third of placebo (100%) ( $n=13$ ).

and insomnia (Bastien et al., 2003; Merica et al., 1998), and these reductions may reflect abnormalities in sleep homeostasis in these individuals.

In contrast to other studies (Brunner et al., 1991; Dijk et al., 1989; Walsh et al., 2007) no significant enhancement in the sigma frequency range (12.0–14.75 Hz) was observed following administration of zolpidem or temazepam when compared to placebo. However, visual inspection of the power density spectrum showed that there was a tendency for temazepam and zolpidem to increase power at 13 Hz and 13–14 Hz for temazepam and zolpidem respectively when compared to placebo; In addition, statistically significant enhancement of sigma activity was not always observed (Dijk et al., 1989). The differences between our study

and others' could undoubtedly be related to age effects on the EEG, whereby EEG power decreases with age, particularly at frequencies below 14 Hz (Landolt et al., 1996). Despite subjects being older in our study compared with most previous studies, the majority of the effects of the study medications on the spectral composition of the EEG were similar to those previously observed by others; in addition, the effects of temazepam and zolpidem on the EEG serve to indicate that the small effects of prolonged-release melatonin on the EEG cannot be due to a lack of sensitivity of the spectral analysis method or absence of effects of hypnotics on the EEG of middle-aged subjects. It should also be acknowledged that temazepam has a longer terminal half-life compared with zolpidem (3.5–18.4 h and 2.5 h respectively) and this may have contributed to the more pronounced effects of temazepam on the entire night EEG power spectrum compared to zolpidem. The absence of major effects of prolonged-release melatonin administration on EEG power spectra is reminiscent of the effects of a dual orexin receptor antagonist, where neither the NREM nor REM sleep EEG was affected, however, measures of hypnotic efficacy such as latency to persistent sleep and total sleep time were significantly affected (Bettica et al., 2012). In our analyses we have used definitions for frequency ranges based on our previous work (Aeschbach et al., 1994, 1997; Dijk and Czeisler, 1995) allowing comparison with these previous publications. These definitions are slightly different from the American Academy of Sleep Medicine (AASM) manual (Iber et al., 2007) but our analyses with a 1 Hz resolution of the EEG power spectrum (see Figure 3 and Figure 4) and the behaviour of the EEG in the low frequency range (see Table 2 and Table 3) provide a detailed description of the changes in the EEG, independent of the somewhat arbitrary frequency band definitions.

Prolonged-release melatonin did not significantly affect PSG sleep measures compared with placebo. This is in accordance with the observations by others that exogenous melatonin administration either minimally or does not at all affect PSG sleep measures in healthy individuals (Cajochen et al., 1997; Wyatt et al., 2006) or insomnia sufferers (Luthringer et al., 2009) when administered during the phase of endogenous melatonin secretion. It appears from studies in healthy individuals that exogenous melatonin administration only affects sleep when endogenous melatonin levels are low (Rajaratnam et al., 2004; Stone et al., 2000; Wyatt et al., 2006). Temazepam and zolpidem improved sleep by increasing TST, NREM sleep duration and sleep efficiency, amongst other variables. The observed increase in sleep stage 4 sleep following zolpidem treatment compared with temazepam and prolonged-release melatonin, and an increase in SWS after zolpidem compared with prolonged-release melatonin, resembles the differential effects of zolpidem on the low-delta frequency band, even though the effects of zolpidem on the slow-wave and theta frequencies is very similar to the effects of temazepam. Spectral analysis revealed the complexity of the effects of these medications on the EEG and our data gives further support to the notion that spectral analysis is a more sensitive tool for assessing drug efficacy.

There was significant subjective improvement of sleep quality following temazepam and zolpidem compared with placebo and prolonged-release melatonin on several of the SQQ questions. The morning KSS did not reveal any significant subjective differences between treatments and placebo and therefore suggests that there were no residual effects of treatment in

**Table 4.** Polysomnography variables derived from visual scoring of the electroencephalogram (EEG) during nocturnal sleep.

PSG variable	Placebo (n=15) mean (SE)	PR melatonin (n=14-15) mean (SE)	Temazepam (n=15) mean (SE)	Zolpidem (n=15) mean (SE)	Temazepam-PR melatonin (n=14-15) mean (SE)	Zolpidem-PR melatonin (n=14-15) mean (SE)	Zolpidem-temazepam (n=15) mean (SE)
SOL (min)	8.47 (1.76)	6.97 (1.74)	8.87 (1.81)	10.03 (1.95)	1.90 (1.36)	3.07 (1.55)	1.17 (1.41)
LPS (min)	15.70 (6.01)	14.17 (3.77)	12.17 (2.49)	17.00 (4.37)	-2.00 (3.21)	2.83 (2.24)	4.83 (3.62)
REM latency (min)	87.03 (11.94)	87.14 (12.56)	100.67 (12.85)	115.30 (15.12) <sup>a</sup>	16.21 (15.64)	30.89 (15.96)	14.63 (15.12)
Stage 1 (min)	53.47 (4.79)	57.00 (4.70)	45.33 (3.59)	52.57 (5.52)	-11.96 (4.17) <sup>a</sup>	-6.54 (4.24)	7.23 (5.28)
Stage 2 (min)	179.30 (10.44)	179.04 (9.46)	229.70 (10.44) <sup>c</sup>	215.63 (7.34) <sup>c</sup>	50.61 (7.58) <sup>c</sup>	38.71 (5.48) <sup>c</sup>	-14.07 (8.72)
Stage 3 (min)	36.77 (4.49)	34.04 (2.37)	39.43 (2.89)	37.07 (4.41)	4.07 (3.08)	1.57 (4.76)	-2.37 (5.25)
Stage 4 (min)	43.93 (7.71)	41.00 (9.62)	39.97 (9.09)	51.33 (8.25)	-0.50 (5.65)	11.79 (4.68) <sup>a</sup>	11.37 (4.35) <sup>a</sup>
Stages 3+4 (min)	80.70 (8.08)	75.04 (9.88)	79.40 (9.65)	88.40 (7.66)	3.57 (6.32)	13.36 (5.74) <sup>a</sup>	9.00 (6.89)
NREM sleep (min)	313.47 (11.80)	311.07 (7.27)	354.43 (6.59) <sup>b</sup>	356.60 (5.79) <sup>c</sup>	42.21(8.01) <sup>c</sup>	45.54 (6.65) <sup>c</sup>	2.17 (7.79)
REM sleep (min)	88.83 (7.92)	94.54 (5.30)	85.97 (7.40)	76.50 (5.28)	-8.64 (7.35)	-18.11 (7.00) <sup>a</sup>	-9.47 (7.86)
TST (min)	402.30 (15.77)	405.61 (10.59)	440.40 (5.21) <sup>b</sup>	433.10 (6.17) <sup>a</sup>	33.57(8.55) <sup>b</sup>	27.43 (7.71) <sup>b</sup>	-7.30 (5.16)
SPT (min)	467.37 (2.63)	465.25 (3.80)	465.43 (3.52)	464.87 (4.14)	-0.46 (5.59)	-1.32 (6.36)	-0.57 (4.39)
Sleep efficiency (%)	83.81 (3.28)	84.51 (2.21)	91.75 (1.09) <sup>b</sup>	90.22 (1.29) <sup>a</sup>	6.99 (1.78) <sup>b</sup>	5.69 (1.61) <sup>b</sup>	-1.53 (1.08)
WASO (min)	69.23 (14.41)	66.96 (10.02)	30.73 (4.38) <sup>b</sup>	36.87 (4.98) <sup>a</sup>	-35.32 (8.32) <sup>c</sup>	-30.68 (7.89) <sup>b</sup>	6.13 (4.50)
NAW	22.00 (1.86)	24.79 (2.43)	15.87 (1.82) <sup>b</sup>	20.93 (2.20)	-8.36 (2.28) <sup>b</sup>	-3.57 (3.19)	5.07 (2.27) <sup>a</sup>

LPS: latency to persistent sleep (lights off to 10 minutes continuous sleep); NAW: number of awakenings (number of blocks of consecutive epochs of wake from LPS until lights on); NREM: non-rapid eye movement; PR melatonin: prolonged-release melatonin; PSG: polysomnography; REM: rapid eye movement; SE: standard error; SOL: sleep onset latency; SPT: sleep period time; TST: total sleep time; WASO: wake after sleep onset. Treatment columns display mean and markers indicate significant change from placebo. Contrast columns display mean difference between treatments and markers indicate a significant change between treatments. Significant change at  $\leq 0.05$ ,  $\leq 0.01$  and  $\leq 0.001$  respectively.

the morning. Although we did not find that prolonged-release melatonin improved subjective sleep quality, it has been reported that prolonged-release melatonin is effective in the subjective improvement of insomnia in patients (Lemoine et al., 2007, 2011; Lemoine and Zisapel, 2012; Wade et al., 2007, 2010, 2011). The present study was powered to detect effects on EEG power spectra in the slow-wave frequency ranges and not to detect effects on PSG or subjective measures of sleep and therefore the results from these variables should be interpreted with caution. Nevertheless, the dissonance observed between our prolonged-release melatonin results in healthy subjects and those reported in insomnia patients implies that it may be informative to conduct further PSG studies of the effects of melatonin in well-screened insomnia patients.

Whilst it is beyond the scope of the research presented here to advise upon treatment options for insomnia in older people, the well documented impracticalities of the use of benzodiazepines and non-benzodiazepine z-drugs including withdrawal symptoms and rebound insomnia, provide substantial reason to consider other treatment options for insomnia. In addition, prolonged-release melatonin use is not associated with the residual effects which are often observed following administration of benzodiazepine and other allosteric modulators of the GABA<sub>A</sub> receptor. A study of the effects of prolonged-release melatonin in healthy men and women aged 55 years and over reported that prolonged-release melatonin was not associated with impaired memory recall, driving performance or psychomotor function (Otmani et al., 2008). Along these lines, prolonged-release melatonin has been recommended as a first line therapy in older insomnia patients (Wilson et al., 2010), and a large surveillance study recently reported that following withdrawal from Circadin, sleep quality and alertness remained improved and rebound insomnia incidence was low for older insomnia patients (Hajak et al., 2014). Whilst we only reported very few changes in the EEG induced by prolonged-release melatonin, greater responses to exogenous melatonin administration have been observed at other times of day and given the role of melatonin in sleep regulation, there would be a rational basis for pursuing medications that target these receptor systems in the development of treatments for insomnia. The exact physiological roles of the melatonin receptors is not well understood and indeed, further understanding of the mechanisms by which melatonin receptor agonists exert their effects is required in order to develop nocturnally beneficial therapies for insomnia that target these receptors. Newer approaches to melatonergic therapies appear to be focussing on the development of drugs which target several receptor systems in order to improve efficacy (Carocci et al., 2014).

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### Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article:

D-JD has received funding from pharmaceutical companies and has acted as consultant to several pharmaceutical companies, including H Lundbeck A/S. All other authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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