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Quantitative Electroencephalography Within Sleep/Wake States Differentiates $GABA_A$ Modulators Eszopiclone and Zolpidem From Dual Orexin Receptor Antagonists in Rats

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Dual orexin receptor antagonists (DORAs) induce sleep by blocking orexin 1 and orexin 2 receptor-mediated activities responsible for regulating wakefulness. DORAs represent a potential alternative mechanism to the current standard of care that includes the γ-aminobutyric acid $(GABA)_A$ receptor-positive allosteric modulators, eszopiclone and zolpidem. This work uses an innovative method to analyze electroencephalogram (EEG) spectral frequencies within sleep/wake states to differentiate the effects of $GABA_A$ modulators from DORA-22, an analog of the DORA MK-6096, in Sprague–Dawley rats. The effects of low, intermediate, and high doses of eszopiclone, zolpidem, and DORA-22 were examined after first defining each compound's ability to promote sleep during active-phase dosing. The EEG spectral frequency power within specific sleep stages was calculated in 1-Hz intervals from 1 to 100 Hz within each sleep/wake state for the first 4 h after the dose. Eszopiclone and zolpidem produced marked, dose-responsive disruptions in sleep stage-specific EEG spectral profiles compared with vehicle treatment. In marked contrast, DORA-22 exhibited marginal changes in the spectral profile, observed only during rapid eye movement sleep, and only at the highest dose tested. Moreover, while eszopiclone- and zolpidem-induced changes were evident in the inactive period, the EEG spectral responses to DORA-22 were absent during this phase. These results suggest that DORA-22 differs from eszopiclone and zolpidem whereby DORA-22 promotes somnolence without altering the neuronal network EEG activity observed during normal sleep.
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INTRODUCTION

Insomnia is estimated to affect 10–15% of the general population (Ohayon, 2002) and many patients are treated with the γ-aminobutyric acid $(GABA)_A$ receptor modulators eszopiclone (Sunovion Pharmaceuticals) and zolpidem (Sanofi) to help induce and maintain sleep. In recent years, orexin (hypocretin) receptor antagonism has been shown to be an effective mechanism of modulating arousal and promoting somnolence (Brisbare-Roch *et al*, 2007; Herring *et al*, 2012). Dual orexin receptor antagonists (DORAs) reversibly block both orexin 1 and orexin 2 receptors (OX_1R and OX_2R) to promote sleep by a mechanism consistent with suppressing wakefulness (Bettica *et al*, 2012a; Brisbare-Roch *et al*, 2007; Winrow *et al*, 2011, 2012). It is currently unclear whether sleep produced by $GABA_A$-positive allosteric modulators differs quantitatively from that induced by DORAs.

Spectral electroencephalogram (EEG) frequency analysis is a useful quantitative analytical technique for measuring neuronal network activity. Clinically, zolpidem and zopiclone (a racemic mixture of eszopiclone and its inactive enantiomer) have been shown to reduce wakefulness and increase non-rapid eye movement (NREM) sleep while modifying the EEG frequency distribution in delta (slow-wave or NREM) sleep (Landolt *et al*, 2000; Lundahl *et al*, 2012; Brunner *et al*, 1991; Trachsel *et al*, 1990). $GABA_A$ modulators have also been associated with central nervous system-related adverse events (AEs) including sleep walking, sleep driving, sleep eating, amnesia, and cognitive impairment (Hindmarch *et al*, 2006; Hoque and Chesson, 2009; Otmani *et al*, 2008; Rush *et al*, 1998). Orexin receptor antagonism has emerged as an alternative mechanism for the potential treatment of insomnia that specifically targets neuronal orexinergic networks involved in wakefulness. It is

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as yet unknown whether these distinct pharmacological mechanisms can be quantitatively differentiated based on unique EEG signatures within the central nervous system (CNS).

DORA-22 is an analog of MK-6096, an orally bioavailable, potent and selective reversible antagonist of OXR1 and OXR2 currently in clinical development for insomnia (Winrow *et al*, 2012). To better understand the potential differences in sleep architecture between the $GABA_A$ modulators eszopiclone and zolpidem and DORA-22, we developed an analysis technique to evaluate quantitative EEG (qEEG) spectral power changes in CNS activity across rat sleep/wake states at sleep-effective doses of these compounds.

MATERIALS AND METHODS

Rat Sleep Architecture and qEEG Data Collection

All animal studies were performed in accordance with The National Research Council's Guide for the Care and Use of Laboratory Animals and were approved by the Merck Institutional Animal Care and Use Committee. All efforts were made to minimize animal use and suffering.

Rat sleep and qEEG studies were conducted in ambulatory animals via radio telemetry in a manner similar to that described previously (Renger *et al*, 2004; Winrow *et al*, 2011). Cortical EEG/electrocorticogram (ECoG), electromyogram (EMG), and generalized locomotor activity recordings were collected in male Sprague–Dawley rats ($n=16$ per study; age: 6–9 months; weight: 500–750 *g*). Telemetric physiological monitors were implanted subcutaneously (4ET; Data Sciences International, St Paul, MN), and included electrodes for EEG monitoring and electrodes implanted beneath the neck muscle for EMG recording. The animals were singly housed with food and water available *ad libitum*, and on a 12 : 12 light : dark cycle with lights off at 0400 hours (zeitgeber time; ZT12) and on at 1600 hours (ZT0).

Rat sleep/qEEG studies were performed on animals treated with DORA-22, eszopiclone (Myoderm USA, Norristown, PA), and zolpidem (Myoderm USA). Compounds were dosed orally in independent studies in a counterbalanced, vehicle-controlled, crossover design. In the first arm of the study, half the animals were treated with vehicle and the other half were treated with a single dose of compound daily for 3 consecutive days. After a 3-day washout period, the groups were subsequently reversed in the second arm of the experiment in which they received the alternative treatment condition for 3 consecutive days such that all animals ultimately received both vehicle and compound treatments over the course of the entire experiment. The study design included a 1-day vehicle only run-in period, a 3-day between-crossover washout, and a 2-day end-of-study washout (11 total oral dosings per animal).

To identify doses of eszopiclone, zolpidem, and DORA-22 that could achieve similar reductions in active wake, dose–response curves were generated for each compound in independent 3-day crossover studies following their daily oral administration during the active phase. Reduction of active wake over the first 4 h following dosing was used as the primary measure of sleep-promoting efficacy.

Effects of each compound were analyzed and compared with within-animal controls using 20% vitamin E *d*-α-tocopheryl polyethylene glycol 1000 succinate (TPGS; Eastman Chemical, Kingsport, TN) as vehicle; all dosing was performed orally (per os). Data were averaged by animal for each 3-day treatment crossover condition and subsequently analyzed for within subject comparisons of vehicle to compound treatment as described previously (Cox *et al*, 2010; Renger *et al*, 2004; Winrow *et al*, 2011). EEG/ECoG, EMG, and generalized activity signals were automatically classified in 10 s epochs for each 24 h recording using the Somnologica Science software (Embla, Denver, CO) for four sleep/wake states: active wake, delta sleep, REM sleep, and light sleep/quiet wake (reported here as light sleep). Active wake was classified as low amplitude, high-frequency EEG, and high amplitude EMG (muscle tone) with or without locomotor activity. Light sleep was classified as having higher delta and theta EEG frequency components and decreased EMG amplitude relative to active wake, and no locomotor activity. Scoring epochs classified as delta sleep exhibited delta power as the major EEG component, decreased EMG muscle tone, and no locomotor activity. REM sleep included high theta power with increased higher frequencies similar to active wake in the EEG, with little EMG muscle tone and no locomotor activity. Active-phase (wake) dosing studies occurred 5 h into active-phase (ZT17); inactive-phase (sleep) dosing studies occurred 1 h before lights on (ZT23). The MK-6096 analog, DORA-22, has well-characterized rodent pharmacokinetic properties (Coleman *et al*, 2012; Gotter *et al*, 2012; Winrow *et al*, 2012).

The effects of compounds on EEG frequencies were determined by comparing the EEG spectral frequency changes relative to vehicle within each sleep/wake state. The spectral ratio data (response to compound divided by vehicle response) indicate where responses to the compound result in an EEG frequency that increases (>1), decreases (<1), or has no effective change ($=1$).

Quantitative EEG Analysis

A custom-developed qEEG analysis algorithm was coded and compiled in Matlab (R2007a; MathWorks, Natick, MA) to perform artifact rejection and short-time Fourier transform (spectrogram function) on EEG signals; qEEG data were analyzed from 1 to 100 Hz in 1 Hz increments and aligned with sleep epoch data based on sleep/wake state epoch assignment. qEEG spectral powers were log-transformed and averaged by sleep/wake state by animal. Data from the first 4 h after the dose (ZT17–ZT21, active-phase dosing; ZT23–ZT03, inactive-phase dosing) for each animal following compound administration were divided by within-subject vehicle data to normalize spectral power measures (expressed as a compound/vehicle spectral ratio of treatment effect size). A locally weighted regression with a linear polynomial fit (Matlab, LOWESS) was applied and 95% confidence bounds were calculated using a bootstrap method to model the resulting spectral ratio using Matlab R2011a. Significance was determined by non-overlapping 95% confidence interval segments of the model with the

threshold value 1 ± 5% given a baseline (non-dosing)/vehicle dosing effect.

Area Over/Under Spectral Frequency Curve as a Function of Decreased Active Wake

Further analysis of the area over or under the spectral frequency curves (AUC) (outside of the ± 5% dose effect) for 1–100 Hz by sleep state and dose using trapezoidal integration (Matlab R2011a) as a function of decreases in active wake reveals linear trends and correlations. Decreases in active wake for all treatments at each dose were used to normalize sleep time for the first 4 h after the dose (ZT17–ZT21; ZT23–ZT03). Standard error of the means were calculated for both the AUC (y axis) and decreases in active wake time (x axis) for each data point.

RESULTS

Sleep Promotion

The respective low, intermediate, and high doses of eszopiclone, 3, 6, and 10 mg/kg, zolpidem, 10, 30, and 60 mg/kg, and DORA-22, 3, 10, and 30 mg/kg, promoted sleep to a similar extent as measured by compound-induced reductions in active wake (Figure 1, Active Wake panel). At 3, 6, and 10 mg/kg, eszopiclone dose-dependently decreased active wake relative to vehicle by 8.1 ± 6.5, 28.3 ± 4.5, and 46.0 ± 7.5 min, respectively, with significant reductions achieved by the 6 and 10 mg/kg doses ($P<0.01$). Zolpidem at 10, 30, and 60 mg/kg decreased active wake by 9.1 ± 5.0, 30.6 ± 4.7, and 49.4 ± 6.8 min, respectively; both the 30 and 60 mg/kg doses significantly decreased active wake ($P<0.01$). DORA-22 significantly reduced active wake at all three doses tested by 18.7 ± 5.7 ($P<0.05$), 29.1 ± 4.5 ($P<0.01$), and 39.4 ± 5.3 min ($P<0.01$), respectively. Subsequent analyses relied on these previously determined pharmacologically normalized doses to conduct side-by-side comparisons of sleep-stage and qEEG spectral profile changes.

Further examination of sleep states indicated that eszopiclone and zolpidem modulated sleep states differently compared with DORA-22. Both $GABA_A$ modulators dose-dependently increased the amount of time spent in light and delta sleep during the active phase, whereas DORA-22 increased delta sleep, but had no significant effect on light sleep in the 4 h following treatment (Figure 1, Light Sleep panel Figure 1 and Delta Sleep panel). Marked differences were seen particularly in REM sleep: eszopiclone dose-dependently decreased REM sleep, zolpidem nonsignificantly

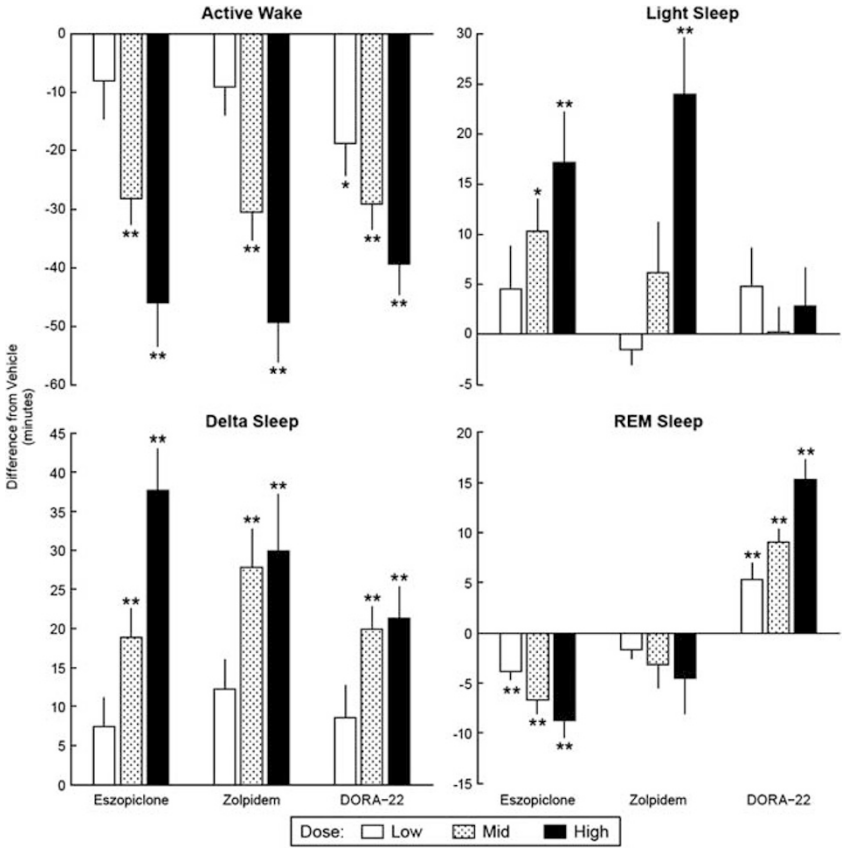

Figure 1 γ-Aminobutyric acid ($GABA_A$) modulators and dual orexin receptor antagonist (DORAs) dose-responsively increase sleep time in active-phase dosing (zeitgeber time (ZT) 17). Average time (min ± standard error of mean (SEM)) male Sprague–Dawley rats ($n = 16$ per study) spent in sleep/wake state compared with vehicle treatment for respective low, mid, and high (eszopiclone: 3, 6, and 10 mg/kg; zolpidem: 10, 30, and 60 mg/kg; DORA-22: 3, 10, and 30 mg/kg) oral (per os) doses during 3-day crossover design studies during active phase. Each treatment dose was conducted independently and compared with within-subject vehicle. Statistics were calculated within each study using two-way analysis of variance (ANOVA) with repeated measure (factors for treatment and day); $^*P<0.05$, $^{**}P<0.01$.

trended towards a dose-responsive decrease in REM sleep, whereas DORA-22 dose-dependently increased REM sleep (Figure 1, REM Sleep panel). When similar studies were conducted during the inactive phase (by administering the study compounds 1 h before 'lights on' (ZT23)), DORA-22 showed no significant changes in any of the sleep/wake states. Similarly, neither zolpidem nor eszopiclone significantly altered active wake or delta sleep in the inactive phase studies, both $GABA_A$ modulators did significantly decrease REM sleep (both $GABA_A$ modulators at the intermediate and high doses; $P<0.001$) with a concurrent increase in light sleep (eszopiclone and zolpidem intermediate and high doses, respectively; $P<0.01$) (Figure 2).

Alteration of Sleep Stage-Specific EEG Spectral Frequency

Spectral EEG records spontaneous brain activity in terms of rhythmic neural firing. When dosed during the active phase, both eszopiclone and zolpidem produced significant and dose-dependent changes in EEG spectral frequencies within sleep/wake vigilant states compared with normal vehicle controls, while DORA-22 had significantly smaller effects across all doses. Administration of eszopiclone or zolpidem was associated with increased mid (12–55 Hz) and concurrent decreased high (70–100 Hz) frequencies during active wake and light, delta, and REM sleep (Figure 3). At low and mid doses, zolpidem and eszopiclone increased the 1–3 Hz spectral power and decreased 4–12 Hz power during delta sleep. In contrast, no changes in sleep stage-dependent frequencies were observed with the lowest dose of DORA-22 (3 mg/kg), a dose exhibiting significant active wake reduction exceeding that observed in response to the lowest doses of eszopiclone and zolpidem. The small deviations in frequencies at this dose were no different in magnitude from the ±5% fluctuations associated with vehicle alone (yellow shaded area, Figure 3). Small changes in spectral frequency power during REM sleep were observed relative to vehicle at the intermediate and highest doses of DORA-22 (decreases at 1–4, 10–12, and 21–23 Hz for 10 mg/kg, and decreases at 1–25 Hz and increases at 42–82 Hz for 30 mg/kg). At 30 mg/kg, marginal decreases in spectral frequency power were observed during active wake and delta sleep (increases at 12–20 Hz and decrease at 80–100 Hz).

Consistent with polysomnographic data, the effects of eszopiclone and zolpidem on EEG spectral power were observed in the inactive phase, whereas DORA-22-induced changes were even further diminished relative to active-phase treatments. Dosing 1 h (ZT23) before normal sleep resulted in eszopiclone- and zolpidem-induced EEG spectral

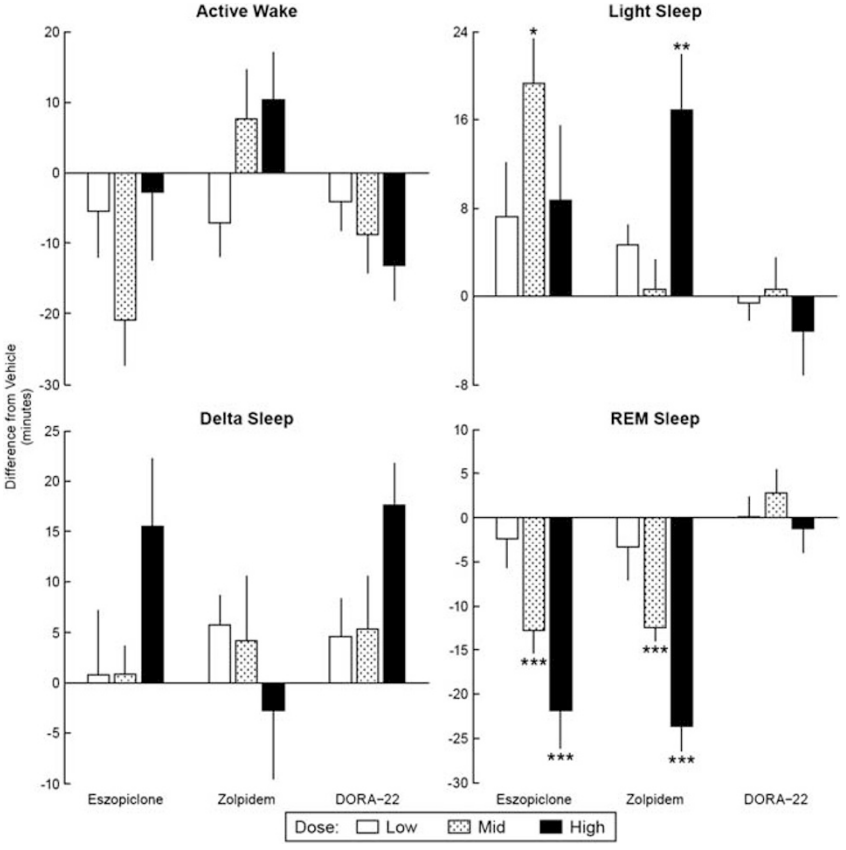

Figure 2 γ-Aminobutyric acid ($GABA_A$) modulators but not dual orexin receptor antagonist (DORAs) decrease rapid eye movement (REM) sleep in inactive-phase dosing (zeitgeber time (ZT)23). Average time (min ± standard error of mean (SEM)) male Sprague–Dawley rats ($n=16$ per study) spent in sleep/wake state compared with vehicle treatment for respective low, mid, and high (eszopiclone: 3, 6, and 10 mg/kg; zolpidem: 10, 30, and 60 mg/kg; DORA-22: 3, 10, and 30 mg/kg) oral (per os) doses during 3-day crossover design studies during active phase. Each treatment dose was conducted independently and compared with within-subject vehicle. Statistics were calculated within each study using two-way analysis of variance (ANOVA) with repeated measure (factors for treatment and day); $*P<0.05$, $**P<0.01$, and $***P<0.001$.

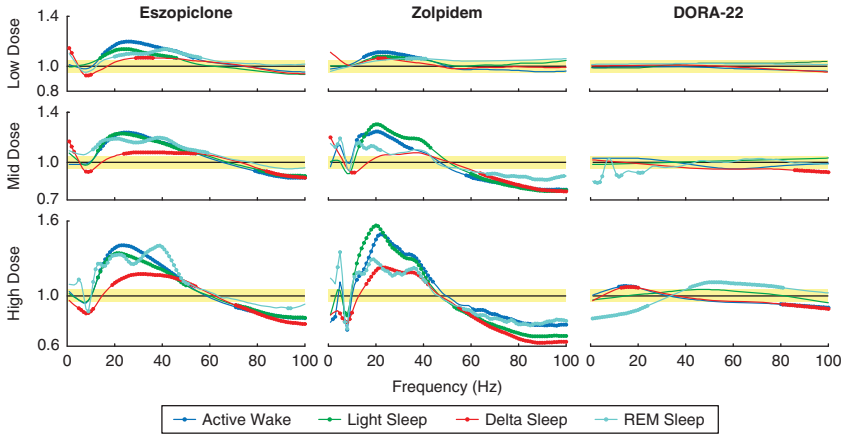

Figure 3 γ-Aminobutyric acid ($GABA_A$) modulators dose-responsively alter electroencephalogram (EEG) spectral frequency of sleep/wake states in active-phase dosing (zeitgeber time (ZT)17). EEG spectral changes (as a ratio of treatment over vehicle) from 1 to 100 Hz within each sleep/wake state for each treatment at respective doses (low, mid, and high). Yellow shaded area represents 1 (no change from vehicle) ± 5% as observed baseline dosing effect. 95% Confidence bounds were calculated and filled circles indicate non-overlapping areas above/below baseline dosing effect and 95% confidence intervals for each sleep/wake state. All treatments and doses were collected as independent 3-day crossover study designs in male Sprague–Dawley rats ($n = 16$) during the active phase (ZT17).

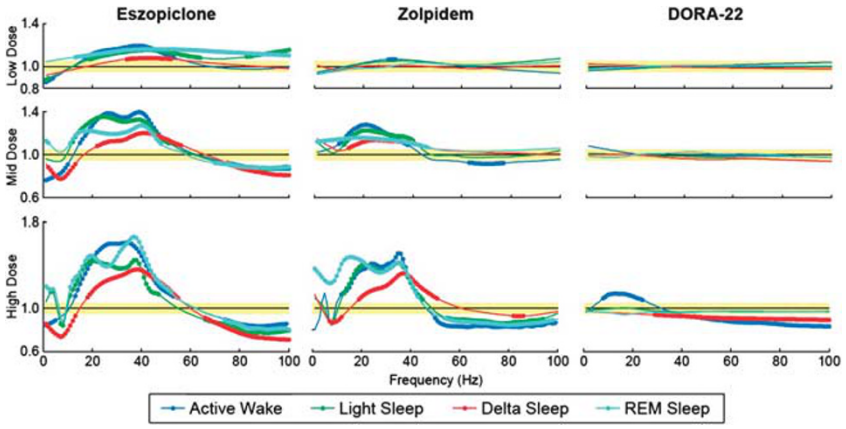

Figure 4 γ-Aminobutyric acid ($GABA_A$) modulators dose-responsively alter electroencephalogram (EEG) spectral frequency of sleep/wake states in inactive-phase dosing (zeitgeber time (ZT)23). EEG spectral changes (as a ratio of treatment over vehicle) from 1 to 100 Hz within each sleep/wake state for each treatment at respective doses (low, mid, and high). Yellow shaded area represents 1 (no change from vehicle) ± 5% as observed baseline dosing effect. 95% Confidence bounds were calculated and filled circles indicate non-overlapping areas above/below baseline dosing effect and 95% confidence intervals for each sleep/wake state. All treatments and doses were collected as independent 3-day crossover study designs in male Sprague–Dawley rats ($n = 16$) during the inactive phase (ZT23).

power changes reminiscent of those that occurred during the active phase and were again dose-responsive (Figure 4). Marginal changes *vs* vehicle in the spectral profile of delta and active wake reached significance in response to DORA-22 at the high dose (Figure 4).

Effects on Normal Rat Sleep

The correlation between the magnitude of compound-induced EEG spectral changes within each sleep stage and sleep-promoting efficacy (as measured by decreases in active wake) was calculated to better quantify the difference between agents. Eszopiclone and zolpidem produced a strong relationship between changes in EEG spectral frequency (y axis) and decreases in active wake time (x axis) across all sleep/wake states (Figure 5). DORA-22, however, exhibited no correlation between changes in the EEG spectral frequency and decreases in active wake time except in REM sleep, which was associated with a decrease in the lower frequencies and an increase in the higher frequencies at the high dose. When the EEG spectral frequency was analyzed separately by sleep/wake state, eszopiclone and zolpidem exhibited significant correlations between most of these measures during active wake and light, delta, and REM sleep ($P<0.05$ through $P<0.001$; Supplementary Table S1); the exception was the correlation between changes in spectral frequency and delta sleep with zolpidem, which was not significant. DORA-22 showed a significant correlation between spectral power changes and sleep efficacy only during REM sleep (R^2 correlation value, 0.13; $P<0.05$) (Supplementary Table S1).

DISCUSSION

To better understand the different effects on sleep architecture of the $GABA_A$ modulators eszopiclone and zolpidem and the orexin receptor antagonist DORA-22, we

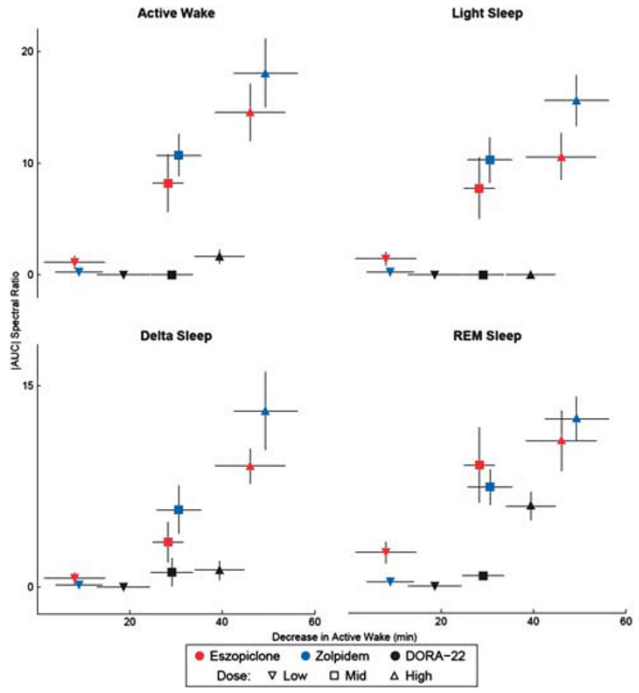

Figure 5 Dual orexin receptor antagonist (DORA-22) minimally alters EEG frequency as a function of sleep. Area under/over the electroencephalogram (EEG) spectral frequency (Figure 3) as a function of decreases in active wake (Figure 1) for each sleep/wake state (different panes). Inverted triangle (▼) indicates low dose, square (■) indicates mid dose, and triangle (▲) indicates high dose. Symbols represent average decrease in active wake (x axis) ± standard error of mean (SEM), and average area under/over the EEG frequency curve outside of the ± 5% dosing effect area (y axis) ± SEM.

developed an innovative analysis technique to evaluate qEEG spectral power changes across rat sleep/wake vigilance states at dose ranges with sleep-promoting effects.

We report that at doses producing similar amounts of sleep during the active phase (as measured by decreases in active wake), eszopiclone and zolpidem substantially and dose-dependently altered EEG spectral frequencies. Generalized decreases in spectral power at frequency ranges of 1–10 and 50–100 Hz along with increases in power at frequencies of 20–40 Hz relative to within-subject vehicle control were seen across sleep/wake stages with these two $GABA_A$ modulators. These effects were even observed at the lowest doses, which also did not promote sleep. The lowest DORA-22 dose tested (3 mg/kg) significantly increased sleep during the active phase, but had no effect on EEG spectral frequency as compared across matching sleep/wake states. The intermediate dose (10 mg/kg) of DORA-22 promoted substantially more sleep than low-dose DORA-22 and proportionally as much sleep as the intermediate doses of eszopiclone (6 mg/kg) and zolpidem (30 mg/kg). Minor EEG spectral changes were observed with the intermediate dose of DORA-22 that were of a lesser magnitude compared with those observed with eszopiclone and zolpidem during active-phase dosing.

The more targeted distribution of orexin receptors, *vs* the broader distribution and more diverse actions of GABA receptors, is likely to have a substantial role in the distinct downstream effects of their respective modulators, including the changes in EEG spectra observed with eszopiclone and zolpidem. Distribution of target receptors may have important clinical implications in terms of their specificity to desired sleep effects with reduced concerns of off-target effects.

Bettica and co-workers (2012b) reported similar findings to those described herein with the DORA SB-649868 (10 and 30 mg doses) and zolpidem (10 mg); zolpidem and both doses of SB-649868 significantly ($P<0.05$) increased total sleep time in human subjects exposed to a traffic noise model of situational insomnia. However, while zolpidem significantly increased slow-wave sleep and disrupted EEG power spectra during NREM (non-REM) sleep compared with placebo, neither dose of SB-689698 was found to have these effects (Bettica *et al*, 2012b). This similarity between humans and rats suggests that animal models can be used as translational models of EEG spectral frequency assessment within and among sleep/wake states.

Eszopiclone and zolpidem minimally affected active wake when administered during the inactive phase; however, both compounds significantly reduced REM sleep, and moreover, substantially disrupted EEG spectral patterns during all sleep stages in this phase. In common with eszopiclone and zolpidem, DORA-22 did not alter sleep in the inactive phase when compared with within-subject vehicle control. In contrast with the $GABA_A$ modulators, however, DORA-22 did not alter the EEG spectra within sleep/wake states at the low dose and mid-dose.

As noted, DORA-22 has a different mechanism of action to that of the $GABA_A$ modulators, specifically targeting orexin signaling responsible for arousal in neuronal pathways controlling sleep/wake. Orexin peptides cycle with a circadian rhythm, reaching their nadir during prolonged natural sleep. Therefore, blocking of orexin receptor signaling by administration of DORA-22 during the inactive (sleep) phase would be expected to differ little from vehicle administration because of naturally lower endogenous orexin levels at this time (Desarnaud *et al*, 2004; Kiyashchenko *et al*, 2002; Martinez *et al*, 2002; Saper *et al*, 2005).

Alteration of EEG frequencies to a similar extent during the active and inactive phases by eszopiclone or zolpidem suggests that $GABA_A$ modulators that induce sleep have consistent effects on neuronal activity within the brain, and that brain activity in rats during compound-induced sleep differs from brain activity occurring during physiologically normal sleep. Furthermore, it appears that the compound-induced EEG spectral pattern of sleep in the active or inactive phase is similar with $GABA_A$ modulators, irrespective of the natural wake drive during the active phase, thus demonstrating a consistent effect on neuronal network activity independent of circadian phase. The consistency of this effect on EEG activity during both the active and inactive phases, and across sleep/wake states, suggests a general and widespread $GABA_A$ receptor activity enhancement throughout the brain. In contrast to eszopiclone and zolpidem, DORA-22 did not suppress REM sleep at any dose during the active or inactive phases, suggesting a potential adverse effect of $GABA_A$ modulators with respect to memory consolidation and learning (Karni *et al*, 1994; Mintzer *et al*, 1997; Smith *et al*, 2004). Furthermore, DORA-22 did not reduce the duration of time spent in REM sleep,

nor did it substantially change the EEG spectral frequency within REM sleep compared with normal vehicle controls, again suggesting that DORA-22 may not have the same memory-related adverse effects as the $GABA_A$ modulators.

Taken together, these data suggest that DORAs promote sleep that is more similar to physiological sleep and quantitatively different from the sleep induced by $GABA_A$ modulators. There is no specific evidence that the observed spectral EEG changes with $GABA_A$ modulators are a precursor to or are direct evidence of the AEs associated with these compounds. However, it has been reported that changes in the frequencies involved in sleep spindles (or sigma power bands; bursts of oscillatory brain activity during stage 2 sleep) are related to amnesia, changes in the beta band are related to sensorimotor control and cognition, and changes in the slower delta band are related to subjective sleep complaints (Espa *et al*, 2000; Krystal *et al*, 2002; Spiegelhalder *et al*, 2012). In humans, the distinct typical spectral EEG change in delta sleep induced by benzodiazepine and benzodiazepine-like hypnotics, including zolpidem and zopiclone, have been well characterized (Achermann and Borbely, 1987; Aeschbach *et al*, 1994; Feige *et al*, 1999; Lundahl *et al*, 2012; Trachsel *et al*, 1990).

CONCLUSION

In summary, spectral EEG frequency analysis is a useful analytical technique for differentiating sleep/wake state changes for sleep-inducing insomnia treatments. It has been demonstrated here that DORAs promote sleep that is more similar in spectral frequency distribution characteristics to normal sleep in rats after administration of a vehicle control, and is different to the sleep induced by the GABA modulators eszopiclone and zolpidem. Further clinical studies will reveal the translatability of these preclinical findings to compound-induced changes in human frequency distributions within sleep/wake states and the underlying meaning of these EEG frequency changes.

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