EEG POWER SPECTRAL DENSITY OF THE OREXIN RECEPTOR ANTAGONIST SUVOREXANT

Electroencephalographic Power Spectral Density Profile of the Orexin Receptor Antagonist Suvorexant in Patients with Primary Insomnia and Healthy Subjects

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Study Objectives: Suvorexant, an orexin receptor antagonist, improves sleep in healthy subjects (HS) and patients with insomnia. We compared the electroencephalographic (EEG) power spectral density (PSD) profile of suvorexant with placebo using data from a phase 2 trial in patients with insomnia. We also compared suvorexant's PSD profile with the profiles of other insomnia treatments using data from 3 HS studies

Design: Phase 2 trial—randomized, double-blind, two-period (4 w per period) crossover. HS studies—randomized, double-blind, crossover.

Setting: Sleep laboratories.

Participants: Insomnia patients (n = 229) or HS (n = 124).

Interventions: Phase 2 trial—suvorexant 10 mg, 20 mg, 40 mg, 80 mg, placebo; HS study 1—suvorexant 10 mg, 50 mg, placebo; HS study 2—gaboxadol 15 mg, zolpidem 10 mg, placebo; HS study 3—trazodone 150 mg, placebo.

Measurements and Results: The PSD of the EEG signal at 1–32 Hz of each PSG recording during nonrapid eye movement (NREM) and rapid eye movement (REM) sleep were calculated. The day 1 and day 28 PSD profiles of suvorexant at all four doses during NREM and REM sleep in patients with insomnia were generally flat and close to 1.0 (placebo) at all frequencies. The day 1 PSD profile of suvorexant in HS was similar to that in insomnia patients. In contrast, the other three drugs had distinct PSD profiles in HS that differed from each other.

Conclusions: Suvorexant at clinically effective doses had limited effects on power spectral density compared with placebo in healthy subjects and in patients with insomnia, in contrast to the three comparison insomnia treatments. These findings suggest the possibility that antagonism of the orexin pathway might lead to improvements in sleep without major changes in the patient's neurophysiology as assessed by electroencephalographic.

Keywords: gaboxadol, orexin, polysomnography, qEEG, suvorexant, trazodone, zolpidem

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INTRODUCTION

Insomnia remains a significant and undertreated medical disorder despite a long history of drug development efforts.¹ Comorbidity of insomnia with a variety of other disorders is high, further complicating treatment. Most current insomnia drugs act through the inhibitory gamma-aminobutyric acid (GABA) receptor in the brain, typically either as an agonist or as a positive allosteric modulator of the benzodiazepine receptor at the GABA_A receptor complex.² One effect of increasing inhibitory tone in the brain is to induce sleep, but this mechanism of sleep induction differs from the normal physiological sleep-inducing mechanisms and can cause side effects because of general central nervous system suppression. Other non-GABA receptor-mediated hypnotic agents have widespread activity on multiple neurotransmitters, and in the case of trazodone, in addition to its antihistaminergic effect, its antagonist activity on the serotonin (5-HT_{2A}) and adrenergic (α 1) receptors also leads to its antidepressant and anxiolytic action.^{3,4}

The orexin (hypocretin) system, in contrast, is a normal physiological mechanism for inducing wakefulness, acting via the action of orexin peptides that bind to two orexin excitatory receptors (OX1R, OX2R) present in key parts of the brain.^{5,6} High endogenous levels of orexins are found during the active

phase of the circadian cycle and low levels are found during the inactive phase across many mammalian species.^{7,8} Antagonists of OX1R and OX2R elicit somnolence in rodents, dogs, and humans.⁹

Suvorexant (MK-4305) is a selective orexin receptor antagonist, with similar potency at both orexin receptors.¹⁰ It promotes sleep as measured by polysomnographic (PSG) sleep scoring of electroencephalographic (EEG) data in rats, dogs, and non-human primates.¹¹ In humans, suvorexant has been shown to promote sleep in both healthy subjects at doses of 10–100 mg¹² and insomnia patients at doses of 10–80 mg.^{13–15} In elderly or nonelderly patients with insomnia, suvorexant was effective at improving sleep induction and maintenance, and these benefits were maintained over 3 mo of nightly dosing in two pivotal phase 3 trials,^{14,15} and up to 1 y in a long-term phase 3 trial.¹⁶ In these studies suvorexant was well tolerated and did not show rebound or withdrawal upon discontinuation.

Studies of insomnia therapies may use PSG recordings to objectively quantitate sleep. The usual method of analysis is to review the EEG signals in the PSG recordings, and assign a sleep-wake stage score to each 30-sec epoch using standardized sleep scoring rules.^{17,18} The sleep stage scores are then used to compute standard sleep endpoints such as latency to persistent sleep and wake after sleep onset. These types of sleep measurements are referred to as PSG endpoints. EEG activity during sleep can also be quantitated by objectively calculating the power spectral density (PSD) directly from the EEG signal. These types of sleep measurements are referred to as quantitative EEG (qEEG) endpoints. The conventional thinking in the sleep community is that sleep drugs generally reduce the “wake EEG signal”, exemplified by increasing the PSD in the

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Table 1—Summary of the randomized, double-blind, placebo-controlled studies included in the power spectral density analyses.

Study	Subjects	Number in analysis	Design	Treatments	Duration	Time administered	PSG recording
Phase 2 trial ¹³	Insomnia patients (men and women)	229	Two-period crossover in four subgroups	SG1: suvorexant 10 mg, placebo SG2: suvorexant 20 mg, placebo SG3: suvorexant 40 mg, placebo SG4: suvorexant 80 mg, placebo	1 dose per night for 28 nights	30 min before lights-off	8 h starting at usual bedtime on day 1 and day 28
HS study 1 ¹²	Healthy men	18	Four-period crossover	Suvorexant 10mg, 50mg, 100mg, placebo	Single dose	60 min before lights-off	8 h starting at usual bedtime
HS study 2 ²²	Healthy men and women	82	Five-period crossover using a 4-h phase advance model of transient insomnia	Gaboxadol 5 mg, 10 mg, 15 mg, zolpidem 10 mg, placebo	Single dose	30 min before lights-off	8 h starting at a bedtime 4 h earlier than usual bedtime
HS study 3 (unpublished)	Healthy men and women	24	Two-period crossover	Trazodone 150 mg, placebo	Single dose	30 min before lights-off	8 h starting at 23:00 bedtime

HS, healthy subjects; PSG, polysomnography; SG, subgroup.

low frequency band (i.e., delta band). To date, insomnia medications that have effects on PSG endpoints have usually been found to have effects on qEEG endpoints during sleep. Several pharmacological studies with the GABA-modulating drugs zolpidem and gaboxadol, and the orexin receptor antagonist SB-649868 (see Van Bommel et al.¹⁹ for trazodone), have analyzed the EEG PSD during nonrapid eye movement (NREM) sleep.^{20–22} The results of these studies show changes in the PSD of the EEG signals with the GABA-modulating drugs during NREM sleep. Zolpidem changes the EEG PSD differently from gaboxadol, potentially reflecting their different binding activity and selectivity on the GABA receptor, but both drugs still have significant effects at frequencies less than 15 Hz.^{21,22} In contrast, minimal changes in the EEG PSD profile during NREM sleep have been reported with the orexin antagonist SB-649868,²⁰ although orexin antagonists have clearly demonstrated efficacy in improving both objective (PSG) and subjective (patient-reported) sleep measurements.^{13–15,23,24}

The primary objective of the current study was to describe the effect of suvorexant on the PSD profile in patients with primary insomnia, using data from a previous phase 2 clinical trial. Next, we compared the PSD profile of suvorexant with those of other insomnia treatments in healthy subjects (HS), using data from three separate studies. The effects of gaboxadol and zolpidem on the PSD profiles during NREM sleep from one of these studies have been previously published.²²

METHODS

The current study comprised a *post hoc* analysis of data from four separate randomized, double-blind, placebo-controlled studies (Table 1). A consistent processing and analytic approach was applied to data from all studies to facilitate comparisons. The first dataset was from a 4-w, phase 2 trial of four doses of suvorexant (10 mg, 20 mg, 40 mg, and 80 mg) in patients with insomnia.¹³ The PSD profiles of the four suvorexant doses were evaluated on the first night and at the end of 4 w of treatment

during NREM and during rapid eye movement (REM) sleep. The other three datasets, i.e. HS study 1,¹² HS study 2,²² and unpublished HS study 3, were 1-night studies evaluating different sleep drugs in HS. Suvorexant 10 mg, 50 mg, and 100 mg were evaluated in HS study 1, zolpidem 10 mg and gaboxadol 5 mg, 10 mg, and 15 mg were evaluated in HS study 2, and trazodone 150 mg was evaluated in HS study 3. The current analysis of HS study-1 focused on the 10 mg and 50 mg doses of suvorexant that approximate the doses of 20 mg and 40 mg evaluated in phase 3 clinical trials.^{14,15} The analysis of HS study 2 focused on the 15-mg dose of gaboxadol shown to be effective in phase 3 clinical trials.^{25,26} Summary information for each study is provided in Table 1. Further details are provided in the corresponding publications. Each study was approved by the appropriate institutional review boards and regulatory agencies for each site. Informed consent was obtained from all patients and subjects.

Power Spectral Density Calculation

PSG recordings were obtained from the four studies conducted at sites in North America and Europe. Sites often had different PSG recording equipment, software, and system settings. However, all sites were required to follow a technical procedure detailed in a standard PSG operation manual, which specified the key technical parameters, such as the acceptable minimal sampling rates of the EEG channels, the impedance of each EEG electrode, the settings of the antialiasing low-pass filters, etc. Placement of electrodes was per the International 10-20 system.

PSG recordings were saved in European Data Format. Each PSG recording includes multiple channels of different types of physiological signals, such as EEG, electro-oculogram (EOG), electromyogram (EMG), and electrocardiogram (EKG) signals. EEG signals were our primary interest, because they are an indicator of brain activity.

Segments of EEG signals containing severe artifacts/interference were rejected using high-throughput software that was

previously validated in six clinical sleep studies with sample sizes ranging from 18 to more than 1,000 and has been used in published spectral analyses.^{27,28} Different types of EEG artifacts exist in the sleep EEG recordings, such as muscle activity-induced artifacts, eye movement-induced artifacts, EKG-induced artifacts, artifacts caused by loose electrodes, etc. The different origins of these artifact types and that of the EEG signals lead to distinguishable features between different artifacts and the true EEG signals, which enable a high-throughput software system to detect and reject each type of artifact separately using an algorithm specially designed for that artifact type.

After the artifact rejection stage, Welch's method²⁹ was used to calculate the PSD of the EEG signals recorded on the C3-A2 EEG channel by using a 4-sec Hamming window, and a 1-sec overlap between two adjacent windows. Both technical and neurophysiological reasons suggest that it is robust and sufficient to use only C3-A2 data for analysis. Among the six EEG channels usually recorded in PSG, data from the two frontal channels are subject to strong ocular artifacts, especially during REM sleep, whereas data from the two occipital channels are sometimes subject to strong muscle or sweating-induced artifacts. In contrast, data at the central channels, e.g., C3-A2, are less contaminated by artifacts and more robust for detecting drug effects. In addition, our previous investigations consistently revealed that, during sleep, EEG signals became spatially homogeneous and EEG recorded at different channels showed similar effects. Therefore, EEG effects at C3-A2 are representative of those at other recording sites.

The sequence of PSD over the frequencies ranging from 1 Hz to 32 Hz forms a PSD profile. Each PSG recording produces a PSD profile over a given period of time (8 h for the current analyses). The calculated PSD profiles of each subject, represented as curves over the frequency domain, were then reviewed by EEG experts, blinded to treatment, to determine if any PSG recordings should be completely rejected because of severe artifacts undetected by the automated high-throughput procedure. This human review generally led to about 5–8% of the PSG recordings being labeled as abnormal recordings. After unblinding with the treatment information, the EEG experts rechecked these abnormal recordings to make sure the “abnormalities” were not likely caused by treatment. The rejection rates were similar for placebo and active treatments.

Sleep PSD profiles were defined as the PSD calculated separately from EEG recorded during NREM sleep (i.e., sleep stage 1, 2, 3, or 4) and during REM sleep. Not distinguishing NREM sleep stages to produce a single PSD profile is justified by studies showing that the effects of sleep manipulations, such as sleep deprivation and pharmacological intervention, on EEG PSD are similar across stages.^{30,31} Thus, limited extra information is likely to be derived from analyzing PSD separately for each stage. In addition, combining all NREM sleep leads to a more reliable calculation of the PSD profile.

A PSD profile is composed of the PSDs for the individual 1 Hz frequency bin from 1 Hz to 32 Hz. The large number of elements poses methodological challenges to statistical analysis so, in addition to analyzing each frequency separately, the PSD data obtained from each EEG recording were also represented as the average PSD at six frequency bands. The definitions of frequency bands used in this study were as follows: delta band

(1–4 Hz), theta band (5–8 Hz), alpha band (9–12 Hz), sigma band (13–15 Hz), beta band (16–24 Hz), and gamma band (25–32 Hz). Although drug effects by frequency bands are regularly reported in the literature, such reports may be problematic. First, if a drug effect peaks at a frequency that is around the border of two frequency bands, the size of drug effect based on frequency bands can be underestimated. Second, different publications have used different frequency band definitions, which can cause confusion in reported drug effects. Therefore, it is desirable to report drug effects on the entire PSD profile as well as by prespecified frequency bands.

Statistical Analysis

The current analysis was performed on a *post hoc* basis. A consistent approach was applied to the data from all four studies to facilitate and enhance the validity of the comparisons. The PSD data directly calculated from the EEG signals, also called the absolute PSD, were the focus of the analysis. Relative PSD data, which are calculated by dividing the absolute PSD over the mean-square values of the PSD across a range of frequencies or at certain frequency bands, have been reported in some publications, but were not analyzed here because it has been widely observed that the relative PSD can obscure effects that cause changes in overall EEG amplitude. Prior to statistical analysis, a log-transformation was applied to the absolute PSD data to approximate a gaussian distribution, as required by the statistical models used. For each study dataset, a separate statistical model was fitted. The estimates of the relevant drug effects were obtained from the fitted models. The estimated drug effects are presented in the same table or overlaid in the same plot to facilitate the comparison between different doses of suvorexant, or between suvorexant and other insomnia drugs. Among the four study datasets, some had baseline recordings, whereas the others did not. In order to make the models comparable, baselines were not included in the models.

As previously mentioned, each EEG recording can produce two types of PSD vectors: a six-element PSD vector of six frequency bands, or a 32-element PSD profile of 32 1 Hz frequency bins. These two types of PSD data were analyzed with two separate statistical models. The major difference between these two models is that, when modeling the former type, the frequency factor is treated as a six-level categorical variable, and each level represents a frequency band. In contrast, when modeling the latter type, the frequency is treated as a 32-level categorical variable, and each level represents an individual 1 Hz frequency bin.

The PSD data obtained from each study dataset form a complex hierarchical data structure. Each PSD vector is composed of either six or 32 numbers, and several PSD vectors can be produced from the same subject in the study. Thus, the linear mixed-effects approach³² was used to better model this hierarchical structure. The model contained both fixed-effect factors, which are equivalent to the independent variables in a linear model, and the random effects that explain the known variability in the data. In the model, the fixed-effect factors included frequency, treatment, frequency-by-treatment interaction, period, and sex, if applicable. The random effect modeled the between-subject variability at different frequencies. The frequency was treated as a categorical variable; because the relationship between PSD

and frequency is nonlinear, frequency was not included as a single numerical variable in the model. Model convergence issues occurred in some models when the frequency variable was treated as a 32-level categorical variable during modeling of the PSD profile, because of the large number of model parameters to estimate. Therefore, following statistical approaches similar to those previously reported,³³ a set of B-spline functions³⁴ were incorporated in the model to replace the 32-level categorical individual frequency variable.

The drug effects on qEEG power estimated from the fitted models were computed in two ways: (1) the mean differences (on a logarithmical scale) between PSD profiles of the drug and those of the placebo for the six frequency bands in Tables 2–4, and (2) the ratio of mean PSD profile of drug to that of placebo in Figures 1–3 for the 32 individual frequencies. In both cases, a multiple comparison issue arose. In order to address this, the covariance matrix of the simultaneous comparisons for each drug was extracted from the fitted statistical models. Under the asymptotically gaussian assumption, the multiple comparison issue was then addressed by controlling the family-wise error rate with the simultaneous confidence intervals in the figures or adjusted P values for significant claims in the results tables.³⁵

RESULTS

Subjects

In the phase 2 trial, 254 primary insomnia patients were enrolled and 229 patients (94 men, 135 women) with a mean age of 45 y (range, 19–65 y) were included in the PSD analyses of suvorexant 10 mg, 20 mg, 40 mg, and 80 mg; 25 patients were excluded from analyses because of either missing PSG data or sleep scores. In HS study 1, 22 men were enrolled, and 18 men with a mean age of 30 y (range, 18–44 y) were included in the PSD analyses of suvorexant 10 mg and 50 mg; four subjects were excluded from analyses because of either failing to complete the study per protocol or missing PSG data. In HS study 2, 109 subjects were enrolled and 82 subjects (37 men, 45 women) with a mean age of 32 y (range, 18–58 y) were included in the PSD analyses of gaboxadol 15 mg and zolpidem 10 mg; 27 subjects were excluded from analyses because of failure to complete the study, a positive urine drug screen during treatment, or missing PSG data. In HS study 3, 24 subjects (11 men, 13 women) with a mean age of 29 y (range, 19–45 y) were enrolled and all were included in the PSD analyses of trazodone 150 mg during REM sleep, whereas one was excluded from analyses during NREM sleep because data quality was extremely poor.

PSD Profiles of Suvorexant in Patients with Primary Insomnia

The estimated effects of four doses of suvorexant on EEG PSD profiles in patients with primary insomnia during NREM and during REM sleep on treatment day 1 are shown in Figure 1. Their effects on the mean difference of PSDs at six frequency bands on day 1 are shown in Table 2. The day 1 PSD profile of the suvorexant effect at all four doses during both NREM and REM sleep was generally flat and close to 1.0 at all frequencies. Except for the highly variable, very low frequency range (i.e., 1–2 Hz), the estimated effects of suvorexant on EEG PSD for all four doses during NREM sleep were no larger or smaller than 7% of those of the placebo, and during REM sleep no larger or

smaller than 10% of those of the placebo. These results suggest that suvorexant's effect on sleep EEG PSD profile on day 1 was comparable to that of placebo at all four doses, and no dose response was apparent.

The estimated effects of the four doses of suvorexant on EEG PSD profiles in patients with primary insomnia during NREM and REM sleep after 28 days of treatment are shown in Figure 2. The effects of suvorexant on the mean difference of PSDs at six frequency bands after 28 days of treatment are shown in Table 3. The day 28 PSD profiles of suvorexant were generally flat, although they showed greater variability than on day 1. Results in Figure 2 and Table 3 suggested that suvorexant might lower PSD at the higher frequency range. Note that, although the estimated drug effects in Table 3 regarding PSD at frequency bands, and those in Figure 2 regarding PSD at individual frequencies, show the same overall drug effect direction, the conclusions regarding statistical significance do not fully align. Results in Table 3 show that suvorexant 40 mg statistically significantly lowered PSD at the sigma frequency band, whereas those in Figure 2 show that suvorexant 40 mg significantly lowered PSD at 14 Hz with a larger drug effect, and suvorexant 20 mg significantly lowered PSD at many frequencies above 15 Hz with a relatively smaller drug effect. This discrepancy in significance is caused by differences in data types (i.e., mean PSD at frequency bands versus those at individual frequencies), statistical models, and multiplicity adjustment procedures. Even where significant findings were observed for suvorexant, the estimated EEG effects for all four doses were still no larger or smaller than 12% of those of the placebo. Also, no dose response was apparent.

Effects of Insomnia Drugs on PSD Profiles in HS

The effects of suvorexant 10 mg and 50 mg, trazodone 150 mg, zolpidem 10 mg, and gaboxadol 15 mg on PSD profile in HS during NREM and during REM sleep are shown in Figure 3. Their effects on PSD at frequency bands are shown in Table 4. The treatment day 1 spectral profile of suvorexant in HS was generally similar to the day 1 profile observed in patients with primary insomnia in Figure 1 and Table 2, being flat and close to placebo at all frequencies at both doses. In contrast, the day 1 spectral profiles of the other three drugs were not flat. The results demonstrated that each drug significantly increased or decreased the PSD of sleep EEG relative to placebo at different frequency ranges. During NREM sleep, trazodone 150 mg significantly increased (> 15%) the EEG PSD at the sigma frequency band, zolpidem 10 mg significantly decreased (> 25%) the PSD around the theta band and significantly increased (> 15%) the PSD at the sigma band, and gaboxadol 15 mg significantly increased (> 20%) the PSD at the delta and theta frequency bands. During REM sleep, zolpidem 10 mg significantly decreased (> 16%) the PSD around the alpha band, and gaboxadol 15 mg significantly increased (> 25%) the PSD at the delta and theta frequency bands.

DISCUSSION

In this study, we analyzed qEEG data in human subjects undergoing different pharmacologic treatments for insomnia. All studies used randomized, double-blind, placebo-controlled, and crossover designs and PSG data were acquired according

Figure 1—Power spectral density ratio of four doses of suvorexant to placebo in patients with primary insomnia: day 1. NREM, nonrapid eye movement; REM, rapid eye movement.

Table 2—Day 1 quantitative electroencephalogram power spectral density difference of suvorexant versus placebo in patients with insomnia.

Sleep state	Frequency bands	Estimated suvorexant effect, estimate [SE]			
		10 mg	20 mg	40 mg	80 mg
NREM sleep	Delta (1–4 Hz)	0.07 [0.07]	-0.12 [0.07]	0.07 [0.07]	-0.01 [0.08]
	Theta (5–8 Hz)	-0.01 [0.03]	-0.02 [0.05]	-0.04 [0.06]	-0.01 [0.06]
	Alpha (9–12 Hz)	-0.02 [0.04]	-0.03 [0.04]	-0.01 [0.05]	-0.01 [0.06]
	Sigma (13–16 Hz)	0.01 [0.04]	-0.04 [0.04]	-0.02 [0.05]	0.04 [0.06]
	Beta (17–24 Hz)	-0.01 [0.04]	0.01 [0.05]	-0.05 [0.04]	0.01 [0.05]
	Gamma (25–32 Hz)	-0.02 [0.05]	-0.02 [0.06]	-0.05 [0.04]	0.03 [0.05]
REM sleep	Delta (1–4 Hz)	-0.03 [0.08]	-0.08 [0.09]	0.05 [0.1]	-0.08 [0.1]
	Theta (5–8 Hz)	-0.04 [0.04]	-0.01 [0.05]	-0.03 [0.07]	0.03 [0.06]
	Alpha (9–12 Hz)	-0.04 [0.04]	0.01 [0.05]	-0.03 [0.06]	-0.01 [0.07]
	Sigma (13–16 Hz)	0.00 [0.04]	-0.01 [0.04]	-0.03 [0.05]	0.01 [0.05]
	Beta (17–24 Hz)	0.00 [0.04]	-0.01 [0.04]	0.01 [0.04]	0.02 [0.04]
	Gamma (25–32 Hz)	0.02 [0.04]	0.03 [0.07]	0.02 [0.04]	0.04 [0.06]

No estimates are statistically significant after adjustment for multiplicity. The power spectral density data are on a logarithmic scale. NREM, nonrapid eye movement; REM, rapid eye movement.

Table 3—Day 28 quantitative electroencephalogram power spectral density difference between suvorexant and placebo in patients with insomnia.

Sleep state	Frequency bands	Estimated suvorexant effect, estimate [SE]			
		10 mg	20 mg	40 mg	80 mg
NREM sleep	Delta (1–4 Hz)	-0.04 [0.06]	0.03 [0.08]	0.03 [0.07]	0.03 [0.07]
	Theta (5–8 Hz)	-0.03 [0.04]	-0.04 [0.05]	-0.05 [0.05]	0.05 [0.05]
	Alpha (9–12 Hz)	0.00 [0.04]	-0.06 [0.04]	-0.04 [0.04]	0.00 [0.05]
	Sigma (13–16 Hz)	-0.04 [0.05]	-0.08 [0.04]	-0.15 [0.04]	-0.02 [0.04]
	Beta (17–24 Hz)	-0.02 [0.04]	-0.08 [0.04]	-0.09 [0.04]	-0.02 [0.04]
	Gamma (25–32 Hz)	-0.03 [0.04]	-0.11 [0.05]	-0.05 [0.05]	-0.06 [0.06]
REM sleep	Delta (1–4 Hz)	-0.04 [0.09]	-0.01 [0.07]	-0.01 [0.1]	0.03 [0.09]
	Theta (5–8 Hz)	-0.04 [0.05]	0.01 [0.05]	-0.07 [0.05]	0.13 [0.07]
	Alpha (9–12 Hz)	0.02 [0.04]	-0.05 [0.04]	-0.05 [0.04]	0.08 [0.06]
	Sigma (13–16 Hz)	0.01 [0.04]	-0.07 [0.04]	-0.06 [0.04]	0.01 [0.05]
	Beta (17–24 Hz)	-0.03 [0.04]	-0.05 [0.04]	-0.09 [0.05]	0.05 [0.05]
	Gamma (25–32 Hz)	0.00 [0.05]	0.00 [0.05]	-0.10 [0.07]	0.01 [0.06]

The bolded estimate is statistically significant ($P < 0.05$, adjusted for multiplicity). The power spectral density data are on a logarithmic scale. NREM, nonrapid eye movement; REM, rapid eye movement.

to a common standard operating procedure. PSG was recorded at nighttime when subjects were sleeping or attempting to sleep. Furthermore, a consistent data processing and analytic approach was used over all four datasets to enhance the validity of the comparisons. The resulting PSD profiles provide a measure of brain activity, controlled for sleep state (NREM and REM). The drug doses used in the studies have all been shown to improve sleep compared to placebo.^{4,12–16,25,26,36} The evidence base for trazodone efficacy in the treatment of insomnia, which is often used off-label, is weaker than for the other drugs but a relatively high dose of 150 mg was evaluated.^{4,37,38} Although good quality data from randomized controlled trials directly comparing each treatment is lacking, the efficacy of the treatments and doses we studied on improving sleep appears broadly comparable.

Following treatment with each of four doses of suvorexant, the PSD profiles were largely unchanged compared to placebo during both NREM and REM sleep in patients and HS. The amount of REM sleep has been reported to be increased by higher doses of orexin antagonists in several studies,^{13,23} but no effect of suvorexant on the PSD profile during REM sleep was observed here. A range of suvorexant doses were evaluated. Evidence from clinical trials and HS suggests that higher doses of suvorexant are more effective than lower doses at improving sleep.^{13–15} In the current analyses in both patients and HS, there was no suggestion of a dose response in changes in suvorexant PSD profiles when compared with placebo. The lack of effect of suvorexant on PSD during sleep in humans is supported by data from a study of another orexin receptor antagonist, SB-649868.²⁰

Figure 2—Power spectral density ratio of four doses of suvorexant to placebo in patients with primary insomnia: day 28. Bolded segments with triangle markers are the frequency ranges where the difference between drug and placebo are statistically significant ($P < 0.05$, adjusted for multiplicity). NREM, nonrapid eye movement; REM, rapid eye movement.

Figure 3—Power spectral density ratio of suvorexant, gaboxadol, zolpidem, and trazodone to placebo in healthy subjects: day 1. Bolded segments with triangle markers are the frequency ranges where the difference between drug and placebo are statistically significant ($P < 0.05$, adjusted for multiplicity). NREM, nonrapid eye movement; REM, rapid eye movement.

Table 4—Day 1 quantitative electroencephalogram power spectral density difference of suvorexant, gaboxadol, zolpidem, and trazodone versus placebo in healthy subjects.

Sleep state	Frequency bands	Estimated drug effect, estimate [SE]				
		SUV 10 mg	SUV 50 mg	GAB 15 mg	ZOL 15 mg	TRA 150 mg
NREM sleep	Delta (1–4 Hz)	0.01 [0.06]	0.06 [0.06]	0.22 [0.04]	0.02 [0.04]	0.00 [0.08]
	Theta (5–8 Hz)	0.05 [0.04]	0.06 [0.04]	0.23 [0.04]	-0.27 [0.04]	0.11 [0.05]
	Alpha (9–12 Hz)	0.05 [0.08]	0.08 [0.07]	-0.03 [0.03]	-0.15 [0.03]	-0.05 [0.06]
	Sigma (13–16 Hz)	0.04 [0.05]	0.06 [0.05]	-0.10 [0.04]	0.16 [0.03]	-0.17 [0.05]
	Beta (17–24 Hz)	0.02 [0.07]	0.05 [0.06]	-0.02 [0.04]	-0.04 [0.04]	0.06 [0.06]
	Gamma (25–32 Hz)	0.01 [0.08]	-0.02 [0.08]	0.03 [0.04]	0.01 [0.04]	0.24 [0.09]
REM sleep	Delta (1–4 Hz)	0.13 [0.07]	0.08 [0.07]	0.25 [0.05]	0.05 [0.05]	0.03 [0.09]
	Theta (5–8 Hz)	0.06 [0.04]	0.08 [0.04]	0.35 [0.04]	-0.07 [0.04]	0.06 [0.05]
	Alpha (9–12 Hz)	0.09 [0.06]	0.10 [0.05]	0.24 [0.04]	-0.17 [0.04]	-0.02 [0.04]
	Sigma (13–16 Hz)	0.06 [0.05]	0.07 [0.05]	0.07 [0.04]	0.11 [0.04]	-0.07 [0.04]
	Beta (17–24 Hz)	0.04 [0.04]	0.08 [0.04]	0.07 [0.04]	0.09 [0.04]	-0.09 [0.04]
	Gamma (25–32 Hz)	0.03 [0.04]	0.09 [0.04]	0.12 [0.04]	0.09 [0.04]	0.02 [0.06]

The bolded estimates are statistically significant ($P < 0.05$, adjusted for multiplicity). The power spectral density data are on a logarithmic scale. GAB, gaboxadol; NREM, nonrapid eye movement; REM, rapid eye movement; SUV, suvorexant; TRA, trazodone; ZOL, zolpidem.

In contrast to the lack of effect of suvorexant on PSD, the sleep drugs that act by nonorexinergic mechanisms (modulation of GABA or serotonin and adrenergic receptors) all showed characteristic PSD profiles that differed from placebo, and from each other, during both NREM and REM sleep in HS. As previously reported,^{21,22} the two drugs acting on the GABA system, zolpidem and gaboxadol, both had marked effects on EEG activity in the lower frequency ranges: zolpidem reduced activity in the lower frequency ranges whereas gaboxadol increased activity in the same ranges. The differing effects of gaboxadol and zolpidem may be reflective of their differing subunit receptor specificities and binding patterns on the GABA_A receptor complex.^{21,39} The changes in qEEG during NREM in a phase-advanced model of insomnia in HS, reported here for gaboxadol and zolpidem, match well with results for these two drugs in patients with insomnia at regular bedtime.^{21,28} Similarly, the effect of a single nighttime dose of 10 mg zolpidem in a traffic noise model of insomnia in HS²⁰ is similar to the results reported here. Zolpidem has also been shown to reduce low-frequency activity in HS during normal sleep.⁴⁰ The current analysis shows that zolpidem and gaboxadol altered REM PSD profiles in a similar manner to NREM PSD profiles, raising the question as to whether these are sleep effects mediating qEEG changes or direct effects on EEG.

The antidepressant trazodone has previously shown a characteristic reduction of midfrequencies in the qEEG signal during nighttime NREM sleep in depressed patients.¹⁹ The trazodone data from the current study showed a similar pattern of change during NREM sleep in normal subjects, but minimal effects during REM sleep. Thus, it appears that the three nonorexinergic insomnia drugs showed effects consistent with previous reports, regardless of whether subjects were healthy or patients, and regardless of the design (e.g., phase-advanced).

Although the PSD profile of patients with insomnia has not yet been adequately characterized, recent data have suggested that patients with insomnia may show increases in the higher

frequencies of the PSD profile during NREM sleep relative to controls without insomnia.^{41,42} These findings have been interpreted as being supportive of a hyperarousal model of insomnia.^{41,42} Other parameters associated with sleep, such as restfulness, dreams, etc., have not been strongly tied to specific frequencies in the EEG. It is important to note that many studies on PSD differences between patients with and controls without insomnia have been based on relatively small samples with varying inclusion criteria, and have not typically examined the effects of important factors such as age, sex, and sleep period of the night. These limitations may be responsible for the conflicting results reported not only with respect to the magnitude but also in the direction of differences. Recent results^{43,44} based on large samples and diverse populations of patients and controls suggest that there is no single PSD profile “signature” defining the PSD differences between patients with insomnia and controls without insomnia. Rather, these profiles may vary depending on at least three major factors: sex, age, and sleep period of night. For example, the increase in the high-frequency range of the aforementioned PSD profile was found in female but not male patients with insomnia when compared with controls without insomnia matched by age and sex. Another example is that the lower frequency range of the PSD profile is suppressed in patients with insomnia but only in the early sleep periods. In both examples the magnitude of the PSD differences between patients with insomnia and controls without insomnia strongly depends on the subject’s age. Overall, the magnitude of the PSD difference between patients with insomnia and controls without insomnia is smaller than that observed between sexes or ages within each group. Furthermore, the clinical implications of the specific changes in the PSD are not clear.⁴⁵ It can be speculated that altering PSD by a drug may not be beneficial overall for improving the sleep of a general insomnia population. A sleep-enhancing drug that significantly alters PSD irrespective of the patients’ age, sex, and sleep period could alter it in

the “wrong” direction for some patient subpopulations or at certain sleep periods. That is, the drug-altered PSD could deviate from the PSD pattern of non-insomnia healthy sleepers. Further research is necessary to address this hypothesis. Currently, all we can conclude is that different sleep-enhancing drugs have different sleep PSD profiles, with orexin receptor antagonists appearing to have little effect on the sleep PSD profile. This conclusion in humans is supported by preclinical observations.⁴⁶

There are a number of limitations to interpretation of the current data. First, this was an exploratory analysis and was subject to the limitations associated with such approaches. Second, the comparison of the HS profiles of the four drugs in HS subjects included data from three different studies, although steps were taken to ensure consistent gathering of PSG data across studies and data were analyzed in a consistent fashion. Finally, as noted previously, the clinical significance, if any, of the differing PSD profiles of each treatment is unknown.

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