

The Effects of Eszopiclone on Sleep Spindles and Memory Consolidation in Schizophrenia: A Randomized Placebo-Controlled Trial

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Study Objectives: In schizophrenia there is a dramatic reduction of sleep spindles that predicts deficient sleep-dependent memory consolidation. Eszopiclone (Lunesta), a non-benzodiazepine hypnotic, acts on γ -aminobutyric acid (GABA) neurons in the thalamic reticular nucleus where spindles are generated. We investigated whether eszopiclone could increase spindles and thereby improve memory consolidation in schizophrenia.

Design: In a double-blind design, patients were randomly assigned to receive either placebo or 3 mg of eszopiclone. Patients completed Baseline and Treatment visits, each consisting of two consecutive nights of polysomnography. On the second night of each visit, patients were trained on the motor sequence task (MST) at bedtime and tested the following morning.

Setting: Academic research center.

Participants: Twenty-one chronic, medicated schizophrenia outpatients.

Measurements and Results: We compared the effects of two nights of eszopiclone vs. placebo on stage 2 sleep spindles and overnight changes in MST performance. Eszopiclone increased the number and density of spindles over baseline levels significantly more than placebo, but did not significantly enhance overnight MST improvement. In the combined eszopiclone and placebo groups, spindle number and density predicted overnight MST improvement.

Conclusion: Eszopiclone significantly increased sleep spindles, which correlated with overnight motor sequence task improvement. These findings provide partial support for the hypothesis that the spindle deficit in schizophrenia impairs sleep-dependent memory consolidation and may be ameliorated by eszopiclone. Larger samples may be needed to detect a significant effect on memory. Given the general role of sleep spindles in cognition, they offer a promising novel potential target for treating cognitive deficits in schizophrenia.

Keywords: Schizophrenia, procedural learning, motor skill, sleep spindles, memory consolidation, automation

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INTRODUCTION

Cognitive deficits are the strongest predictor of functional outcome in schizophrenia,¹ and antipsychotic drugs (APDs) are relatively ineffective in treating them (e.g., Sergi²). Amelioration of cognitive deficits has thus become a priority of the schizophrenia research community and is the focus of large-scale studies.³ A limitation of these efforts is that cognition is measured in cross-section. While this provides a valid snapshot of function, it misses critical aspects of learning and memory that happen offline, over time and with sleep. Following encoding, memories undergo “consolidation” processes that stabilize, enhance, integrate, and reorganize memory in the brain. A wealth of evidence from molecular, cellular, neural network, regional brain activation, and behavioral studies of birds,⁴ rodents,⁵ cats,⁶ and humans⁷ demonstrates that sleep plays a critical role in memory consolidation. This work suggests an evolutionarily conserved function for sleep in memory consolidation. In schizophrenia, there is evidence of both abnormal

sleep⁸ and impaired memory consolidation,⁹ yet little is known about how abnormal sleep contributes to memory consolidation deficits. Understanding this contribution may open new avenues to treatment.

Recent studies find marked impairments (reduced by 67% to 100%) of sleep-dependent consolidation of motor procedural memory¹⁰⁻¹² and dramatically reduced sleep spindle activity¹¹⁻¹⁵ in schizophrenia. Sleep spindles are a defining characteristic of stage 2 NREM sleep, evident in the EEG as brief powerful bursts of 12-15 Hz synchronous activity.¹⁶ We recently reported that the sleep spindle and memory deficits are correlated and hypothesized that the reduction in sleep spindles impairs memory consolidation in schizophrenia.¹⁵ This hypothesis is consistent with evidence from animal studies that sleep spindles are a key mechanism of synaptic plasticity that mediates memory consolidation during sleep¹⁷ and from human studies that link spindles to the sleep-dependent consolidation of both procedural and declarative memory.¹⁸ In the present study, we investigated whether eszopiclone (Lunesta), a non-benzodiazepine hypnotic agent that acts on γ -aminobutyric acid (GABA)ergic neurons in the thalamic reticular nucleus (TRN),¹⁹ where sleep spindles are generated,²⁰ could increase sleep spindles and thereby improve memory consolidation in schizophrenia.

While the cause of the spindle deficit in schizophrenia is unknown, reduced spindle activity may reflect TRN dysfunction, consistent with evidence of TRN abnormalities in schizophrenia.²¹

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The TRN is comprised entirely of GABAergic neurons²² that primarily project to glutamatergic thalamic neurons, which then project to the cortex. Cortical neurons, in turn, send glutamatergic inputs back to N-methyl-D-aspartate acid (NMDA) receptors on TRN neurons. Thus, spindles are mediated by a thalamocortical feedback loop that is regulated by both GABAergic and NMDA-receptor mediated glutamatergic neurotransmission.²³ Eszopiclone acts on GABA_A receptors,²⁴ particularly on subunits preferentially expressed in the TRN.¹⁹ It has demonstrated safety and sustained efficacy in treating insomnia²⁵ and is approved for long-term treatment. The potential for eszopiclone to increase spindles without causing physiologic tolerance or serious withdrawal effects,²⁶ and its apparent lack of detrimental effects on cognition and psychomotor function the following day, make it a viable potential adjunctive agent.^{27,28}

To test the hypothesis that eszopiclone would increase sleep spindles and thereby improve memory consolidation in schizophrenia, we conducted a double-blind, placebo-controlled study. Medically and clinically stable outpatients with schizophrenia participated in Baseline and Treatment visits, each consisting of 2 consecutive nights of polysomnography. We employed the same simple, well-characterized test of motor procedural memory as in our previous studies

of schizophrenia, the finger tapping motor sequence task (MST).^{29,30} Improvement of MST performance occurs after sleep—but not after an equivalent period of wake and MST improvement—as well as overnight improvement on other simple motor skill tasks correlates with the amount of stage 2 NREM sleep³⁰ and with the number and density of sleep spindles.³¹⁻³³ In schizophrenia, overnight MST improvement is markedly reduced,^{10,11} and in the data from the Baseline visit of the present study reported elsewhere,¹⁵ this reduction correlated with spindle number and density, which were also markedly reduced (by 36% and 38%, respectively). Here we report the findings from the Treatment visit examining the effects of eszopiclone versus placebo on stage 2 sleep spindles and overnight MST improvement in patients.

METHODS

Participants

Twenty-five schizophrenia outpatients were recruited from an urban mental health center; 21 completed the study. All had been maintained on stable doses of second-generation APDs for ≥ 6 weeks, and 12 took diverse adjunctive medications for anxiety, agitation, and/or concurrent mood disturbance (see Table S1 in the supplemental material). There were no medication changes during study participation. Potential participants were screened to exclude those with diagnosed sleep disorders, treatment with sleep medications, a history of significant head injury or neurological illness, and a history of substance abuse or dependence within the past 6 months based on patient interview, chart review, and clinician report. Diagnoses were confirmed with Structured Clinical Interviews for DSM-IV³⁴ and symptoms were rated with the Positive and Negative Syndrome Scale.³⁵ All participants gave written informed consent. The study was approved by the institutional review boards of Massachusetts General Hospital and the Massachusetts Department of Mental Health. Participants were randomly assigned in a double-blind fashion to either eszopiclone (3 mg; $n = 10$) or placebo ($n = 11$) groups. The groups did not differ significantly in age, sex, parental education, symptom ratings, or APD dose as measured by chlorpromazine equivalents (Table 1).

Table 1—Demographic characteristics and description of study samples

	Placebo (n = 11)	Eszopiclone (n = 10)	P
Age (years)	34 ± 9	35 ± 10	0.82
Sex (M/F)	10/1	7/3	0.24
Parental Education (years)	15 ± 5	15 ± 5	0.74
Length of Illness (years)	10 ± 8	11 ± 9	0.80
PANSS Positive	12 ± 4	13 ± 6	0.69
PANSS Negative	13 ± 5	16 ± 6	0.22
PANSS General	26 ± 9	31 ± 10	0.27
CPZ equivalents	533 ± 499	290 ± 278	0.19

Means ± SD; PANSS, Positive and Negative Syndrome Scale; CPZ, chlorpromazine.

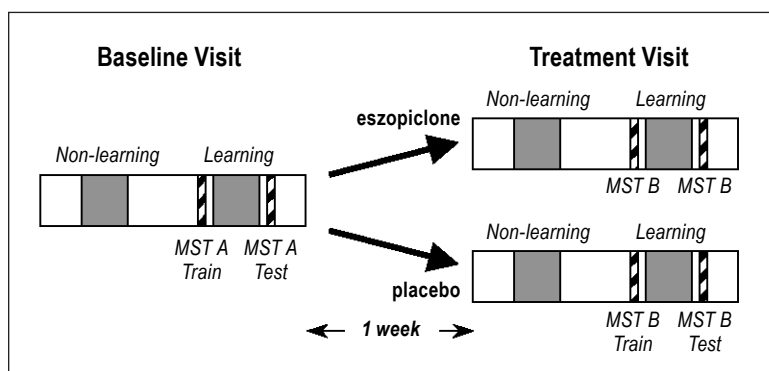


Figure 1—Time course of study. Twenty-one schizophrenia patients participated in Baseline and Treatment visits each consisting of 2 consecutive nights (shaded areas). The visits were separated by one week. On the second (learning) night of each visit they trained on alternate versions of the finger tapping motor sequence task (MST) prior to bedtime and were tested the following morning. For the Treatment visit, they were randomized to placebo or eszopiclone.

Procedures

In the week prior to their Baseline visit to the Clinical Research Center (CRC), participants completed informed consent, demographic questionnaires, and rating scales, and toured the CRC. Baseline and Treatment visits to the CRC were separated by one week (Figure 1). Each visit involved polysomnography (PSG) on 2 consecutive weeknights, with the first night of each visit serving as the non-learning night. On the second (learning) night of each visit, participants were trained on the finger tapping motor sequence task (MST) 1 h prior to their usual bedtime, wired for PSG, and allowed to sleep for up to 10 h. They were tested on the MST 1 h after awakening. Participants engaged in their usual activities during the day. During the Treatment visit, 3 mg of eszopiclone or an identical capsule containing placebo was administered double-blind on both nights after PSG wiring and approximately 30 min prior to retiring.

Polysomnography (PSG)

PSG data were acquired at 100 Hz using an Embla N7000 system (Medcare Systems, Buffalo NY), with a standard montage of EEG (electroencephalography; F3, F4, C3, Cz, C4, Pz, O1, O2) referenced to the linked mastoids, EMG (electromyography), and EOG (electrooculography).

Finger Tapping Motor Sequence Task (MST)

The MST involves pressing 4 numerically labeled keys on a standard computer keyboard with the fingers of the left hand, repeating a 5-element sequence “as quickly and accurately as possible” for 30 s. Different sequences (e.g., 4-1-3-2-4 and 1-4-2-3-1) were employed for the Baseline and Treatment visits in a counter-balanced order across subjects. There is no transfer of learning between sequences on this task.³⁶ The numeric sequence was displayed at the top of the screen, and dots appeared beneath it with each keystroke. During both training and test sessions, participants alternated tapping and resting for periods of 30 s for a total of 12 tapping trials. The primary outcome measure was the number of correct sequences per trial, which reflects both the speed and accuracy of performance. Overnight improvement was calculated as the percent increase in correct sequences from the last 3 training trials to the first 3 test trials the following morning.³⁰ Prior to each MST administration, participants completed the Stanford Sleepiness Scale (SSS) to measure subjective alertness.³⁷

Data Analysis

Sleep Architecture

Each 30-s epoch of PSG sleep was visually classified into stages (Wake, 1, 2, 3, 4, and REM) according to standard criteria³⁸ by raters blind to visit, group, and night. Sleep stages are reported as total min spent in each stage. Sleep efficiency was computed as the total sleep time (TST) divided by the total time in bed. Wake time after sleep onset (WASO) is the number of minutes spent awake between sleep onset and arising from bed. Following AASM criteria, an arousal from sleep was scored whenever there was a shift to waking EEG frequencies lasting ≥ 3 s. Total number of arousals per night, arousals per hour, and the mean duration of arousals were quantified.

Spindle and Stage 2 Spectral Power Measurements

We measured spindles during stage 2 sleep as previously described.¹⁵ PSG data were preprocessed and analyzed using BrainVision Analyzer (v. 2.0, BrainProducts, Munich Germany) and MatLab (v. R2009b, The MathWorks, Natick MA) software. Artifacts were automatically detected and removed and EEG data filtered at 0.5-35 Hz. Artifact rejection was confirmed by visual inspection.

Power spectral density ($\mu\text{V}^2/\text{Hz}$) was calculated by fast Fourier transform (FFT), applying a Hanning window to successive 3-s epochs of stage 2 sleep with 50% overlap. Sigma band (12-15 Hz) spectral power, which correlates with sleep spindle activity, was divided into low (12-13.5 Hz) and high (13.5-15 Hz) sigma band power.^{39,40} We also examined delta (1-4 Hz), theta (4-7 Hz), alpha (8-12 Hz), and beta (15-35 Hz) band spectral power within stage 2 sleep.

Sleep spindles were automatically detected at all electrodes using a wavelet-based algorithm, previously validated against

hand-counted spindles in both patients with schizophrenia and healthy individuals.¹⁵ For each spindle identified at Cz, amplitude, sigma power, frequency, and duration were calculated based on analysis of 2-s EEG epochs centered on the point of spindle detection. Spindle *amplitude* is the maximal voltage following 12-15 Hz band pass filtering of the spindle; *frequency* is the peak spectral power of the spindle following FFT decomposition; *sigma power* is the mean FFT-derived power spectral density across the 12-15 Hz range; and *duration* is calculated as the half-height width of wavelet energy within the spindle frequency range.

Finally, we assessed the sigma coherence during spindles across EEG channels. Coherence (*Coh*) across EEG channels (c_i) was assessed for each 2-s epoch during which a spindle was auto-detected in any channel. Coherence values range from 0-1, calculated as the cross-spectrum (*CS*)-to-autospectrum coherence ratio within the full 12-15 Hz sigma frequency band (f) according to the formula:

$$Coh(c1, c2)(f) = |CS(c1, c2)(f)|^2 / (|CS(c1, c1)(f)| |CS(c2, c2)(f)|)$$

Eszopiclone Effects

We examined the effects of eszopiclone on sleep parameters and overnight improvement of MST performance using repeated measures ANOVAs with factors for Drug Group (Placebo, Eszopiclone) and Visit (Baseline, Treatment) and planned post hoc contrasts. The analysis of sleep parameters also included a factor for Night (Non-learning, Learning), but as there were no significant main effects or interactions with Night, unless otherwise indicated, we report results as the average of the 2 nights of each Visit. For both spindle parameters and MST improvement, we expected to find Drug Group by Visit interactions reflecting that, relative to baseline values, treatment with eszopiclone increased sleep spindles and memory consolidation significantly more than placebo.

To test the relationship between sleep spindles and memory consolidation, spindle number and density were correlated with overnight MST improvement. ANCOVAs were used to test for effects of Drug Group (placebo vs. eszopiclone) on the slope of these relations.

APD Effects

We examined the correlations of APD dosage measured in chlorpromazine equivalents⁴¹ with spindle parameters and overnight MST improvement. Because participants taking clozapine ($n = 9$) exhibited significantly lower spindle densities at Baseline,¹⁵ we examined the effects of eszopiclone on spindle parameters and overnight MST improvement in patients divided by clozapine treatment using repeated measures ANOVAs with factors for Clozapine (No, Yes), Drug Group (Placebo, Eszopiclone), and Visit (Baseline, Treatment).

RESULTS

Sleep Quality and Architecture

There were no significant effects of eszopiclone compared with placebo on any measure of sleep quality or architecture (Table 2). Patients generally slept better during the Treatment visit as indicated by significant increases in TST and

Table 2—Changes in sleep quality, architecture, spindle parameters, and individual spindle characteristics in the eszopiclone compared with the placebo group

	Eszopiclone (n = 10)			Placebo (n = 11)			Visit	Group × Visit
	Baseline	Treatment	P	Baseline	Treatment	P	P	P
Sleep Quality and Architecture								
TST (min)	443 ± 87	487 ± 51	0.07	443 ± 75	491 ± 74	0.02*	0.003*	0.86
WASO (min)	77 ± 61	56 ± 48	0.28	64 ± 59	40 ± 23	0.11	0.06	0.89
Stage 1 (min)	33 ± 16	36 ± 17	0.69	43 ± 24	40 ± 19	0.65	0.92	0.55
Stage 2 (min)	242 ± 53	276 ± 52	0.03*	233 ± 68	280 ± 63	0.01*	0.001*	0.54
SWS (min)	76 ± 34	63 ± 23	0.27	79 ± 59	71 ± 46	0.28	0.12	0.75
REM (min)	86 ± 40	109 ± 25	0.04*	87 ± 20	100 ± 38	0.23	0.02*	0.50
Sleep Efficiency (%)	85 ± 11	90 ± 8	0.20	88 ± 11	92 ± 5	0.08	0.04*	0.92
Total Arousals	99 ± 35	120 ± 49	0.04*	88 ± 66	95 ± 44	0.52	0.06	0.31
Arousal per hour	14.1 ± 5.1	15.0 ± 6.1	0.49	12.1 ± 7.9	11.7 ± 4.9	0.76	0.81	0.49
Arousal duration (sec)	8.3 ± 1.8	8.5 ± 1.3	0.62	8.7 ± 1.3	8.2 ± 1.6	0.30	0.58	0.26
Spindle Parameters								
Spindle Number	370 ± 143	496 ± 207	0.001*	251 ± 125	294 ± 124	0.01*	< 0.001*	0.01*
Spindle Density	1.57 ± 0.56	1.76 ± 0.55	0.01*	1.08 ± 0.43	1.07 ± 0.45	0.91	0.04*	0.03*
Sigma Power (µV ² /Hz)	0.041 ± 0.02	0.050 ± 0.02	0.11	0.034 ± 0.01	0.030 ± 0.01	0.12	0.34	0.02*
Low	0.045 ± 0.02	0.057 ± 0.03	0.08	0.042 ± 0.02	0.038 ± 0.02	0.23	0.25	0.03*
High	0.039 ± 0.02	0.050 ± 0.02	0.17	0.030 ± 0.01	0.027 ± 0.01	0.12	0.54	0.04*
Coherence (0-1)	0.36 ± 0.10	0.37 ± 0.11	0.43	0.34 ± 0.09	0.32 ± 0.01	0.39	0.99	0.23
Characteristics of Individual Spindles								
Amplitude (µV)	17.3 ± 3.7	17.9 ± 3.8	0.01*	16.2 ± 3.2	16.3 ± 2.8	0.76	0.19	0.25
Frequency (Hz)	13.6 ± 0.6	13.4 ± 0.8	0.52	13.0 ± 0.8	12.8 ± 1.0	0.41	0.40	0.62
Duration (sec)	100 ± 16	106 ± 3	0.33	101 ± 17	106 ± 4	0.32	0.56	0.87
Sigma Power (µV ² /Hz)	0.34 ± 0.15	0.35 ± 0.15	0.81	0.28 ± 0.12	0.26 ± 0.10	0.19	0.94	0.48

Means ± SD averaged over the learning and non-learning nights of each visit. TST, total sleep time; WASO, wake time after sleep onset; SWS, slow wave sleep; REM, rapid eye movement sleep. *P < 0.05.

sleep efficiency and a trend towards decreased WASO. There was a trend towards increased arousals at the Treatment visit, which likely reflected the increased TST, since the number of arousals per hour did not change. Patients also showed significant increases in the durations of both stage 2 and REM sleep at the Treatment visit.

Spindle Number, Density, and Coherence

Eszopiclone significantly increased sleep spindle number and density during stage 2 sleep compared to placebo (Figure 2, Table 2). Although greater in the eszopiclone group (34% increase; P = 0.001), the placebo group also showed a significant increase in spindle number (17%, P = 0.01). This likely reflects the increased duration of stage 2 sleep during the Treatment visit, since only the eszopiclone group also showed a significant increase in spindle density (eszopiclone: 11%, P = 0.01; placebo: -1%, P = 0.91).

The effects of eszopiclone on spindle number and density did not appear to be topographically specific, increasing at all electrodes from the Baseline to the Treatment visit (paired t-tests for spindle number: P < 0.02 for all electrodes; spindle density: P < 0.05 at all electrodes except F4, P = 0.14 and O2, P = 0.32). Additionally, the distribution of spindles across the scalp in the eszopiclone group was similar to placebo, with maximal spindle densities at Pz.

Eszopiclone did not significantly affect spindle coherence (Table 2).

Characteristics of Individual Sleep Spindles

Eszopiclone did not affect spindle frequency, duration, amplitude, or sigma power significantly more than placebo (Table 2); there were no significant Drug Group by Visit interactions and no significant main effects of Visit. Although spindle amplitude increased significantly from Baseline only in the eszopiclone group (3.2%, P = 0.01), this increase did not differ significantly from that in the placebo group (0.7%, P = 0.76).

Stage 2 EEG Spectral Power

Consistent with its effects on sleep spindles, eszopiclone significantly increased sigma power during stage 2 sleep compared with placebo. Significant Drug Group by Visit interactions were seen for total spectral power in the sigma-frequency band, as well as for both slow and fast sigma power (Table 2). Although eszopiclone did not significantly alter theta, alpha, or beta power during this same period, it did produce a significant decrease in delta power compared with placebo (Drug Group × Visit interaction: $F_{1,19} = 7.97$, P = 0.01; eszopiclone: $t_9 = -3.09$, P = 0.01, placebo: $t_{10} = -0.004$, P = 0.99).

Overnight MST Improvement

The key finding was that relative to Baseline levels, treatment with eszopiclone did not increase overnight MST improvement significantly more than placebo (Figure 3, Drug Group by Visit interaction, P = 0.55). At the Treatment visit, however, patients taking eszopiclone showed significant overnight MST

improvement (bedtime vs. morning: $21\% \pm 25\%$ SD, $t_9 = 2.64$, $P = 0.03$) that was numerically, but not significantly, greater than their own overnight improvement at the Baseline visit ($8\% \pm 19\%$, $t_9 = 1.39$, $P = 0.20$; Baseline vs. Treatment, $P = 0.12$) as well as that of healthy controls at the Baseline visit ($15\% \pm 13\%$, $t_{16} = 4.92$, $P = 0.0002$ as previously reported¹⁵; eszopiclone patients vs. controls, $P = 0.47$). Patients taking placebo, in contrast, showed no significant overnight improvement during either the Baseline ($-5\% \pm 25\%$, $t_9 = 0.61$, $P = 0.55$) or Treatment visit ($2\% \pm 22\%$, $t_{10} = 0.23$, $P = 0.83$; Baseline vs. Treatment, $P = 0.40$). At the Treatment visit, the placebo group differed from the eszopiclone group at a trend level ($t_9 = 1.89$, $P = 0.07$). There were no Group \times Visit interactions for SSS ratings indicating that eszopiclone did not affect subjective alertness prior to MST administrations differently from placebo (bedtime: $P = 0.81$; morning: $P = 0.80$). Moreover, the eszopiclone and placebo groups did not differ in SSS alertness ratings at either the bedtime (placebo = 2.5 ± 1.4 , eszopiclone = 2.5 ± 1.6 , $P = 0.53$) or morning (placebo = 2.1 ± 0.9 , eszopiclone = 2.4 ± 1.0 , $P = 0.89$) MST sessions.

Relations of Overnight Improvement with Spindle Parameters

On the learning night of the Treatment visit, both spindle number ($r_{21} = 0.45$, $P = 0.04$) and density ($r_{21} = 0.44$, $P = 0.05$) predicted overnight MST improvement in the combined eszopiclone and placebo groups (Figure 4). The slopes of these relations did not differ between groups and the slopes of each group were similar to the slopes of the combined group data. These correlations are similar in magnitude to those observed on the learning night of the Baseline visit (spindle number: $r_{21} = 0.38$, $P = 0.09$; spindle density: $r_{21} = 0.39$, $P = 0.08$). Correlations were not significant when spindle number and density were averaged across the 2 nights of the Treatment visit, or on the non-learning night alone.

APD Effects

APD dosage did not correlate with any spindle parameter or with overnight MST improvement. Whether or not patients took clozapine however, significantly modulated the effect of eszopiclone on spindle density (Group \times Visit \times Clozapine interaction: $F_{1,17} = 4.34$, $P = 0.05$). Eszopiclone had no effect on spindle density in clozapine patients (Group \times Visit interaction: $P = 0.95$), but had a large effect in non-clozapine patients (Group \times Visit interaction: $F_{1,10} = 11.94$, $P = 0.006$). Clozapine status did not significantly modulate the effects of eszopiclone on overnight MST improvement (Group \times Visit \times Clozapine interaction, $P = 0.37$) and neither the clozapine patients (Group \times Visit interaction, $P = 0.71$) nor the non-clozapine patients (Group \times Visit interaction, $P = 0.36$) showed a significant effect of eszopiclone vs. placebo on overnight MST improvement.

DISCUSSION

Patients with schizophrenia show profound reductions in sleep spindles¹¹⁻¹⁴ and sleep-dependent memory consolidation.¹⁰⁻¹² Here, in a

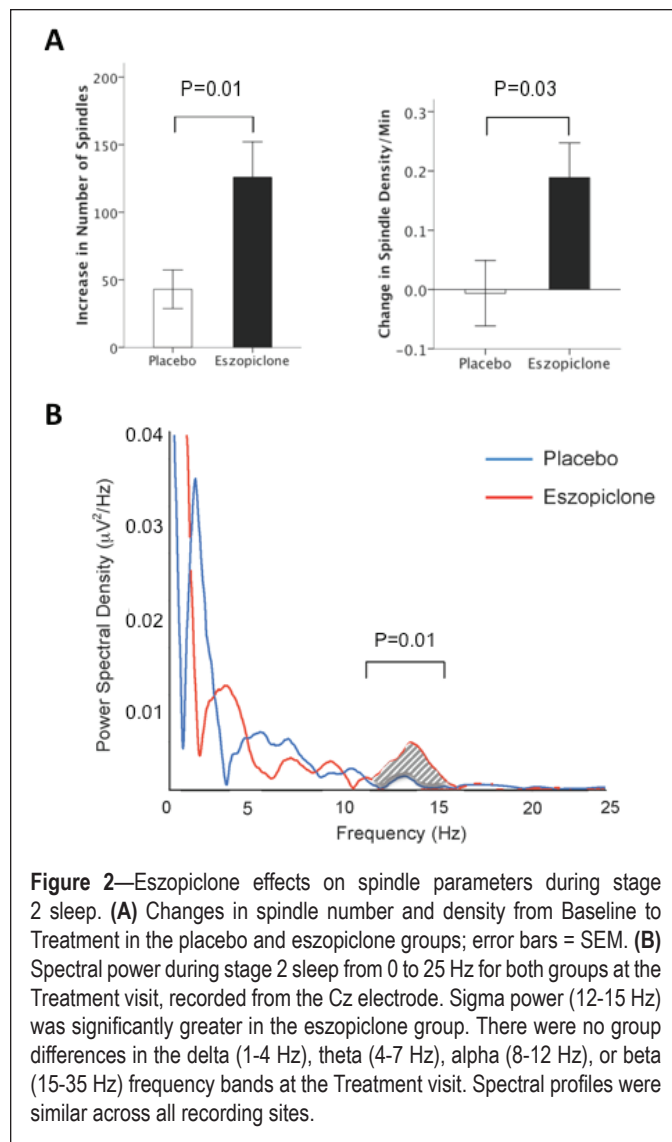


Figure 2—Eszopiclone effects on spindle parameters during stage 2 sleep. **(A)** Changes in spindle number and density from Baseline to Treatment in the placebo and eszopiclone groups; error bars = SEM. **(B)** Spectral power during stage 2 sleep from 0 to 25 Hz for both groups at the Treatment visit, recorded from the Cz electrode. Sigma power (12-15 Hz) was significantly greater in the eszopiclone group. There were no group differences in the delta (1-4 Hz), theta (4-7 Hz), alpha (8-12 Hz), or beta (15-35 Hz) frequency bands at the Treatment visit. Spectral profiles were similar across all recording sites.

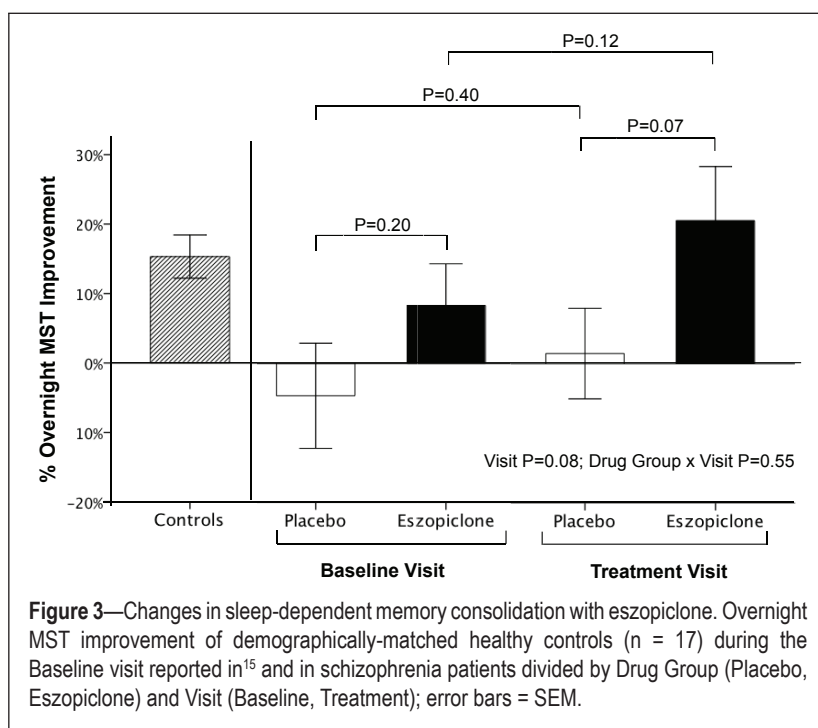


Figure 3—Changes in sleep-dependent memory consolidation with eszopiclone. Overnight MST improvement of demographically-matched healthy controls ($n = 17$) during the Baseline visit reported in¹⁵ and in schizophrenia patients divided by Drug Group (Placebo, Eszopiclone) and Visit (Baseline, Treatment); error bars = SEM.

placebo-controlled, double-blind study, eszopiclone increased sleep spindles significantly more than placebo in schizophrenia. Eszopiclone, however, did not significantly enhance overnight improvement of motor procedural memory. As in our Baseline data¹⁵ and prior studies of healthy individuals,^{31,33} sleep spindle density predicted overnight MST improvement at the treatment visit. The strength of this relation ($r = 0.44$) was remarkably similar to that of a prior study of schizophrenia ($r = 0.46$ ¹¹). Thus, the present findings provide partial support for the theory that the spindle deficit in schizophrenia impairs sleep-dependent memory consolidation and can be effectively treated. These findings, along with evidence that sleep spindles play a more general role in cognition,¹⁸ suggest that the sleep spindle deficit in schizophrenia represents a promising novel potential target for the treatment of cognitive deficits.

The observed effects of eszopiclone were limited to a marked increase in sleep spindles and sigma power accompanied by a decrease in delta power during stage 2 sleep. In contrast, there were no significant effects on sleep architecture or quality, thus excluding the possibility that the effects of eszopiclone on spindles are secondary to its more general effects on sleep quality or architecture. The lack of a significant effect on sleep quality contrasts with prior reports that eszopiclone significantly reduces WASO while increasing sleep efficiency in placebo-controlled clinical trials in primary insomnia.²⁶ These differences may reflect both the relatively small sample size and the exclusion of individuals with insomnia in the present study. It is also possible that schizophrenia, its treatment, or other related factors interact with eszopiclone to modify its effects. While eszopiclone has been shown to increase the duration of stage 2

sleep,²⁶ to our knowledge, this is the first report that it increases spindles. It is therefore unknown whether this effect would generalize to other populations.

Whether our findings are specific to eszopiclone or reflect a more general property of sedative-hypnotic agents also remains unknown. Benzodiazepines and other sedative-hypnotic agents that act on GABA_A receptors have been shown to increase sigma-frequency activity and spindle number.^{42,43} One study that examined memory consolidation found that triazolam, which has been found to increase spindles,⁴⁴ resulted in an overnight *deterioration* of MST performance in healthy individuals despite improved sleep quality, while zolpidem did not differ from placebo in its effects on sleep and memory.⁴⁵ The overnight deterioration of MST performance on triazolam may reflect its effect on response time (slowing of 20 ms on a separate continuous performance task), which may have obscured any effects of increased spindles if present. It is also possible that benzodiazepine-induced increases in sleep spindles would only lead to increased memory consolidation when spindles are deficient, as is the case in schizophrenia. A third possibility is that the spindles induced by benzodiazepines differ from those produced by eszopiclone and are, for some unknown reason, ineffective in improving memory consolidation. In support of this possibility, prior studies have reported that benzodiazepine-induced spindles differ from typical spindles in frequency, amplitude, and duration.^{46,47} In contrast, eszopiclone did not alter the frequency, amplitude, duration, and topographical distribution of sleep spindles. Finally, the differential effect of eszopiclone may reflect its unique GABA_A receptor subunit profile. While its subunit selectivity profile has not been established, eszopiclone has greater affinity for $\alpha_3\beta_3\gamma_2$ GABA_A receptors, which are expressed on TRN neurons, than for the $\alpha_1\beta_2\gamma_2$ subtype found on thalamocortical relay neurons, where zolpidem exerts greater effects.¹⁹ As a result, eszopiclone has preferential effects on GABAergic neurons in the TRN. Further study is necessary to determine whether other GABAergic hypnotics also affect sleep-dependent memory.

While the mechanism of eszopiclone-mediated increases in spindles has not been investigated, the unique, voltage-dependent firing properties of TRN neurons have been well-described.⁴⁸ Like most neurons, TRN cells fire in “tonic” mode at resting membrane potential. However, when TRN neurons are relatively hyperpolarized at rest (to roughly -70 mV)—due to increased inhibitory inputs, decreased excitatory post-synaptic potentials, or both—the low threshold Ca²⁺ conductance is de-inactivated, and the neurons fire in “burst” mode. During burst mode, a depolarizing input activates T-type Ca²⁺ channels, leading to a low threshold Ca²⁺ spike (LTS), and to rhythmic bursts of action potentials. Rhythmic bursting in TRN neurons produces powerful and prolonged inhibition in target thalamocortical relay neurons, and is critical for generating and synchronizing sleep spindles. Neurons in the TRN primarily express

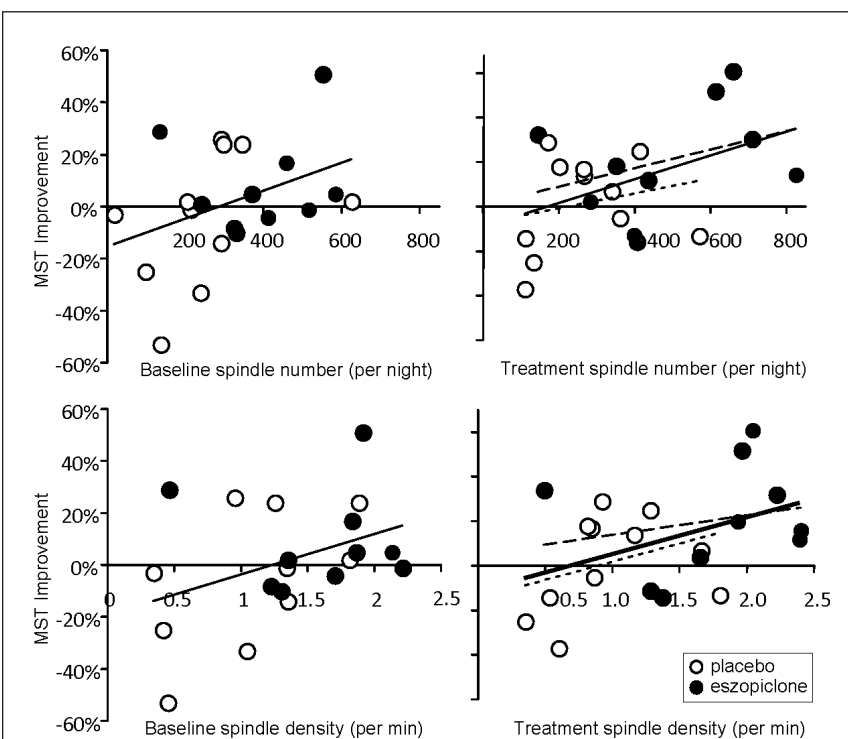


Figure 4—Overnight MST improvement as a function of spindle number and density on the learning night in patients divided by Drug Group (Placebo, Eszopiclone) and Visit (Baseline, Treatment). Long dashed regression lines: eszopiclone group; short dashed lines: placebo; solid lines: combined groups.

the $\alpha_3\beta_3\gamma_2$ subtype of GABA_A receptors,⁴⁹ and eszopiclone slows the decay phase of inhibitory post-synaptic potentials (IPSPs) in these neurons.¹⁹ We speculate that eszopiclone increases spindles by prolonging these IPSP's thereby increasing the time during which TRN neurons can fire in burst mode.

While these initial findings are promising, and demonstrate that eszopiclone increases spindles in schizophrenia, there are several important limitations that temper our conclusions. First, replication is necessary due to the small sample size, which likely limited our power to detect significant effects on memory consolidation. Although eszopiclone significantly increased sleep spindles, which correlated with memory consolidation, and despite the significant overnight MST improvement during treatment with eszopiclone, the increased memory consolidation seen on eszopiclone was not statistically different from placebo. If, as hypothesized, eszopiclone improves memory via its effects on spindles, the failure to reach significance may reflect that memory consolidation is a more distal, and therefore less sensitive index of eszopiclone effects than spindles, requiring a larger sample size to reach significance. It is noteworthy, however, that the eszopiclone-treated group is the first published sample of schizophrenia patients to show significant sleep-dependent improvement of motor procedural memory.¹⁰⁻¹²

A second limitation is that despite the randomization of participants to treatment groups, the eszopiclone group had significantly greater spindle density than the placebo group at the Baseline visit (Table 2). As spindle density at Baseline did not correlate with the change in spindle density from the Baseline to the Treatment visit ($P = 0.70$), the greater increase in spindle density in patients treated with eszopiclone is unlikely to reflect Baseline differences.

A third limitation is that eszopiclone was added to a stable, clinically indicated medication regimen that differed across participants. All patients were treated with a variety of second-generation APDs, and twelve also took adjunctive agents, primarily antidepressants, but also benzodiazepines, stimulants, and other mood stabilizers. While APD dosage was not related to any measure of spindle activity and the effects of chronic APD treatment are unknown, a prior study reported decreased spindle activity following a single dose of olanzapine in schizophrenia.⁵⁰ Benzodiazepines are known to increase spindles (as reported above) and one study suggested that selective serotonin reuptake inhibitors (SSRIs) increase spindle density,⁵¹ but data on SSRIs and other antidepressants are very limited. We lacked adequate power to fully examine the effects of these different medication classes in our sample. As all patients were maintained on a stable medication regimen during study participation, the increases in spindle density and number in the eszopiclone group were most likely due to the addition of eszopiclone. Although patients taking clozapine showed significantly fewer spindles than non-clozapine patients at both visits, the spindle reduction remained significant in non-clozapine patients, and the effects of eszopiclone on spindles were only present in non-clozapine patients. It is unclear whether the more pronounced spindle deficit and the diminished response to eszopiclone in clozapine patients reflect medication effects or that the patients taking clozapine were more severely ill and treatment refractory.

Finally, as our studies have been limited to chronic, medicated patients, it is unknown whether early course and unmedicated

patients also have sleep-dependent memory and spindle deficits. Evidence of intact spindle activity in non-schizophrenia psychiatric patients taking APDs suggests that the spindle deficit in schizophrenia is unlikely to be an APD side effect.¹⁴ Further, findings of correlations between sigma power during NREM sleep and measures of attention and reasoning in APD-naïve patients with recent-onset psychosis suggest that spindle activity is also relevant to cognitive function in early course untreated individuals.⁵²

Sleep spindles correlate with a wide range of cognitive measures including declarative and procedural memory consolidation, learning potential, and general intelligence.¹⁸ In addition, in two prior reports, reduced spindle activity predicted more severe positive symptoms in schizophrenia.^{14,15} These findings raise intriguing questions about whether long-term pharmacological enhancement of spindles can improve memory consolidation, cognition more generally, and positive symptoms in schizophrenia. In summary, the present findings link a specific cognitive deficit in schizophrenia (sleep-dependent memory consolidation) to a particular mechanism (sleep spindles) and raise the possibility of effective intervention. The investigation of the role of sleep in memory consolidation in schizophrenia has the potential to substantially expand current models of cognitive deficits and to lead to interventions that improve quality of life.

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Table S1—Patient medications

	Medication Class			
	Antipsychotic	Antidepressant	Benzodiazepine	Other
Placebo Patients (n = 11)				
P1	Clozapine (100 mg)	Bupropion (150 mg)		
P2	Clozapine (900 mg)	Sertraline (300 mg)		Ritalin (30 mg)
P3	Clozapine (100 mg); Aripiprazole (15mg)			
P4	Clozapine (100mg)	Paroxetine (60 mg)	Klonopin (2 mg)	
P5	Clozapine (100 mg)	Escitalopram (20 mg)		
P6	Clozapine (500 mg); Aripiprazole (15 mg)			Lamictal (200 mg); Lithium (300 mg)
P7	Olanzapine (20 mg)	Fluoxetine (20 mg)		
P8	Aripiprazole (10 mg); Quetiapine (300 mg)	Fluoxetine (40 mg)	Lorazepam (1 mg)	Benztropine (1 mg)
P9	Aripiprazole (20 mg)			
P10	Olanzapine (20 mg)	Sertraline (100 mg)		
P11	Risperidone (37.5 mg biweekly)			
Eszopiclone Patients (n = 10)				
E1	Clozapine (500 mg)	Fluoxetine (20 mg)		
E2	Clozapine (200 mg)		Klonopin (0.5 mg)	
E3	Clozapine (200 mg)			
E4	Risperidone (25 mg biweekly); Aripiprazole (5 mg)		Lorazepam (0.5 mg prn)	
E5	Aripiprazole (10 mg)			Modafinil (200 mg)
E6	Aripiprazole (10 mg)			
E7	Olanzapine (15 mg)			
E8	Aripiprazole (10 mg)			
E9	Risperidone (25 mg biweekly)	Escitalopram (20 mg)		
E10	Aripiprazole (4 mg)			

Unless otherwise indicated, dosage = mg/day. Patients are grouped by Drug Group and clozapine status.