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Coordination of Slow Waves with Sleep Spindles Predicts Sleep-Dependent Memory Consolidation in Schizophrenia

Charmaine Demanuele, Ph.D[1,2,3], Ullrich Bartsch[4], Ph.D[1], Bengi Baran, Ph.D[11,2,3], Sheraz Khan, Ph.D[12], Mark G. Vangel, Ph.D[12,3], Roy Cox, Ph.D[13,5], Matti Hämäläinen, Ph.D[12], Matthew W. Jones, Ph.D[14], Robert Stickgold, Ph.D[13,5], Dara S. Manoach, Ph.D[11,2,3,*]

[1]Department of Psychiatry, Massachusetts General Hospital, Charlestown, MA 02215; [2]Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA 02129; [3]Harvard Medical School, Boston, MA 02115; [4]School of Physiology and Pharmacology, University of Bristol, Bristol, United Kingdom, BS8 1TD; [5]Department of Psychiatry, Beth Israel Deaconess Medical Center, Boston MA 02215.

Address correspondence to:
Dara S. Manoach, Ph.D.
Massachusetts General Hospital
Charlestown Navy Yard
Department of Psychiatry
149 13th Street, Room 1.111
Charlestown, MA 02129
E-mail: dara@nmr.mgh.harvard.edu.

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Institution at which work was performed: Department of Psychiatry, Massachusetts General Hospital and Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA

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Abstract

Study Objectives: Schizophrenia patients have correlated deficits in sleep spindle density and sleep-dependent memory consolidation. In addition to spindle density, memory consolidation is thought to rely on the precise temporal coordination of spindles with slow waves (SWs). We investigated whether this coordination is intact in schizophrenia and its relation to motor procedural memory consolidation.

Methods: Twenty-one chronic medicated schizophrenia patients and 17 demographically-matched healthy controls underwent two nights of polysomnography with training on the finger tapping motor sequence task (MST) on the second night and testing the following morning. We detected SWs (0.5-4Hz) and spindles during non-rapid eye-movement (NREM) sleep. We measured SW-spindle phase-amplitude coupling and its relation with overnight improvement of MST performance.

Results: Patients did not differ from controls in the timing of SW-spindle coupling. In both groups spindles peaked during the SW upstate. For patients only, the later in the SW upstate that spindles peaked and the more reliable this phase relationship, the greater the overnight MST improvement. Regression models that included both spindle density and SW-spindle coordination predicted overnight improvement significantly better than either parameter alone suggesting that both contribute to memory consolidation.

Conclusions: Schizophrenia patients show intact spindle-SW temporal coordination and these timing relationships, together with spindle density, predict sleep-dependent memory consolidation. These relations were seen only in patients suggesting that their memory is more dependent on optimal spindle-SW timing, possibly due to reduced spindle density. Interventions to improve memory may need to both increase spindle density and optimize the coordination of NREM oscillations.

Keywords: sleep spindles; slow waves; schizophrenia; motor; procedural learning; memory consolidation

Statement of Significance

Sleep spindles, a defining electroencephalographic feature of non-rapid eye movement sleep, act in concert with slow waves to consolidate memory. Patients with schizophrenia have a marked reduction in sleep spindles that correlates with impaired memory consolidation. In this first investigation of slow wave-spindle coordination in schizophrenia, we demonstrate that despite markedly reduced spindles, the temporal relationship between spindles and slow waves is largely preserved and that it correlates with memory consolidation. Together, spindle density (spindles per minute) and slow wave-spindle coordination predicted memory consolidation better than either parameter alone. These findings illuminate basic mechanisms of memory consolidation and indicate that interventions to improve memory in schizophrenia may need to both increase spindle density and preserve or improve slow wave-spindle coordination.

Introduction

Cognitive deficits are core features of schizophrenia that often precede the onset of psychosis and are the strongest predictor of poor functional outcome[1,2]. The neural bases of cognitive deficits are poorly understood and, consequently, effective treatments are lacking[3]. Recent work places deficient sleep-dependent memory consolidation among the cognitive deficits of schizophrenia and implicates reduced sleep spindles, a defining electroencephalographic (EEG) feature of Stage 2 non-rapid eye movement sleep (N2), as a potentially treatable mechanism[4]. Schizophrenia patients have deficient sleep-dependent consolidation of both procedural[5–10] and declarative[11] memory that, in some studies, correlates with reduced sleep spindle density (spindles per minute) and number[10,11]. These relations are consistent with a large basic literature showing that spindle activity correlates with measures of intelligence and sleep-dependent memory consolidation[12]. Merely having enough spindles may not be sufficient – intact sleep-dependent memory consolidation is also thought to rely on the precise temporal coordination of spindles with neocortical slow waves (SW) and hippocampal sharp-wave ripples[13–17]. This coordination is disrupted in a rat model of schizophrenia[18] but has not been studied in patients. Since hippocampal ripples are not detectable with surface EEG, we investigated the temporal coordination of spindles with SWs in schizophrenia and its relation to memory.

Sleep spindles, seen in the EEG as brief bursts of 12-15Hz synchronous activity, are initiated by the thalamic reticular nucleus (TRN) and are mediated by thalamocortical networks[19]. Hippocampal sharp-wave ripples, which are associated with memory reactivation[20] preferentially occur in the troughs of spindles[21], which preferentially occur during SW upstates[13–16]. SWs are generated within thalamocortical networks and arise from the rhythmic depolarization and hyperpolarization of cortical pyramidal neurons[22]. Human and animal studies[23–25] report that SWs synchronize neuronal activity in structures relevant to memory including the hippocampus and thalamus. Coordinated SW-spindle-ripple activity is theorized to redistribute recently encoded memories from temporary dependence on the hippocampus to longer-term representation in the cortex[14,16,21,26–29].

To investigate SW-spindle coordination, we analyzed polysomnography (PSG) data from a previously reported study of chronic medicated schizophrenia patients and demographically-matched healthy controls[10,30]. Using this dataset, we previously reported that in the context of normal sleep architecture and quality, patients showed significant reductions in N2 sleep spindle number and density, low sigma (12-13.5Hz) power and cortical spindle coherence. In patients only, spindle density correlated with overnight improvement on the finger tapping motor sequence task (MST), the most extensively validated probe of sleep-dependent memory consolidation[31–34]. In the present study, we investigated whether patients show impaired SW-spindle coordination by measuring both the SW phase at the spindle peak and SW-spindle phase amplitude coupling. We then examined whether SW-spindle coordination contributes to the sleep-dependent consolidation of motor procedural memory using the MST.

Methods and Materials

Participants

Twenty-one schizophrenia outpatients and 17 healthy control participants matched for age, sex and parental education completed the study (Supplemental Tables S1, S2 provide patient medications and participant characteristics respectively). All participants gave written informed consent and were paid for participation, including a monetary bonus ($.05) for each correctly typed MST sequence. The study was approved by the Institutional Review Boards of Massachusetts General Hospital (MGH), the Massachusetts Department of Mental Health, and Beth Israel Deaconess Medical Center. Data will be made available upon request.

Procedures

See[10] for procedural details. Participants spent two consecutive weeknights in the MGH Clinical Research Center and were allowed to sleep up to 10 hours with PSG. They engaged in their usual daytime activities but were asked not to nap, which was confirmed by sleep diary and actigraphy. On the second night, participants trained on the MST one hour before their usual bedtime and were tested

the following morning, one hour after awakening.

Finger Tapping Motor Sequence Task (MST)

The MST involves pressing four numerically labeled keys on a standard computer keyboard with the fingers of the left hand, repeating a five element sequence (*4-1-3-2-4*) "as quickly and accurately as possible" for 30s. The numeric sequence is displayed at the top of the screen, and dots appear beneath it with each keystroke. During both training and test sessions, participants alternate tapping and resting for 30s for a total of 12 tapping trials. The primary outcome measure is the number of correct sequences typed per trial, which reflects both the speed and accuracy of performance. *Overnight improvement* is calculated as the percent increase in correct sequences from the last three training trials at night to the first three test trials the following morning[33].

Polysomnography

Data were digitally acquired at 100Hz using an Embla N7000 system (Medcare Systems, Buffalo, New York) with 8 EEG electrodes (F3, F4, C3, Cz, C4, Pz, O1, O2) placed according to the 10-20 system. Electrodes were referenced to the linked mastoids. Electromyography and electrooculography were also acquired. Recordings were divided into 30s epochs and scored according to standard criteria[35] as WAKE, REM, N1, N2 and N3 by expert scorers blind to night and diagnosis. PSG data were preprocessed and analyzed using BrainVision Analyzer 2.0 (BrainProducts, Germany), MATLAB R2014a (The MathWorks, Massachusetts) and R[36]. Data were filtered at 0.5-35Hz. Artifacts identified using automated algorithms were visually confirmed and removed.

Spindle detection

Spindles were automatically detected using a wavelet-based algorithm that has been validated against hand-counted spindles and 12-15Hz sigma power in healthy and schizophrenia participants [10], and against expert consensus spindle counts[37].

SW detection

SWs were detected using a modified version of a published algorithm[14] (Supplemental Methods, Fig. S1). SW number, density (number per minute), mean duration and mean peak-to-peak amplitudes were calculated for each electrode.

SW measures were compared across electrodes, groups and nights using linear mixed model regression[38]. Subject and night were random effects, and electrode, group, night and their interactions were fixed effects. Night was a fixed effect to account for the systematic order of the two nights, and also a random effect to account for differences between nights unrelated to order (e.g., different electrode impedances). We examined group differences in the spatial distribution of SW-spindle parameters with electrode by group interactions and *post hoc* generalized linear hypothesis tests as implemented by the *multcomp* R package[39]. These tests correct for multiple comparisons while accounting for any dependencies between electrodes.

Data from O1 and O2 were deemed unreliable and omitted from analyses since SW amplitude was significantly lower than other electrodes (controls: $t(1,15)=22.9$, $p<10^{-6}$; patients: $t(1,19)=13.25$, $p<10^{-6}$) and the density of suprathreshold SWs was significantly lower than other electrodes and highly variable across participants (Table 1).

SW-spindle coordination analyses

Overview: EEG analyses were conducted for each night (non-learning, learning) and for N2 and N3 separately. (A) First to visualize sigma power (12-15Hz, spindle frequency band) in relation to SWs we plotted spectrograms. (B) Next, we quantified the timing of spindles with SWs by identifying the SW phase at the spindle peak, which is the most reliable measure of spindle timing. We also examined the consistency of this relationship for each participant. We determined whether these parameters -- SW phase at spindle peak and consistency -- differed by group and, to test our main hypothesis, we correlated them with sleep dependent memory consolidation. (C) As a secondary analysis to confirm timing results we used the standard technique, phase amplitude coupling (PAC)[40–42], which uses all spindle time points to identify the SW phase at which sigma amplitude is maximal and tests the

significance of this coupling.

A. Spectrograms of sigma power during SWs

Sigma power was derived for a time-window of ±1.5s centered on the negative peaks (x) of all detected SWs from multi-tapered spectrograms (2 tapers, 10ms sliding window, 1Hz bandwidth) using the Chronux toolbox in MATLAB [43]. Spectrograms were time-locked to SW downstates, which are typically sharper and more distinct than the longer and more attenuated upstates[14]. From these spectrograms we computed the relative sigma power $RP_{12\text{-}15Hz}$ within these SW time-windows, $RP_{12\text{-}15Hz} = P_{12\text{-}15Hz} \Big/ \left(P_{0.5\text{-}25Hz} - P_{12\text{-}15Hz} \right)$. This relative measure corrects for subject-specific baseline differences across the entire power spectrum[37].

B. SW phase at spindle peak

We focused on spindle peak amplitude (Fig. 1a) as our most reliable index of spindle timing, but also identified spindle start and end times (Fig. S2, S3). The EEG signal was filtered in the SW (0.5-4Hz) and sigma frequency bands using a two-way least-squares finite impulse response filter as implemented by *eeglab* function *eegfilt*[44]. A 2s time window around each spindle detection point was considered. The spindle peak was defined as the point of maximum amplitude in the spindle envelope derived from the Hilbert transform[45] of the EEG trace filtered in the sigma band. Spindle start and end times were identified by a threshold of 2 times the average amplitude of the entire signal envelope. The phase of the SW at the start, peak and end of the spindle were derived from the Hilbert transform of the EEG trace filtered in the SW band. Histograms of the SW phase at these three spindle points were computed for each electrode (Figs. 1, S2, S3). For each histogram, the angular mean and variance of the SW phase distribution were found using the circular statistics toolbox in MATLAB[46]. For this and subsequent analyses, we considered only those spindles for which the corresponding peak-to-peak amplitude of the SW was greater than 70μV. This ensured that we were analyzing prototypical SWs as defined by standard sleep scoring criteria[35], and eliminated low amplitude, low frequency fluctuations, which are less likely to represent true up- and down-state activity (Figs. S2, S3). If, after amplitude

thresholding, fewer than 30 SW-associated spindles remained, the electrode was excluded from these analyses.

Measurement of the consistency of the SW phase at spindle peak (and start and end) was based on the deviation of histograms from a uniform distribution (Figs. S2, S3). This "phase consistency" is equal to the area between the curves defined by the SW phase cumulative distribution function (CDF) and the uniform CDF, according to the Cramér-von Mises test[47]. Area between curves was estimated by numerical integration via the trapezoidal method in MATLAB (function *trapz*). This measure tests the degree to which the phase distribution shows a preferential peak relative to a uniform distribution, and would have a value of one when the SW phase at a specific spindle time point is consistent across all spindles.

SW-spindle coordination parameters (x_1:either SW phase at spindle peak or phase consistency) were regressed on overnight MST improvement (y) using a model that included Group (x_2) and its interaction with the coordination parameter (x_1) to test whether relations with MST improvement differed by group: $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2$. Robust regression[48] was used to limit the influence of outliers. To test whether including both spindle density and SW-spindle coordination significantly improved the prediction of overnight MST improvement over either parameter alone, we compared the residual sum of squares of the individual (spindle density or coordination parameter) vs. bivariate models (spindle density, coordination and their interactions) using analysis of variance. Data from Cz was used to be consistent with our previous reports on this dataset[10,30] and because more participants (19 patients, 15 controls) had recordings and quantifiable SW-spindle coordination parameters at Cz than other electrodes.

C. SW-spindle phase amplitude coupling (PAC)

PAC[40,41] measures the degree of coupling between the phase of the SW signal q_{SW}, and the amplitude of sigma activity, A_{sigma}, within the associated spindle[42]. It is defined as a mean complex vector $PAC = mean\left(A_{sigma} e^{jq_{SW}}\right)$, which gives the preferential phase of the SW at which sigma

amplitude is maximal, and the mean vector length, also known as the Modulation Index $MI = abs\left(PAC\right)$, which quantifies the coupling between these two oscillations (Supplemental Methods and Fig. S4 describe PAC computation).

Results

We report N2 findings based on prior studies showing N2 spindle abnormalities in schizophrenia and correlations of N2 spindle activity with sleep-dependent memory consolidation[4,7,11]. N3 results are in the Supplement.

Spindle Characteristics

As previously reported, compared with healthy controls, patients showed reduced spindle number (36%) and density (38%) but did not differ in the amplitude, frequency, sigma power or duration of individual spindles based on data averaged across the two study nights[10]. Spindle characteristics did not differ by night for either group.

SW Characteristics

Patients showed trends to greater SW density, greater SW peak-to-peak amplitude and shorter SW duration relative to controls and this did not differ by night (Table 1, S3). All SW parameters differed as a function of electrode ($p<10^{-6}$). In the combined groups, SW density and peak-to-peak amplitude were higher at Cz and frontal electrodes than at C3, C4 and Pz, consistent with the literature [49,50], and duration was shortest at Cz. There were significant group differences in the spatial variation of SW density (Group by electrode interaction: F(5,277)=3.9, p=.002) and peak-to-peak amplitude (F(5,274)=3.6, p=.003) that were primarily driven by numerically higher SW density at Pz (z=1.6, p=.12) and statistical trend toward higher peak-to-peak amplitude at Cz (z=1.9, p=.09) in patients.

SW-spindle coordination analyses

Except for the spectrograms, which included all detected SWs, SW-spindle coordination analyses included only SWs >70μV peak-to-peak amplitude.

A. SW-sigma spectrograms

As there was no significant night effect on SW-locked sigma power, spectrograms time-locked to the downstate of all detected SWs were averaged across the two nights (Table 1, Fig. 2). For both groups, maximal sigma power occurred during the upstate of SWs. At all electrodes, SW-locked sigma power was significantly reduced in patients compared with controls, likely reflecting the spindle deficit[10]. Results were similar for N3 (Supplement, Fig. S5).

B. SW phase at spindle peak

On average, spindles peaked during the upstate of SWs following the positive peak (0°) irrespective of night and group. Spindles peaked later in the SW upstate on the learning vs. non-learning night ($F(1,28)=5.94$, $p=.02$), but the groups did not differ in the mean SW phase of spindle peaks at Cz on either night (non-learning night: Fig. 3a; Controls: 30°, Patients: 32°; learning night: Fig. 3b; Controls: 41°; Patients: 44°). SW-spindle phase consistency did not differ between groups and was similar at every electrode (Table S4). This was also true for spindle starts and ends (Fig. S2, S3). Similar findings were found for analysis of these relationships during N3 (Fig. S6, Table S5).

We next examined these parameters in relation to MST overnight improvement. We previously reported that MST improvement correlated with spindle density in schizophrenia but not controls[10]. Extending these results, we now see, again in patients only, that SW-spindle coordination on the learning night also correlated with overnight improvement, for both SW phase at spindle peak ($R^2=.31$, $p=.01$) and SW-spindle phase consistency ($R^2=.25$, $p=.03$; Fig. 4b). The later within the SW upstate the spindle peaked and the more consistent this timing, the greater the overnight improvement. Controls, in contrast, showed no correlation of SW phase at spindle peak with MST improvement ($R^2=.008$, $p=.77$) and only a trend-level correlation of phase consistency with MST improvement ($R^2=.29$, $p=.06$; Fig. 4a) that was not in the predicted direction. Regression analyses revealed that the slope of the relations between MST improvement and coordination differed significantly between groups for phase consistency ($p=.03$), with patients having a stronger positive relationship, but did not differ significantly for SW phase at spindle peak ($p=.15$).

Spindle density and SW phase at spindle peak both predicted overnight MST improvement and together they predicted improvement significantly better than either parameter alone (Fig. 4c; spindle density vs. bivariate model: $F(2,15)=4.1$, $p=.04$; SW phase at spindle peak vs. bivariate model: $F(2,15)=4.2$, $p=.04$). The same was true for spindle density and phase consistency (Fig. 4c; spindle density vs. bivariate model: $F(2,15)=4.7$, $p=.03$; phase consistency vs. bivariate model: $F(2,15)=7.8$, $p=.005$). When both SW phase at spindle peak and phase consistency were entered into a bivariate model they each explained a significant proportion of the variance in overnight improvement ($R^2=.65$; SW phase at spindle peak $p=.001$, phase consistency $p=.002$) suggesting that each contributes to memory consolidation.

C. SW-spindle PAC

PAC results were consistent with our primary analyses of SW phase at spindle peak. At each electrode, only participants with significant PAC results (as determined by MI permutations) were included in the mixed model regression (Fig. 5c). Since there was no main effect of night on either the SW phase ($F(1,27.5)=.36$, $p=.55$) or the MI ($F(1,35)=.47$, $p=.49$), PAC results were averaged across nights (Fig. 5). There was a main effect of electrode ($F(5,181)=26$, $p<10^{-6}$), indicating that maximum sigma amplitude occurred earlier within the SW at centroparietal than frontal electrodes. While SW phase at maximum sigma amplitude did not differ by group (mixed model regression $F(1,34.4)=.54$, $p=.47$) and was similar to the SW phase at spindle peak (Fig. 3), there was a significant group by electrode interaction ($F(5,180)=4.6$, $p=.001$) reflecting that patients had a later SW phase than controls at Cz ($z=2.7$, $p=.03$) but did not differ at other electrodes. Finally, the MI (i.e., degree of SW-spindle coupling) did not differ by electrode, group or night. N3 results were similar (Supplement, Fig. S7).

Discussion

In this first investigation of the coordination of sleep spindles with SWs in schizophrenia, we demonstrate that in the context of markedly reduced spindle density and number, the temporal relationship between spindles and SWs is largely preserved. As in prior studies of healthy

individuals[23,25], in both healthy and schizophrenia participants, spindles started on the rising phase of the SW upstate, peaked after its maximum and ended during the downstate. In the schizophrenia group only, the later in the SW upstate that spindles peaked and the more consistent this timing, the greater the sleep-dependent memory consolidation. Each coordination parameter (SW phase at spindle peak and phase consistency) predicted memory consolidation independently of spindle density. Together, spindle density and SW-spindle coordination predicted memory consolidation better than either parameter alone. These findings suggest that both the density of spindles and their coordination with SWs contribute to sleep-dependent memory consolidation in schizophrenia.

The finding of similar SW-spindle coordination between patients and controls was consistent across three methods, two study nights, and N2 and N3. First, using spectrograms, sigma power was highest during SW upstates in both groups. (The reduction in SW-locked sigma power in schizophrenia likely reflects their significantly lower spindle number and density.) Second, spindles peaked at approximately the same phase of the upstate of SWs (~43°) and with similar consistency in both groups. Third, PAC, which uses all spindle time points, but only participants with significant coupling, showed that the SW phase at which sigma amplitude was maximal was similar to the SW phase at spindle peak. While there was no overall group difference in PAC, patients showed a later SW phase at Cz, but did not differ at other electrodes. These findings suggest that SW-spindle coordination is intact in chronic medicated patients with schizophrenia.

The dissociation between intact SW-spindle coordination and deficient spindle number and density suggests that they rely on different neural mechanisms. SWs emerge spontaneously in the neocortex, even after cortical de-afferentation[51], but can also be elicited by optogenetic manipulation of TRN and thalamocortical neurons[52,53] suggesting that thalamic input shapes SW expression[22]. In contrast, spindle frequency rhythms are seen in the isolated TRN[54,55], but not in the isolated cortex or other thalamic nuclei[55,56]. Cortical feedback to the TRN and thalamocortical neurons, however, synchronizes spindles across cortical regions[57,58] and can initiate and terminate spindles[59]. Thus, in the intact brain, both spindles and SWs are the products of thalamocortical circuits. While SWs contribute

to spindle synchronization, initiation and duration, spindles do not appear to trigger SWs or affect their duration[60]. This relative independence of SWs from mediation by spindles may account for why the spindle deficit in schizophrenia is not paralleled by a SW or SW-spindle coordination deficit.

Our findings regarding SW abnormalities in schizophrenia are inconclusive. Patients showed trends to greater SW density and peak-to-peak amplitude and shorter SW duration. There was no deficit in delta power. Previous investigations of SW activity in schizophrenia have been inconsistent reporting no differences during NREM sleep in medicated patients[61,62], reduced N3 SW count and delta power in antipsychotic-naïve and unmedicated patients[63,64] and reduced delta power in N3 but not N2 in medicated patients[65]. These inconsistencies may reflect differences in definitions of delta power, sleep stages considered and medication status of the participants.

Although the groups were similar in SW-spindle coordination, only in patients did coordination predict sleep-dependent memory consolidation. Patients whose spindles peaked later in the upstate of the SW and for whom this temporal relation was more consistent showed better memory consolidation. Adding either coordination parameter to spindle density in a model predicting sleep-dependent memory consolidation resulted in a significantly stronger prediction than either parameter alone suggesting that both SW-spindle coordination and spindle density are important for memory consolidation in schizophrenia. We can only speculate why we did not see similar relations of either spindle density[10] or SW-spindle coordination to memory in healthy controls. This may reflect that controls showed a more restricted range of overnight MST improvement (Fig. 4: controls SD=13%; patients 23%). Another possibility is that patients are more sensitive to variation in SW-spindle coordination due to their spindle deficit. The overall reduction in spindles may lead to fewer spindles that are optimally coordinated with SWs to consolidate memory. For controls, spindle density and the number of coordinated SW-spindle events may be sufficient and not rate limiting for memory consolidation.

These findings raise the question of whether enhancing spindles while preserving or improving SW-spindle coordination might improve sleep-dependent memory consolidation in schizophrenia. In healthy individuals, increasing spindles with zolpidem[66,67], increasing sigma activity with transcranial

stimulation[68,69] and enhancing the synchronization of sigma activity with slow oscillations using auditory closed-loop stimulation[70] all improve memory, while transcranial stimulation that decreases sigma activity impairs memory[71]. Only a few studies have attempted to improve cognition in schizophrenia by manipulating sleep oscillations. In a small sample of patients, .75Hz transcranial stimulation during N2 did not significantly alter sleep but improved word list recall[72]. In a small preliminary study, eszopiclone, which acts on γ-aminobutyric acid (GABA)-ergic neurons in the TRN[73], significantly increased spindles in schizophrenia but not sleep-dependent memory[30]. In another study that did not include PSG, longer-term administration of eszopiclone improved working memory in schizophrenia, but not symptoms[74]. The effects of eszopiclone on SW-spindle coordination in schizophrenia are now being investigated. This body of work provides an impetus to develop and test novel therapies for spindle deficits and synchronization to improve cognition.

A limitation to the interpretation of our findings is that our patients were treated with medications that affect sleep[75]. As previously reported, antipsychotic medications, measured by chlorpromazine equivalents, did not significantly correlate with any spindle parameter[10], nor does it correlate with SW-spindle phase (R=.17, p=.20) or phase consistency (R=.19, p=.52). In addition, we recently reported spindle deficits in antipsychotic-naïve early course patients with schizophrenia (but not to those with other psychotic disorders) and non-psychotic first-degree relatives. These deficits correlated with IQ and executive function[76]. These data suggest that spindle deficits in schizophrenia are not a side-effect of antipsychotic medications or chronicity and may instead be endophenotypes that are linked to risk genes and contribute to cognitive dysfunction[4].

It is possible that larger samples would have revealed clinically meaningful group differences in SW-spindle coordination. Based on the small effect sizes for SW-spindle phase and phase consistency and the consistent lack of significant group differences across electrodes, however, this seems unlikely. Other study limitations include that our sparse EEG array does not allow a fuller exploration of the spatial characteristics of spindles associated with memory. We were also not able to examine the coordination of SWs and spindles with hippocampal ripples since ripples are not seen on scalp

recordings. Hippocampal involvement is more associated with sleep-dependent declarative than procedural memory, which is also impaired in schizophrenia[11]. Future work will investigate the contribution of SW-spindle coordination to declarative memory deficits. Animal models may be necessary to evaluate the contribution of coordination between all three NREM oscillations (SWs, spindles, ripples) to memory and how this is affected by potential interventions. Recent studies suggest distinctions between fast and slow frequency spindles with regard to cortical generators,[77] mnemonic function[58] and relation to SW phase[25,78]. As the present study was restricted to the standard definition of spindle frequency (12-15 Hz; [35,79]), future work is needed to address whether spindles in the fast and slow halves of this range, as well as in lower frequency bands are preserved in schizophrenia and contribute to sleep-dependent memory consolidation.

In summary, despite marked spindle deficits, the temporal coordination of SWs and spindles is preserved in chronic medicated schizophrenia. Moreover, SW-spindle coordination correlated with overnight improvement on a procedural memory task, suggesting a role in sleep-dependent memory consolidation. This implies that interventions aimed at ameliorating deficient sleep-dependent memory consolidation in schizophrenia will need to both increase spindle density and preserve (or improve) the coordination of NREM oscillations. A greater understanding of the role of coordination of NREM oscillations in memory may open new avenues for treating cognitive deficits in schizophrenia.

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Figure Legends

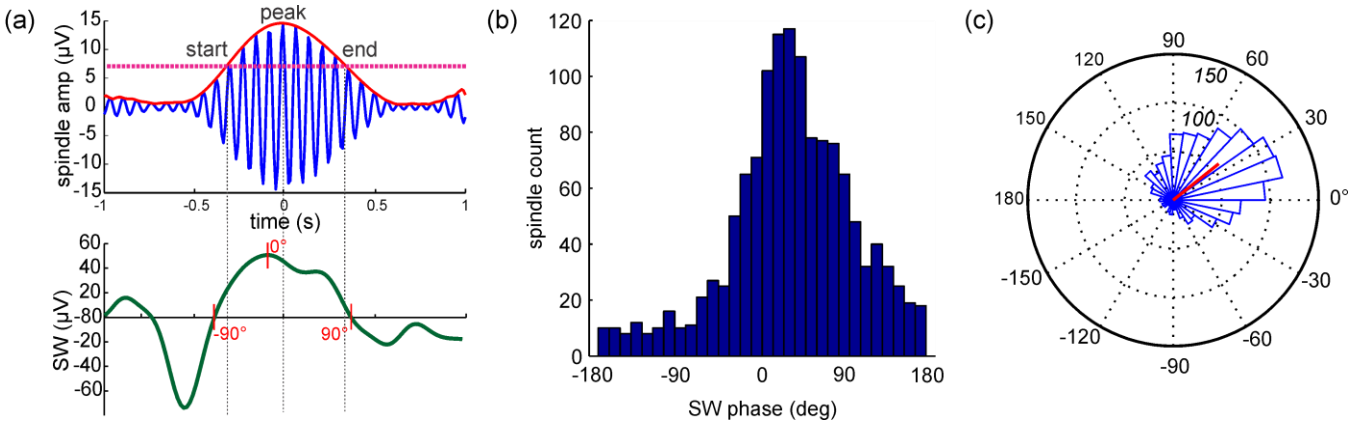

Figure 1. SW phase at spindle peak. (a) Timing of one sleep spindle (top, blue trace, red envelope derived from the Hilbert transform) in relation to the corresponding SW (bottom, green trace). (b) Distribution of SW phases at spindle peaks pooled across control participants at Cz for SWs >70μV, and (c) plotted on an angular histogram (numbers in italics represent spindle counts); red line represents the angular mean and variance of the phase distribution.

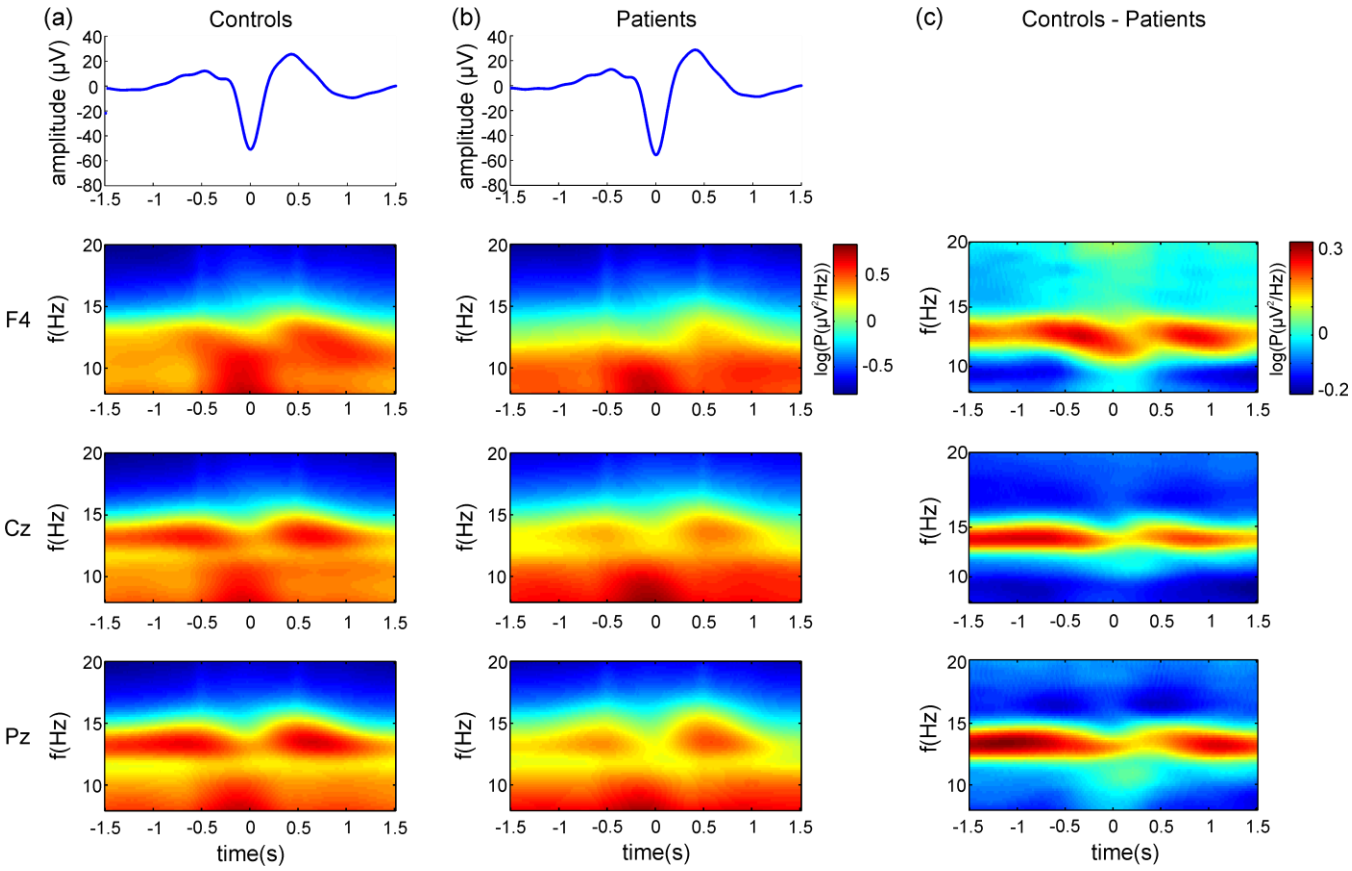

Figure 2. Spectrograms of sigma power during SWs. The blue trace represents the average detected SW at F4. Spectrograms of sigma power locked to each electrode's local SW downstate (0s) averaged across nights for (a) controls and (b) patients. For both groups, maximum sigma power occurred on the upstate of SWs. (c) Spectral difference between controls and patients. Patients showed a specific reduction in SW-locked sigma power compared with controls, reflecting their spindle deficit.

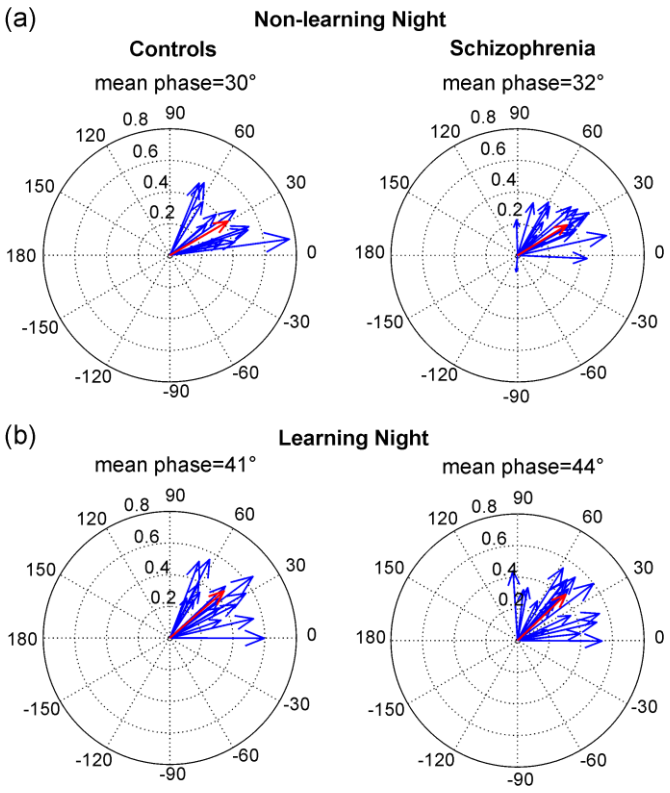

Figure 3. Mean SW phase at spindle peak at Cz. (a) Non-learning and (b) learning nights. Blue arrows represent the mean SW phase for each participant; the length of each arrow (vector) represents the variance for that participant. Red arrows represent group means and their magnitude represents the between-subject variance.

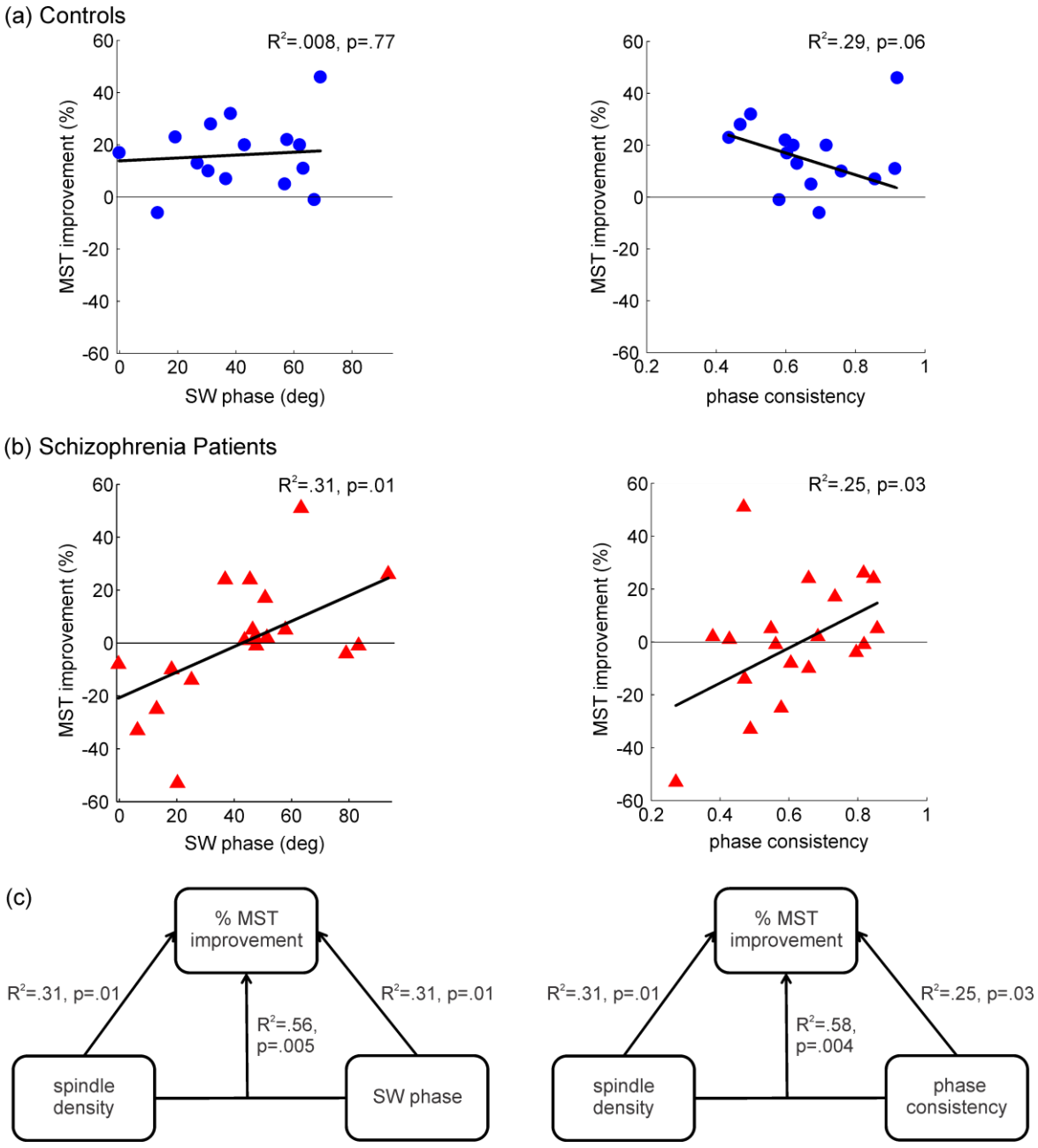

Figure 4. Relations of SW-spindle coordination with sleep-dependent memory consolidation. Data from Cz. (a) Controls: SW phase at spindle peak and phase consistency did not show significant correlations with overnight MST improvement. (b) Patients: Both SW phase at spindle peak and phase consistency predicted overnight MST memory improvement (i.e., memory consolidation). (c) Models that included both spindle density and SW-spindle coordination (either SW phase at spindle peak or phase consistency) predicted overnight MST improvement better than either parameter alone.

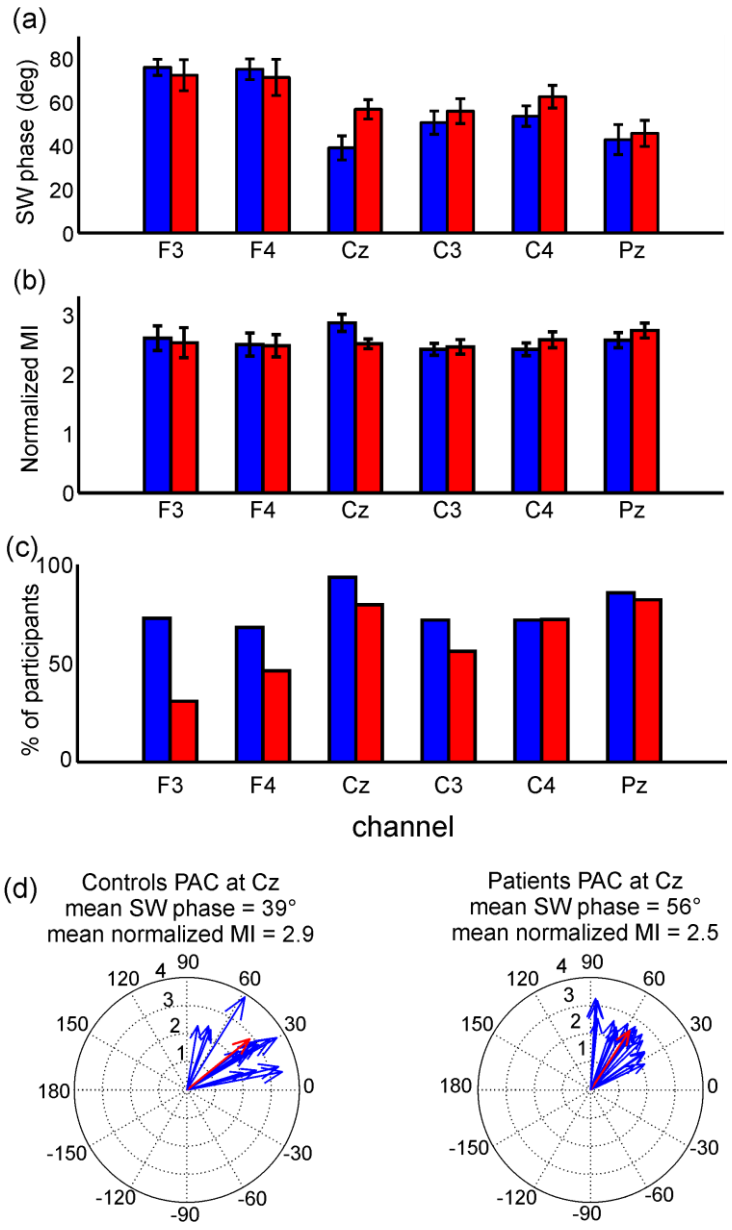

Figure 5. PAC of SWs with spindles. Significant PAC results averaged across nights; Controls (blue), Patients (red). Error bars represent SEM across participants. (a) The SW phase of maximal sigma amplitude during spindle time windows did not significantly differ between groups. (b) Modulation index (MI) normalized by the null distribution did not vary spatially or differ between groups. (c) Percentage of participants with significant PAC as determined by MI permutations. (d) PAC for controls and patients at Cz. Blue arrows represent mean significant PAC for each participant, *i.e.*, the mean SW phase (vector angle) and mean normalized MI (vector length). Red arrows represent group means.

Table 1. SWs during N2. Mean ± SD; F- and p-values were derived from mixed-model regression.

	Electrode*						Night		Group	
	F3	**F4**	**Cz**	**C3**	**C4**	**Pz**	**Non-learning**	**Learning**	**Controls**	**Patients**
SW Density (no./min)	3.90 ± .90	3.96 ± .79	4.15 ± 1.09	3.10 ± .91	3.08 ± .91	3.08 ± 1.32	3.54 ± 1.10	3.48 ± 1.11	3.20 ± 1.01	3.77 ± 1.11
	F(5,277) = 49.77, $p < 10^{-6}$						F(1,37) = .82, p = .37		F(1,34) = 3.11, p = .09	
SW p-p Amp (μV)	100 ± 22	100 ± 22	93 ± 21	90 ± 20	88 ± 20	84 ± 20	91 ± 20	93 ± 22	86 ± 17	97 ± 23
	F(5,274) = 84.49, $p < 10^{-6}$						F(1,35) = .72, p = .40		F(1,34) = 2.80, p = .10	
SW Duration (s)	1.15 ± .07	1.15 ± .08	1.12 ± .07	1.14 ± .08	1.15 ± .08	1.16 ± .07	1.15 ± .07	1.14 ± .07	1.16 ± .07	1.12 ± .07
	F(5,275) = 16.50, $p < 10^{-6}$						F(1,35) = 8.36, p = .007		F(1,34) = 2.98, p = .09	
Relative Sigma Power Locked to SWs	.23 ± .09	.23 ± .09	.31 ± .16	.28 ± .14	.27 ± .13	.37 ± .25	.28 ± .16	.28 ± .16	.37 ± .19	.21 ± .07
	F(5,308) = 41.73, $p < 10^{-6}$						F(1,307) = .002, p = .96		F(1,34) = 16.96, p = .0002	

* O1 and O2 were excluded from analyses (see Methods): SW density: O1: .67±.67, O2: .62±.66.