Journal of Psychiatric Research 44 (2010) 112–120

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Reduced overnight consolidation of procedural learning in chronic medicated schizophrenia is related to specific sleep stages

Dara S. Manoach [a,c,e,*], Katharine N. Thakkar [a,e], Eva Stroynowski [d,e], Alice Ely [d,e], Sophia K. McKinley [d,e], Erin Wamsley [d,e], Ina Djonlagic [d,e], Mark G. Vangel [b,e], Donald C. Goff [a,e], Robert Stickgold [d,e]

[a] Department of Psychiatry, Massachusetts General Hospital, Charlestown, MA 02129, United States
[b] Department of Radiology, Massachusetts General Hospital, Charlestown, MA 02129, United States
[c] Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA 02129, United States
[d] Department of Psychiatry, Beth Israel Deaconess Medical Center, Boston, MA 02215, United States
[e] Harvard Medical School, Boston, MA 02215, United States

ARTICLE INFO

Article history:
Received 27 March 2009
Received in revised form 17 June 2009
Accepted 26 June 2009

Keywords:
Sleep
Schizophrenia
Procedural learning
Motor skill
Memory
Consolidation

ABSTRACT

We previously reported that patients with schizophrenia failed to demonstrate normal sleep-dependent improvement in motor procedural learning. Here, we tested whether this failure was associated with the duration of Stage 2 sleep in the last quartile of the night (S2q4) and with spindle activity during this epoch. Fourteen patients with schizophrenia and 15 demographically matched controls performed a motor sequence task (MST) before and after a night of polysomnographically monitored sleep. Patients showed no significant overnight task improvement and significantly less than controls, who did show significant improvement. While there were no group differences in overall sleep architecture, patients showed significant reductions in fast sigma frequency power (45%) and in spindle density (43%) during S2q4 sleep at the electrode proximal to the motor cortex controlling the hand that performed the MST. Although spindle activity did not correlate with overnight improvement in either group, S2q4 sleep duration in patients significantly correlated with the plateau level of overnight improvement seen at the end of the morning testing session, and slow wave sleep (SWS) duration correlated with the delay in reaching this plateau. SWS and S2q4 sleep each predicted the initial level of overnight improvement in schizophrenia, and their product explained 77% of the variance, suggesting that both sleep stages are necessary for consolidation. These findings replicate our prior observation of reduced sleep-dependent consolidation of motor procedural learning in schizophrenia and link this deficit to specific sleep stages. They provide further evidence that sleep is an important contributor to cognitive deficits in schizophrenia.

1. Introduction

Sleep disturbances in schizophrenia have been described since Kraepelin (1919) and are associated with poor coping skills and diminished quality of life (Goldman et al., 1996; Hofstetter et al., 2005). Accumulating evidence suggests that abnormal sleep also contributes to cognitive deficits in schizophrenia (e.g., Forest et al., 2007; Goder et al., 2004, 2008; Yang and Winkelman, 2006). In a prior study, we reported that chronic medicated patients with schizophrenia failed to demonstrate normal improvements in procedural learning after a night of sleep, in spite of showing intact practice-dependent learning during training the previous day (Manoach et al., 2004). The goal of the present study was to determine whether this reduced overnight consolidation of procedural learning in schizophrenia is associated with alterations in specific sleep stages or their characteristics, which could provide insight into the mechanisms underlying this cognitive deficit.

Subjective sleep disturbance is common in patients with schizophrenia and often presages psychotic decompensation (Benson, 2006; Lieberman et al., 2005). The presence of sleep abnormalities in antipsychotic-naïve and unmedicated patients indicates that abnormal sleep is not merely a side-effect of medications (for meta-analysis see Chouinard et al. (2004)). While there are reports of diverse abnormalities of sleep architecture in schizophrenia, reduced slow wave sleep (SWS) is the most consistent (e.g., Keshavan et al., 1998; Monti and Monti, 2004; Yang and Winkelman, 2006), but not universal (e.g., Chouinard et al., 2004; Lauer et al., 1997), finding. In spite of its ubiquity, abnormal sleep has generally been overlooked as a potential contributor to cognitive deficits in schizophrenia. This neglect may stem from a tendency to regard disturbed sleep as secondary to other factors and from difficulty

* Corresponding author. Address: Department of Psychiatry, Massachusetts General Hospital, 149 13th St., Room 2608, Charlestown, MA 02129, United States. Tel.: +1 617 724 6148; fax: +1 617 726 4078.
E-mail address: dara@nmr.mgh.harvard.edu (D.S. Manoach).

0022-3956/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jpsychires.2009.06.011

specifying the exact nature of the disturbance. There is now overwhelming evidence that sleep plays a critical role in memory consolidation (e.g., Stickgold, 2005) and recent studies of schizophrenia report associations between sleep and cognitive performance in medicated (Goder et al., 2004, 2008) and antipsychotic-naïve (Forest et al., 2007) patients. These findings support the hypothesis that abnormal sleep contributes to cognitive deficits in schizophrenia and highlight the need for further study.

In the present study, we employed the same simple, well-characterized test of motor skill learning, the finger tapping motor sequence test (MST) (Karni et al., 1998; Walker et al., 2002) that we used in our previous study of schizophrenia (Manoach et al., 2004). When healthy young participants are trained on this task, they show significant improvements in speed after a night of sleep, but not after an equivalent period of daytime wake (Walker et al., 2002). Additional nights of sleep lead to more improvement, even with no additional practice (Walker et al., 2003b), but sleep deprivation the first night after training blocks all subsequent non-practice related improvement (Fischer et al., 2002). These findings demonstrate that overnight improvement on this task depends on sleep rather than the mere passage of time. Sleep following MST training also leads to increased functional MRI activation in right primary motor cortex, contralateral to the hand performing the task, and to decreased activation in regions that mediate the conscious monitoring of performance (Walker et al., 2005). These and other findings suggest that sleep-dependent consolidation leads to task automation, resulting in performance that is faster, less variable, and less dependent on voluntary attention (Atienza et al., 2004; Kuriyama et al., 2004; Walker et al., 2005).

Overnight improvement on the MST and other simple motor skill tasks specifically correlates with the amount of Stage 2 sleep in the last quartile of the night (S2q4, Fogel et al., 2007; Smith and MacNeill, 1994; Walker et al., 2002). MST improvement also correlates with the number and density of fast spindles (Rasch et al., 2008), and since the MST is performed with the left hand, it is interesting to note that it is associated with right > left asymmetry of spindle density and power at central electrodes proximal to primary motor cortex (Nishida and Walker, 2007). Sleep spindles are brief, powerful bursts of synchronous neural firing that reach peak density late in the night (De Gennaro et al., 2000) and are hypothesized to mediate the consolidation of procedural memory on the MST (Nishida and Walker, 2007; Rasch et al., 2008; Walker et al., 2002) and other motor tasks (Fogel and Smith, 2006; Tamaki et al., 2008). Studies of schizophrenia show reduced spindle activity (Ferrarelli et al., 2007), and positive relations between Stage 2 spindle density and verbal declarative memory performance (Goder et al., 2008). Here, we expected to replicate our finding of reduced overnight improvement of motor procedural learning in schizophrenia and to correlate it with the duration of S2q4 sleep (Walker et al., 2002), reduced sigma frequency power, which corresponds to sleep spindles, and spindle density during S2q4 sleep, specifically at the right central (C4) electrode, and reduced right > - left sigma asymmetry at central electrodes (C4–C3) during S2q4 sleep (Nishida and Walker, 2007; Rasch et al., 2008; Walker et al., 2005).

2. Methods

2.1. Participants

All participants were screened to exclude substance abuse or dependence within the past six months, diagnosed sleep disorders, or any independent conditions that might affect brain function. Outpatients with schizophrenia ($n = 16$) were recruited from an urban mental health center. Two patients were excluded for failing to type a single correct sequence during training. The remaining 14 patients had all been maintained on stable doses of antipsychotic medications for at least six weeks, 12 on atypicals, one on typicals, and one on both. No patients took anticholinergic medications and ten took diverse adjunctive medications for anxiety, agitation, and/or concurrent mood disturbance. Diagnoses were confirmed with Structured Clinical Interviews for DSM-IV (First et al., 1997). Clinical status was characterized with the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962), the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen 1983).

Healthy control participants ($n = 16$) without a history of psychiatric illness were recruited from the community. One control did not complete the study. The 14 schizophrenia and 15 control participants did not differ in age, sex, handedness (modified Edinburgh Handedness Inventory White and Ashton (1976)), or mean parental education (Table 1). Participants gave written informed consent and the study was approved by the Institutional Review Boards of Massachusetts General Hospital, the Massachusetts Department of Mental Health, and Beth Israel Deaconess Medical Center. Remuneration included a bonus based on the number of correct sequences typed on each MST administration.

2.2. Procedures

Experimental design: In the week prior to their stay in the Mallinckrodt General Clinical Research Center (GCRC) at Massachusetts General Hospital, participants met with study staff to complete informed consent, tour the GCRC, and receive a wrist actigraph to wear. Following admission to the GCRC, participants were Trained on the MST and Tested nine hours later (Fig. 1). They were then Trained on a second MST sequence and Tested on this sequence after an additional 9 h. Finally, they were retested on the first sequence. For one sequence, Training and Testing occurred across the day (Wake interval), and for the second sequence, Training and Testing occurred across a night (Sleep interval). The order of the Wake and Sleep intervals and of the two MST sequences were counterbalanced within each group. Participants were monitored to ensure that they did not nap during the day.

Finger Tapping Motor Sequence Test (MST): The MST is described in detail elsewhere (Manoach et al., 2004). In brief, participants pressed four numerically labeled keys on a standard computer keyboard with the fingers of their left hand, repeating a five element tapping sequence (e.g., 4–1–3–2–4) "as quickly and accurately as possible" for 30 s. Throughout the finger tapping trials, the numeric sequence was displayed at the top of the screen. Each session con-

Table 1
Means, standard deviations, and group comparisons of demographic data and rating scale scores. The Phi value is the result of a Fisher's Exact Test.

Participant characteristics	Healthy ($n = 15$)	Schizophrenia ($n = 14$)	t	p
Age	42 ± 6	41 ± 7	0.6	0.54
Sex	11M/4F	11M/3F	Phi = 0.06	0.99
Laterality Score (Handedness)	65 ± 46	60 ± 59	.27	0.79
Parental Education (years)[a]	13 ± 3	13 ± 3	0.25	0.80
Age of Onset		24 ± 6	Average Level of	
Length of Illness (years)		16 ± 8	Severity	
BPRS		18 ± 12	Minimal	
PANSS positive		15 ± 7	Mild	
PANSS negative		15 ± 5	Mild	
SANS		39 ± 17	Mild	

[a] One healthy and two schizophrenia participants were unable to provide this information.

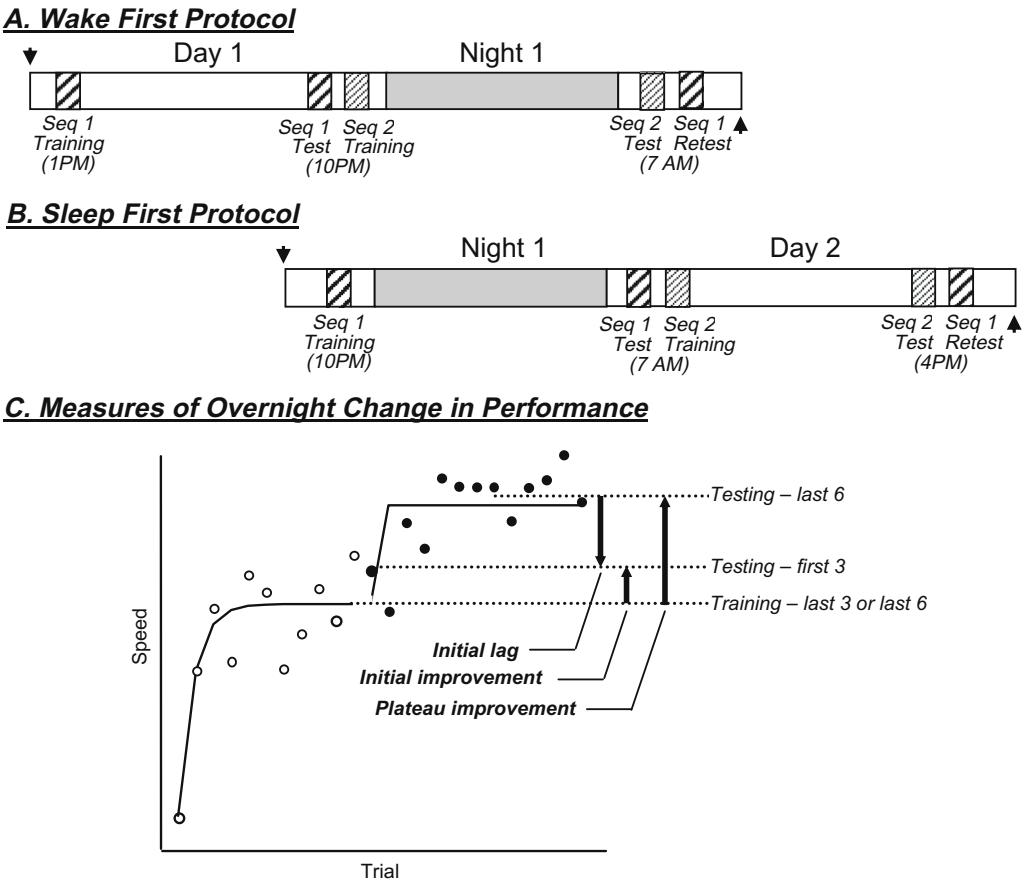

Fig. 1. Experimental protocols. Participants were pseudorandomly assigned to (A) "Wake First" or (B) "Sleep First" protocols. MST sequence order was also counterbalanced within protocol order. Arrows indicate times of admission and discharge from the GCRC. (A) Wake first: Participants arrived at the GCRC at ~10AM. Ten hours prior to their habitual bedtime (~1PM), they Trained on the first MST Sequence (MST Seq 1). Nine hours later, they were Tested on this sequence, and 10 min later, they trained on MST Seq 2. They were subsequently wired for polysomnography and allowed to go to sleep. Seq 2 Test occurred in the morning, approximately nine hours after Training, and after electrodes had been removed and breakfast eaten. Ten minutes later, they were retested on MST Seq 1. (B) Sleep first: Participants arrived at the GCRC 2.5 h prior to their habitual bedtime (approximately 8.30PM), and were Trained on MST Seq 1 90 min later. Prior to going to sleep, they were wired for polysomnography. Testing of MST Seq 1 occurred the next morning after electrodes had been removed and breakfast eaten, approximately nine hours after Training. Ten minutes later, they trained on MST Seq 2. Testing of MST Seq 2 occurred nine hours later, during the afternoon, followed 10 min later by the retest of MST Seq 1. (C) Measurements of Overnight Change in Performance: *Initial* improvement = percent increase from the last three Training trials to the first three Test trials; *Plateau* improvement = percent increase from the last six Training trials to the last six Test trials; *Initial lag* = plateau improvement – initial improvement.

sisted of 12 trials separated by 30-s rest periods. Each of the 12 trials was scored for the number of correct sequences and the number of errors. The primary outcome measure was the number of correct sequences typed, which reflects both the speed and accuracy of performance. We also examined error rates, but no significant changes, across time, conditions, or groups, were observed.

Subjective Alertness: Prior to each MST session, participants completed the Stanford Sleepiness Scale (SSS), a standard measure of subjective alertness (Hoddes et al., 1973).

Actigraphy: Participants wore a Mini-Mitter Actiwatch®-64 actigraph (Mini-Mitter Company, Inc., Bend, OR) on their wrist from enrollment to study completion, including at least three nights prior to their stay in the GCRC. The actigraph monitors and records wrist movement in 15-s epochs, and provides an estimates of sleep and nap time based on periods of wrist immobility.

Polysomnography: Participants were wired for standard polysomnographic (PSG) sleep recordings prior to retiring. EEG (C3, C4, Cz, O1, 02), EOG, and EMG were recorded on an Embla A10 ambulatory monitor (Medcare Systems, Buffalo NY). EEG data were sampled at 200 Hz and band-pass filtered between .3 and 35 Hz for analysis. The spectral data analyses were conducted with a resolution of .25 Hz.

2.3. Data analysis

MST Performance: Schizophrenia and control groups were compared on MST performance using *t*-tests. *Practice-dependent* improvement was calculated as the increase in correct sequences from the first trial to the average of the last three trials of Training. *Initial* improvement was calculated as the percent increase from the last three Training trials to the first three Test trials. *Plateau* improvement was calculated as the percent improvement from the last six Training trials of to the last six Test trials. *Initial lag*, which reflects the delay in expressing the *plateau* level of improvement, was calculated as *plateau* improvement minus *initial* improvement. We examined the relations between measures of overnight improvement and sleep with linear regression models.

Polysomnography: Data were manually scored for sleep stages for each 30 s epoch according to standard criteria (Rechtschaffen and Kales, 1968) by an experienced PSG technician who was blind to MST results. Time spent in Stage 1, Stage 2, S2q4, SWS, and rapid eye movement (REM) sleep were quantified as both the number of minutes and the fraction of the total night's sleep (percent). Sleep efficiency was calculated as total sleep time divided by time spent

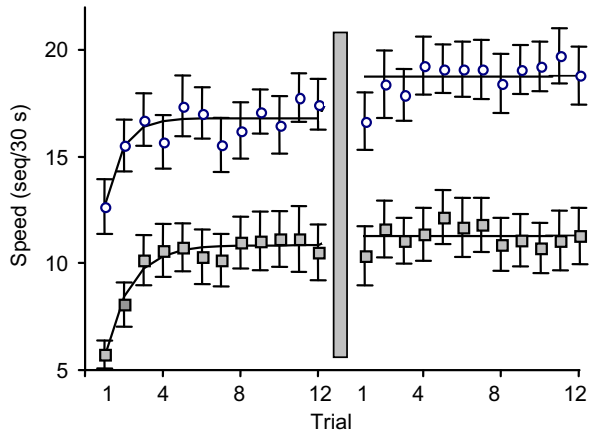

Fig. 2. MST performance. Motor skill learning across Training and Test trials for healthy control ($n = 15$, open circles) and schizophrenia ($n = 14$, filled squares) groups. The data point for each trial represents the group average ± SE. The y-axis represents the number of correct sequences typed in each 30 s trial. The shaded bar represents a night of sleep in the GCRC in between Training and Test trials. The solid lines fit through the data points for Training and Test were derived using an exponential model of motor learning (see Manoach et al. (2004)). While patients and controls did not differ in the absolute amount of learning during Training trials (Table 2), only controls showed significant overnight improvement, which was realized at the plateau of Test.

in bed. Number of awakenings, awakenings per hour, awakenings >60 s, and awakenings >60 s per hour were calculated.

Further analyses were conducted using MATLAB and the EEGLAB signal processing toolbox (MathWorks, Natick MA) (Delorme and Makeig, 2004). Following manual artifact rejection, relative spectral power was examined in the low (12–13.5 Hz) and high (13.5–15 Hz) frequency sigma bands. We also conducted exploratory analyses of power in the delta (1–4 Hz), theta (4–7 Hz), alpha (8–12 Hz), sigma (12–15 Hz), and beta (15–35 Hz) bands via Welch's method, using a Hanning window with 50% overlap. Spindle density during S2q4 was also analyzed using the automated algorithm of Ferrarelli et al. (2007), and calculated as spindles per minute.

3. Results

3.1. MST performance (Fig. 2, Table 2)

Practice-Dependent Improvement: Both groups showed significant improvement across Training. While the groups did not differ in absolute improvement, patients showed greater proportional improvement (45 vs. 115%, $t(27) = 2.27$, $p = .03$).

Table 2
Practice- and sleep-dependent changes in MST performance. Practice-dependent improvement is given in sequences per 30 s trial; *intial*, *plateau*, and *initial lag* values are percent change compared to pre-sleep levels. Within-group *t*-tests are compared to no improvement; between group *t*-tests compare healthy control (HC) and schizophrenia (Sz) participants.

	Practice-dependent improvement	Initial % improvement	Plateau % improvement	Initial % Lag
Healthy controls	4.6 ± 2.8	2.8 ± 13.9	15.2 ± 13.5	12.4 ± 11.4
t(14), *p*	6.32, <.0001	0.74, .47	5.31, <.0001	4.16, .001
Schizophrenia	5.2 ± 3.1	4.7 ± 18.1	5.0 ± 10.7 1.05,	0.3 ± 15.3
t(13), *p*	6.28, <.0001	0.23, .82	.31	0.66, .95
HC vs. Sz	0.56, .58	0.33, .74	2.23, .03	2.4, .02
t(27), *p*				

Overnight Improvement: While controls showed significant *plateau* improvement overnight, patients did not, and improvement was significantly greater in controls (15.2% vs. 5.0%). This is similar to our previous study (Manoach et al., 2004) in which only controls showed significant *plateau* improvement (16.6% vs. 7.6%). Neither group showed significant *initial* overnight improvement, and the group difference was not significant. In controls, the absence of significant *initial* improvement reflects the presence of a significant *initial lag*. *Initial* improvement was 12.4% less than *plateau* improvement. No significant *initial lag* was seen in patients (0.3%) since they did not improve at plateau, and *initial lag* was significantly greater in controls.

3.2. Polysomnography (Table 3)

Although patients spent significantly more time in bed in the GCRC than controls and took twice as long to initiate sleep, they did not show significant differences in either sleep efficiency or total sleep time. Nor were there significant group differences in either the amount or percent of time spent in any sleep stage, although there were trends for increased time in S2q4 and decreased percent time in REM for patients.

As predicted, fast sigma power, which corresponds to sleep spindles, was significantly lower in patients than controls at C4 during S2q4 (45% decrease; Table 3). There were also trends to reduced fast sigma power during S2q4 at the right occipital lead (43% decrease; $t(28) = 1.90$, $p = .07$), and averaged over all leads (40% decrease; $t(28) = 1.76$, $p = .09$). Decreased fast sigma power during S2q4 was accompanied by a shift in the average spindle frequency, which was significantly lower in patients (13.67 vs. 14.04 Hz, $t(27) = 3.62$, $p = .001$) at all leads (all p's < .01). Spindle density during S2q4 also showed a significant 43% reduction in schizophrenia patients at C4 (Table 3), but not at other electrodes (all p's > .19). No group differences were seen in the slow sigma band (all p's > .50). Asymmetry of fast sigma power at central electrodes (C4–C3) during S2q4 did not differ between groups.

Table 3
Sleep stage durations expressed in minutes and percentages of total sleep time and other sleep parameters in healthy control (HC) and schizophrenia (Sz) participants.

	HC ($n = 15$)	Sz ($n = 14$)	*t*	*p*
Stage 1				
min	25 ± 13	34 ± 20	−1.47	.15
%	6 ± 3	8 ± 6	−1.30	.20
Stage 2				
min	247 ± 69	282 ± 70	−1.35	.19
%	62 ± 12	67 ± 11	−1.14	.26
Slow wave sleep				
min	38 ± 23	41 ± 37	−0.26	.80
%	10 ± 7	9 ± 8	0.37	.71
REM				
min	86 ± 40	66 ± 36	1.39	.18
%	22 ± 10	16 ± 8	1.77	.08
Stage 2: 4th Quarter (S2q4)				
min	56 ± 18	79 ± 23	−1.95	.06
%	15 ± 6	17 ± 32	−0.93	.25
S2q4 fast sigma power C4				
%	2.91 ± 2.06	1.61 ± 1.22	2.05	.05
S2q4 fast sigma power C4–C3				
%	.49 ± 2.19	−.02 ± .29	0.87	.39
S2q4 spindle density C4				
min^{-1}	.60 ± .36	.34 ± .31	2.05	.05
Time in bed				
min	492 ± 53	551 ± 72	−2.57	.02
Sleep onset latency				
min	35 ± 33	72 ± 60	2.04	.05
Sleep efficiency				
%	80 ± 9	77 ± 11	0.90	.38
Total sleep time				
min	396 ± 74	423 ± 85	−0.91	.37

Exploratory analyses of spectral power at alpha, beta, delta, theta, and slow oscillation frequencies across all NREM sleep revealed significant reductions in patients in delta power at the left occipital lead (47% reduction, $t(26) = 2.33$, $p = .03$) and in theta power at both occipital leads (left/right 31%/32% reduction, $t(26) = 2.11$, $p = .05$, $t(26) = 2.27$, 32%, $p = .03$), although these differences did not survive correction for multiple comparisons.

3.3. Correlations of Overnight improvement with sleep parameters (Table 4, Figs. 3 and 4)

Initial improvement in schizophrenia, which ranged from 15% deterioration to 45% improvement, was significantly predicted by the amount of S2q4 sleep (Fig. 3A), as well as by the amount of SWS (Fig. 3B). Furthermore, the product of SWS and S2q4 provided an even better fit for *initial* improvement, explaining 77% of the variance (Fig. 3C). SWS and S2q4 were not themselves correlated ($r = -.15$, $p = .61$), and linear regressions showed that while SWS and S2q4 contributed independently to *initial* improvement (SWS $t(13) = 4.01$, $p = .002$; S2q4 $t(13) = 3.08$, $p = .01$), when the product SWS × S2q4 was added to the model, only the product contributed significantly (adjusted $R^2 = .78$; SWS $t(13) = 0.89$, $p = .39$; S2q4 $t(13) = 0.27$, $p = .79$; SWS × S2q4 $t(13) = 2.38$, $p = .04$). When age was added to the regression models, the effects of S2q4, SWS, and their product remained significant.

Since the product of S2q4 and SWS strongly predicted *initial* improvement, and *initial* improvement is the difference between *plateau* improvement and *initial lag*, we investigated whether SWS and S2q4 had separate effects on these parameters. When we did so, a double dissociation emerged. *Plateau* improvement correlated positively with S2q4 (Fig. 4A), but not SWS (Fig. 4B), while *initial lag* correlated negatively with SWS (Fig. 4D), but not S2q4 (Fig. 4C). Thus, SWS strongly predicts *initial lag* and S2q4 strongly predicts *plateau* improvement, and together they even more strongly predict *initial* improvement in schizophrenia.

In controls, *plateau* improvement was negatively correlated with Stage 2 and S2q4 sleep, and positively correlated with REM sleep, but these relations were entirely driven by an outlier whose *plateau* improvement was more than two standard deviations higher than the group mean. Removing this individual from the analyses rendered these correlations non-significant (all p's > .70; see Table 4). Surprisingly, in controls, no other correlations between overnight improvement and the amount of S2q4 sleep or any other sleep stage were observed. Nor were either fast or slow

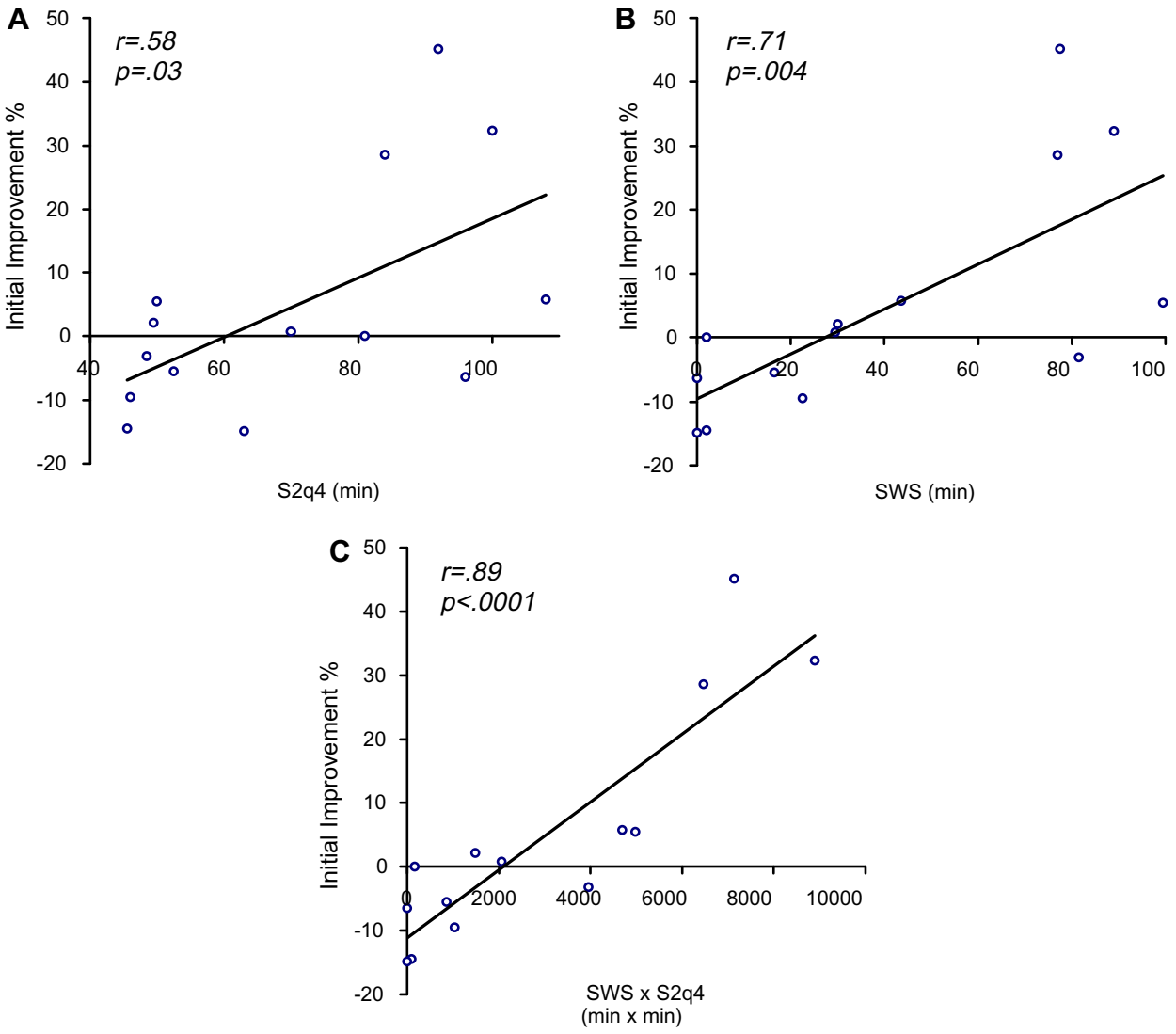

Fig. 3. Correlation of *initial* overnight improvement with minutes spent in SWS and S2q4 in schizophrenia patients. (A) Correlation with S2q4; (B) Correlation with SWS; (C) Correlation with SWS × S2q4.

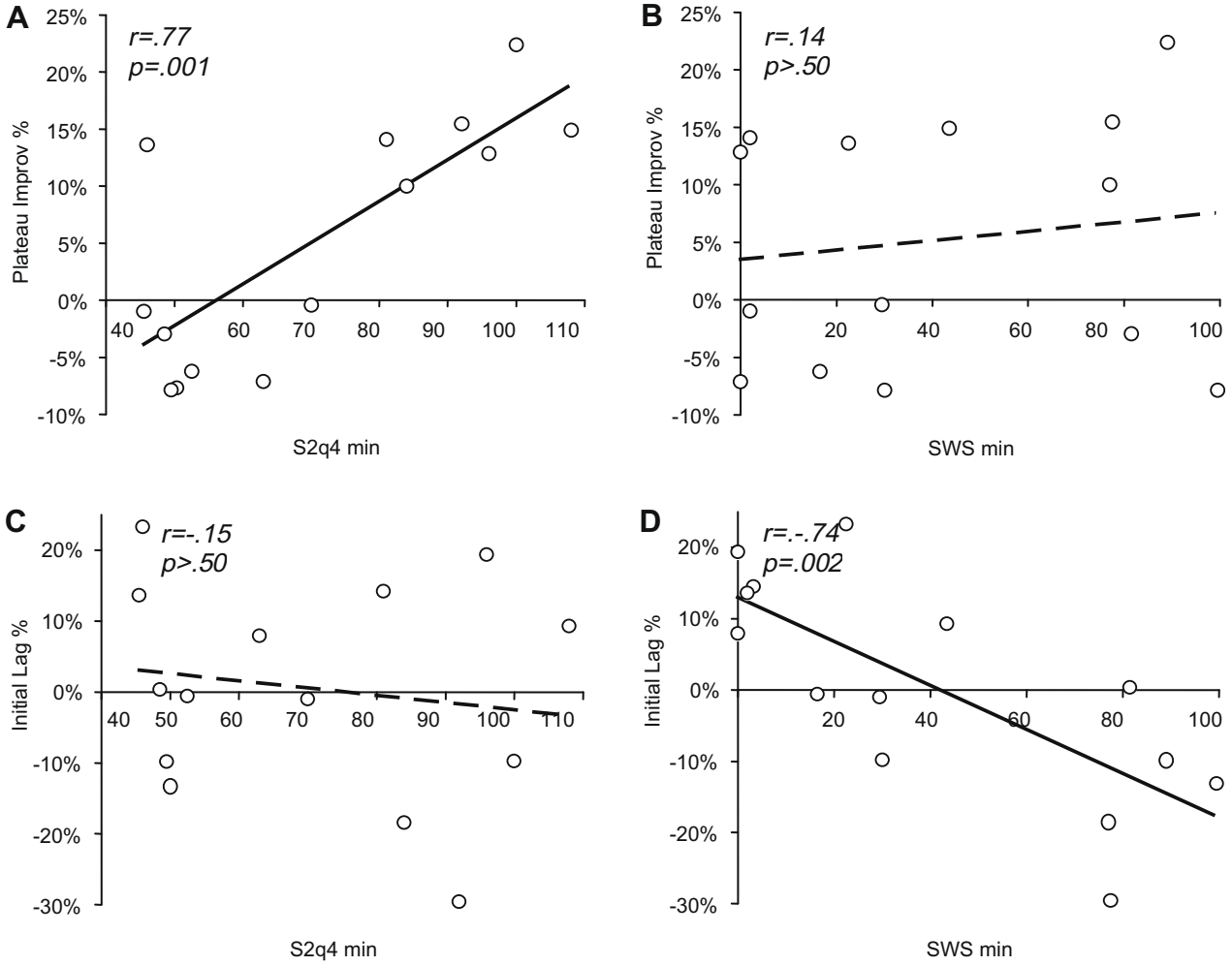

Fig. 4. Correlations of sleep stages with measures of overnight improvement in schizophrenia patients. (A) *Plateau* improvement vs. S2q4; (B) *Plateau* improvement vs. SWS; (C) *Initial Lag* vs. S2q4; (D) *Initial Lag* vs. SWS.

sigma power, or asymmetry of fast or slow sigma power during S2q4 sleep significantly correlated with any measure of overnight improvement in either group. In controls, a higher spindle density at C4 during S2q4 sleep was associated with a smaller *initial lag* ($r = -.68$, $p = .004$). This relation was not expected, and when age, which correlated with *initial lag* ($r = .52$, $p = .05$), but not with *initial* ($r = -.31$, $p = .27$) or *plateau* ($r = .13$, $p = .65$) improvement, was entered as a covariate in the regression model, the effect of age remained significant, but the effect of spindle density was no longer significant ($p = .90$). Age did not correlate with any measure of overnight change in performance in the schizophrenia group (all p's > .60).

Table 4
Relations of minutes in specific sleep stages to overnight improvement in healthy control (HC) and schizophrenia (Sz) participants.

		Initial improvement		Plateau improvement		Initial Lag	
		r	*p*	*r*	*p*	*r*	*p*
Stage 1 NREM	HC	−.04	.88	−.10	.73	−.06	.83
	Sz	.31	.28	.02	.95	−.35	.21
Stage 2 NREM	HC	−.16	.57	−.50	.05*	−.40	.15
	Sz	−.17	.56	−.03	.92	.18	.53
Slow wave sleep (SWS)	HC	.17	.54	.23	.41	.06	.83
	Sz	.71	.003	.14	.63	−.74	.002
REM	HC	.20	.48	.52	.05*	.37	.18
	Sz	.00	.99	−.12	.69	−.09	.77
Stage 2: 4th Quarter (S2q4)	HC	−.31	.27	−.58	.02*	−.31	.28
	Sz	.58	.03	.77	.001	−.15	.61
SWS x S2q4	HC	−.11	.71	−.25	.37	−.17	.55
	Sz	.89	<.0001	.44	.12	−.74	.002

* The significant correlations in controls between plateau improvement and Stage 2, S2q4, and REM sleep were due to a single outlier whose plateau improvement was more than two standard deviations from the mean. When this individual was removed from the analyses, the correlations were no longer significant (Stage 2: $r = -.09$, $p = .76$; S2q4: $r = -.11$, $p = .71$; REM: $r = .05$, $p = .88$).

3.4. Supplemental analyses

Subjective alertness: There were no significant group differences in SSS scores at any MST session (all p's $\geqslant .17$), nor were there any significant differences in alertness in either group between Training and Test (all p's $\geqslant .10$). On the seven point scale (1 being most alert), mean values were as follows: Wake: controls 2.1 ± 0.7, patients 2.2 ± 0.9; Sleep: controls 2.6 ± 1.0, patients 2.2 ± 1.0).

Actigraphy: In controls, simultaneous PSG and actigraphy measurements during the GCRC night showed good correspondence for total sleep time ($r = .83$, $p = .001$), sleep onset latency ($r = .61$, $p = .02$), and sleep efficiency ($r = .54$, $p = .04$). A comparison of actigraphy during home nights with the GCRC night suggests that sleep was similar in both settings. Controls did not differ significantly on measures of time in bed, sleep onset latency, sleep

efficiency, or total sleep time. For patients, in contrast, simultaneous actigraphy and PSG measurements during the GCRC night showed poor correlation (total sleep time: $r = .44$, $p = .12$; sleep onset latency: $r = .08$, $p = .79$; sleep efficiency, $r = -.28$, $p = .33$). This largely reflected that patients lay still in bed for long periods prior to falling asleep leading actigraphy to underestimate sleep onset latency, on which calculations of sleep efficiency and total sleep time are also based. As a result, wrist actigraphy was not a reliable measure of sleep in the patient group, preventing meaningful comparison of measures of sleep in the GCRC and at home.

PSG Awakenings: Patients and controls did not differ on any index of awakenings (all p's > .85).

Medication effects: In patients, antipsychotic medication dose as measured by chlorpromazine equivalents (Woods, 2003) was not significantly correlated with any measure of overnight improvement or with SWS, S2q4, or their product. In addition, the five patients taking benzodiazepines did not significantly differ from the rest of the group on any performance measure.

Order effects: Although the order of the Wake and Sleep intervals was counterbalanced, it is possible that learning two different sequences in close temporal proximity affected the results. This is unlikely as a prior study showed that test performance on the sequences learned first and second did not significantly differ if Training for the second sequence occurred after Testing on the first sequence, as was the case in the present study (Walker et al., 2003a). In addition, ANOVAs showed no significant main effects of order or group by order interaction for any of the outcome measures.

Wake State MST Improvement: To exclude the possibility that reduced overnight improvement in schizophrenia actually reflected deterioration of learning during wake periods rather than a failure to improve overnight, we also Trained and Tested participants across an equivalent period of wake. Patients showed no change in performance across the Wake interval (*initial* improvement, $t(13) = 0.23$, $p = .83$; *plateau* improvement, $t(13) = 1.67$, $p = .12$). While controls showed no *initial* improvement across Wake (0.0 ± 0.1), they did show significant *plateau* improvement ($10.3 \pm 17\%$, $t(14) = 2.4$, $p = .03$), which did not differ significantly from *plateau* improvement over a night of sleep ($15.2 \pm 14\%$, $t(14) = 0.94$, $p = .36$).

4. Discussion

Consistent with our previous report (Manoach et al., 2004), in the context of intact practice-dependent learning, chronic medicated schizophrenia patients failed to demonstrate significant overnight improvement of motor procedural memory. In this respect, they differed significantly from healthy controls, who did show significant improvement. The present study extends these findings by demonstrating that in schizophrenia, overnight improvement is correlated with the amount of time spent in specific sleep stages. As predicted, based on findings in young healthy individuals (Smith and MacNeill, 1994; Walker et al., 2002), the amount of S2q4 sleep significantly predicted *initial* overnight improvement in schizophrenia. But unexpectedly, SWS did as well, and the product of SWS and S2q4 sleep accounted for 77% of the variance in *initial* overnight improvement. These findings demonstrate reduced sleep-dependent consolidation of procedural memory in schizophrenia and support the hypothesis that sleep makes an important contribution to cognitive deficits.

Although patients showed significantly reduced consolidation, there were no significant group differences in the amounts or distribution of time spent in specific sleep stages, or in any index of awakenings. This excludes the possibility that group differences in overnight improvement reflect sleep disturbances secondary to sleep disorders such as apnea or restless leg syndrome. Importantly, we cannot exclude the possibility that these group differences are consequences of medication. All of the patients took antipsychotic medications, which affect neurotransmitter systems that play an important role in sleep regulation and have diverse effects on sleep (Benson, 2008; Krystal et al., 2008; Monti and Monti, 2004). Overall, however, antipsychotic medications tend to improve sleep maintenance and to normalize sleep architecture (e.g., Maixner et al., 1998; Salin-Pascual et al., 1999). While dosage as measured by CPZ equivalent was not correlated with any measure of overnight improvement and antipsychotic medications generally normalize sleep measures, we cannot exclude the possibility that they contributed to variability in improvement. Our sample was too small to allow any firm conclusions regarding the effect of the range of medications used on overnight improvement, although we note that the two subjects taking olanzapine were among the three participants in either group with the highest *initial* improvement (binomial test, $p < .01$). Olanzapine is known to increase Stage 2 and SWS (Monti and Monti, 2004; Salin-Pascual et al., 1999), although a recent study reported that it also decreased spindle density in schizophrenia (Goder et al., 2008). Excluding the two patients on olanzapine, the correlation of *initial improvement* with the product of SWS and S2q4 remained highly significant ($r = .89$, $p < .0001$). The extent to which the deficits in sleep-dependent memory consolidation reported here reflect medication side-effects versus a disease process, thus remains unclear. Another limitation of the present study is that since participants were only studied for one night, we cannot exclude the possibility of first night effects, which may have differentially affected controls and patients. In controls, actigraphy suggested that sleep initiation and maintenance were similar at home and in the GCRC. In patients, however, actigraphy proved to be an unreliable index of sleep. Nevertheless, the fact that the current GCRC findings of a failure to show significant overnight improvement in schizophrenia replicate those seen in our previous home study (Manoach et al., 2004) argues that sleep in the laboratory was not a critical determinant of the failure of sleep-dependent memory consolidation in schizophrenia.

We previously proposed that it might not be the overall architecture of sleep that is culpable in schizophrenia; rather it may be specific memory consolidation processes that are normally activated during sleep (Manoach et al., 2004). Regardless of whether these memory deficits reflect medication side-effects or disease process, the present findings implicate processes occurring during SWS and S2q4 sleep. As predicted, patients showed significant reductions in fast sigma frequency power and in spindle density during S2q4 sleep compared to controls, specifically at the electrode proximal to the motor cortex that controls the hand that performed the MST. This is consistent with the finding of Ferrarelli and colleagues (2007) of decreased spindle activity in medicated patients with schizophrenia. Moreover, in the present study, sleep was recorded following training on a memory task, and the sigma power and spindle density decreases only reached significance at C4, the lead overlying motor cortex contralateral to the hand that performed the task. Contrary to our predictions, however, spindle activity did not correlate with overnight improvement in either group. Recent findings suggest that it may be the change from baseline spindle activity levels that correlates with overnight improvement of motor procedural learning, rather than the absolute level of spindles (Peters et al., 2008). Having only one night of PSG, we were not able to test that possibility here.

Although the duration of SWS and S2q4 both predicted *initial* overnight improvement in schizophrenia they were not themselves correlated, suggesting that their contributions were independent. Furthermore, when their product was added to a regression model of *initial* improvement, their individual contributions were no

longer significant, only their product was. This suggests that both stages are necessary for consolidation and is consistent with the two-stage model of procedural learning proposed in our earlier studies (Stickgold et al., 2000). We previously found that overnight improvement on a visuoperceptual procedural learning task correlated with both SWS early in the night and REM sleep in the last quarter, but even more strongly with their product (Stickgold et al., 2000). A subsequent study of naps showed that while SWS prevented deterioration in visuoperceptual performance over the day, naps with both SWS and REM led to same-day improvement (Mednick et al., 2002). These findings suggest that SWS, which is predominant early in the night, stabilizes visuoperceptual procedural memory, while REM sleep later in the night enhances it. A similar model fits our motor procedural memory findings. While both SWS and S2q4 correlated with *initial* improvement, when *initial* improvement was broken into its component parts, there was a striking double dissociation. SWS appeared to prevent the *initial lag*, while S2q4 appeared to facilitate the sleep-dependent enhancement seen at *plateau*.

Although the present control group did not show the significant *initial* improvement that we observed in our prior study (Manoach et al., 2004), the difference in *initial* improvement between the two control samples did not significantly differ, nor were there significant differences in *plateau* improvement or *initial lag*. Nor did the patient samples from the two studies differ in any measure of overnight change in performance. Our present findings in controls also differ from prior MST studies of healthy college-aged participants in two important respects. First, studies of younger participants (Nishida and Walker, 2007; Walker et al., 2002; Walker et al., 2003b) do not usually show the ramping up of performance over the first 2 or 3 Test trials that characterizes the *initial lag* seen in our healthy middle-aged sample. However, more recent studies of middle-aged (Manoach et al., 2004), and healthy elderly participants (ages 60–79) (McKinley, 2008) revealed similar *initial lags*. The finding in the present study that *initial lag* increased with age ($r = .52$, $p = .05$) suggests that age contributes to the *initial lag*. In addition to age, task difficulty may play a role in the *initial lag*, as healthy young college students do show an initial ramping up of test performance when a longer and more difficult finger tapping sequence is used (Kuriyama et al., 2004). Thus, the finding that only older participants show an *initial lag* with the standard 5-digit sequence may simply reflect the fact that the task is more difficult for them.

A second, and possibly related novel finding is the correlation of SWS with *initial* improvement, and specifically the *initial lag* component of *initial* improvement. No correlations of MST improvement with SWS have been reported in studies of young healthy participants, but this may simply reflect the absence of an *initial lag*. Interestingly, there are reports of increased SWS following learning of motor procedural tasks, one in young adults (Huber et al., 2004), and one in older, but not younger adults (Peters et al., 2008). It is important to note, however, that the correlation with SWS in the present study was only observed in patients.

Although it is unclear why sleep-stage dependencies were only seen in patients, the lack of correlations in controls is consistent with recent findings in an elderly cohort (60–79, McKinley 2008) and may reflect changes in sleep architecture (Ohayon et al., 2004), reductions in Stage 2 sleep spindles (e.g., Peters et al., 2008), and/or reliance of different procedural learning strategies in older individuals (Brown et al., in press). As a result of these differences, consolidation may rely on both wake- and sleep-dependent processes in older cohorts (Robertson et al., 2005). This could help explain our findings of significant improvement across wake as well as sleep in controls, a result not seen in younger individuals. This leaves unresolved the obvious question of why middle-aged schizophrenia patients, who as a group showed no significant overnight improvement, nonetheless showed significant sleep-stage dependencies.

In summary, we have replicated our prior observation of reduced sleep-dependent consolidation of motor procedural learning in schizophrenia (Manoach et al., 2004) and now link variation in the expression of this deficit to specific sleep stages. These correlations with sleep stages ($R^2 = .77$ for SWS $\times$ S2q4), provides the first direct evidence of the sleep-dependency of this deficit in overnight improvement. Insofar as sleep-dependent consolidation of procedural learning reflects task automation (Atienza et al., 2004; Kuriyama et al., 2004; Walker et al., 2005), our findings support the hypothesis of impaired automation in schizophrenia (Manoach, 2003). Since all tasks have procedural components, a fundamental breakdown of sleep-dependent automation in schizophrenia could contribute to a generalized performance deficit (Chapman and Chapman, 1978; Dickinson and Harvey, 2009). Greater allocation of limited-capacity attentional resources to task components that should have been automated would leave fewer available for other, higher-order, task demands. Taken as a whole, these findings provide further evidence that sleep is an important contributor to cognitive deficits in schizophrenia. Understanding this contribution, and clarifying the contribution of medications to this effect, may open new avenues for treatment.

Role of funding sources

Study sponsors had no role in the acquisition, analysis, or presentation of study data.

Contributors

Dara S. Manoach was responsible for all aspects of the present study including the design and execution of the study, data analysis, and manuscript preparation.

Katharine N. Thakkar: data acquisition and analysis of MST findings.

Eva Stroynowski: data acquisition and scoring and analysis of PSG data.

Alice Ely: data acquisition and scoring, analysis, and quality control of PSG data.

Sophia K. McKinley: analysis and interpretation of actigraphy data.

Erin Wamsley: analysis and interpretation of spectral PSG data.

Ina Djonlagic: consultant regarding medication effects on sleep parameters.

Mark G. Vangel: provided statistical consultation to all aspects of data analysis.

Donald C. Goff: responsible for patient recruitment and characterization. Contributed to interpretation of the findings.

Robert Stickgold: participated with Dr. Manoach on all aspects of the present study including the design and execution of the study, data analysis, and manuscript preparation.

Conflict of interest

This was not an industry-supported study. Dr. Manoach has received research funding and consulting fees from Sepracor Inc. Dr. Stickgold has received research funding from Merck & Co., Actelion Pharmaceuticals Ltd., and Sepracor Inc., as well as consulting fees from Actelion Pharmaceuticals Ltd. and Sepracor Inc., speaking fees from Epix Pharmaceuticals, and an educational grant from Takeda Inc. Ms. Stroynowski is presently employed by Alkermes. Dr. Goff has received honoraria or research support over the past year from Organon, Xytis, Wyeth, Forest Labs, Eli Lilly, Pfizer, and

Ortho-McNeil-Janssen. None of the other authors have any conflicts of interest to disclose.

Acknowledgements

Support from: Mallinckrodt General Clinical Research Centers Program at Massachusetts General Hospital (M01-RR-01066); NIMH (MH48832) and NIH T32 training grant HL07901-10 to the Harvard Division of Sleep Medicine. We are grateful to the staff of the Mallinckrodt GCRC program, particularly Mary Sullivan, RN, for their support.

References

Andreasen NC. Scale for the assessment of negative symptoms (SANS). Iowa City: University of Iowa; 1983.
Atienza M, Cantero JL, Stickgold R. Posttraining sleep enhances automaticity in perceptual discrimination. Journal of Cognitive Neuroscience 2004;16:53–64.
Benson KL. Sleep in schizophrenia: impairments, correlates, and treatment. Psychiatric Clinics of North America 2006;29:1033–45. abstract ix–x.
Benson KL. Sleep in schizophrenia. Sleep Medicine Clinics 2008;3:251–60.
Brown R, Robertson E, Press DZ, in press. Sequence skill acquisition and off-line learning in normal aging. Public Library of Science One.
Chapman LJ, Chapman JP. The measurement of differential deficit. Journal of Psychiatry Research 1978;14:303–11.
Chouinard S, Poulin J, Stip E, Godbout R. Sleep in untreated patients with schizophrenia: a meta-analysis. Schizophrenia Bulletin 2004;30:957–67.
De Gennaro L, Ferrara M, Bertini M. Topographical distribution of spindles: variations between and within NREM sleep cycles. Sleep Research Online 2000;3:155–60.
Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. Journal of Neuroscience Methods 2004;134:9–21.
Dickinson D, Harvey PD. Systemic hypotheses for generalized cognitive deficits in schizophrenia: a new take on an old problem. Schizophrenia Bulletin 2009;35:403–14.
Ferrarelli F, Huber R, Peterson MJ, Massimini M, Murphy M, Riedner BA, et al. Reduced sleep spindle activity in schizophrenia patients. The American Journal of Psychiatry 2007;164:483–92.
First MB, Spitzer RL, Gibbon M, Williams JBW, 1997. Structured clinical interview for DSM-IV axis I disorders, research version, patient edition with psychotic screen (SCID-I/P W/PSY SCREEN). New York: Biometrics Research, New York State Psychiatric Institute.
Fischer S, Hallschmid M, Elsner AL, Born J. Sleep forms memory for finger skills. Proceedings of the National Academy of Sciences of the United States of America 2002;99:11987–91.
Fogel SM, Smith CT. Learning-dependent changes in sleep spindles and Stage 2 sleep. Journal of Sleep Research 2006;15:250–5.
Fogel SM, Smith CT, Cote KA. Dissociable learning-dependent changes in REM and non-REM sleep in declarative and procedural memory systems. Behavioural Brain Research 2007;180:48–61.
Forest G, Poulin J, Daoust AM, Lussier I, Stip E, Godbout R. Attention and non-REM sleep in neuroleptic-naive persons with schizophrenia and control participants. Psychiatry Research 2007;149:33–40.
Goder R, Boigs M, Braun S, Friege L, Fritzer G, Aldenhoff JB, et al. Impairment of visuospatial memory is associated with decreased slow wave sleep in schizophrenia. Journal of Psychiatry Research 2004;38:591–9.
Goder R, Fritzer G, Gottwald B, Lippmann B, Seeck-Hirschner M, Serafin I, et al. Effects of olanzapine on slow wave sleep, sleep spindles and sleep-related memory consolidation in schizophrenia. Pharmacopsychiatry 2008;41:92–9.
Goldman M, Tandon R, DeQuardo JR, Taylor SF, Goodson J, McGrath M. Biological predictors of 1-year outcome in schizophrenia in males and females. Schizophrenia Research 1996;21:65–73.
Hoddes E, Zarcone V, Smythe H, Philips R, Dement WC. Quantification of sleepiness: a new approach. Psychophysiology 1973;10:431–6.
Hofstetter JR, Lysaker PH, Mayeda AR. Quality of sleep in patients with schizophrenia is associated with quality of life and coping. BMC Psychiatry 2005;5:13.
Huber R, Ghilardi MF, Massimini M, Tononi G. Local sleep and learning. Nature 2004;430:78–81.
Karni A, Meyer G, Rey-Hipolito C, Jezzard P, Adams MM, Turner R, et al. The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. Proceedings of the National Academy of Sciences of the United States of America 1998;95:861–8.
Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophrenia Bulletin 1987;13:261–76.
Keshavan MS, Reynolds 3rd CF, Miewald MJ, Montrose DM, Sweeney JA, Vasko Jr RC, et al. Delta sleep deficits in schizophrenia: evidence from automated analyses of sleep data. Archives of General Psychiatry 1998;55:443–8.
Kraepelin E. Dementia praecox and paraphrenia. Edinburgh, Scotland: ES Livingston; 1919.
Krystal AD, Goforth HW, Roth T. Effects of antipsychotic medications on sleep in schizophrenia. International Clinical Psychopharmacology 2008;23:150–60.
Kuriyama K, Stickgold R, Walker MP. Sleep-dependent learning and motor-skill complexity. Learning & Memory 2004;11:705–13.
Lauer CJ, Schreiber W, Pollmacher T, Holsboer F, Krieg JC. Sleep in schizophrenia: a polysomnographic study on drug-naive patients. Neuropsychopharmacology 1997;16:51–60.
Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. The New England Journal of Medicine 2005;353:1209–23.
Maixner S, Tandon R, Eiser A, Taylor S, DeQuardo JR, Shipley J. Effects of antipsychotic treatment on polysomnographic measures in schizophrenia: a replication and extension. The American Journal of Psychiatry 1998;155:1600–2.
Manoach DS. Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophrenia Research 2003;60:285–98.
Manoach DS, Cain MS, Vangel MG, Khurana A, Goff DC, Stickgold R. A failure of sleep-dependent procedural learning in chronic, medicated schizophrenia. Biological Psychiatry 2004;56:951–6.
McKinley SK. Sleep learning and memory in the elderly. In: Neurobiology. Cambridge: Harvard University; 2008.
Mednick SC, Nakayama K, Cantero JL, Atienza M, Levin AA, Pathak N, et al. The restorative effect of naps on perceptual deterioration. Nature Neuroscience 2002;5:677–81.
Monti JM, Monti D. Sleep in schizophrenia patients and the effects of antipsychotic drugs. Sleep Medicine Reviews 2004;8:133–48.
Nishida M, Walker MP. Daytime naps, motor memory consolidation and regionally specific sleep spindles. Public Library of Science ONE 2007;2:e341.
Ohayon MM, Carskadon MA, Guilleminault C, Vitiello MV. Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. Sleep 2004;27:1255–73.
Overall JE, Gorham DR. The brief psychiatric rating scale. Psychological Reports 1962;10:799–812.
Peters KR, Ray L, Smith V, Smith C. Changes in the density of stage 2 sleep spindles following motor learning in young and older adults. Journal of Sleep Research 2008;17:23–33.
Rasch B, Pommer J, Diekelmann S, Born J, 2008. Pharmacological REM sleep suppression paradoxically improves rather than impairs skill memory. Nature Neuroscience.
Rechtschaffen A, Kales A, 1968. A manual standardized terminology, techniques and scoring system for sleep stages of human subjects. In: Health UDo editor, Bethesda, MD.
Robertson EM, Press DZ, Pascual-Leone A. Off-line learning and the primary motor cortex. Journal of Neuroscience 2005;25:6372–8.
Salin-Pascual RJ, Herrera-Estrella M, Galicia-Polo L, Laurrabaquio MR. Olanzapine acute administration in schizophrenic patients increases delta sleep and sleep efficiency. Biological Psychiatry 1999;46:141–3.
Smith C, MacNeill C. Impaired motor memory for a pursuit rotor task following Stage 2 sleep loss in college students. Journal of Sleep Research 1994;3:206–13.
Stickgold R. Sleep-dependent memory consolidation. Nature 2005;437:1272–8.
Stickgold R, Whidbee D, Schirmer B, Patel V, Hobson JA. Visual discrimination task improvement: a multi-step process occurring during sleep. Journal of Cognitive Neuroscience 2000;12:246–54.
Tamaki M, Matsuoka T, Nittono H, Hori T. Fast sleep spindle (13–15 hz) activity correlates with sleep-dependent improvement in visuomotor performance. Sleep 2008;31:204–11.
Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R. Practice with sleep makes perfect: sleep-dependent motor skill learning. Neuron 2002;35:205–11.
Walker MP, Brakefield T, Hobson JA, Stickgold R. Dissociable stages of human memory consolidation and reconsolidation. Nature 2003a;425:616–20.
Walker MP, Brakefield T, Seidman J, Morgan A, Hobson JA, Stickgold R. Sleep and the time course of motor skill learning. Learning & Memory 2003b;10:275–84.
Walker MP, Stickgold R, Alsop D, Gaab N, Schlaug G. Sleep-dependent motor memory plasticity in the human brain. Neuroscience 2005;133:911–7.
White K, Ashton R. Handedness assessment inventory. Neuropsychologia 1976;14:261–4.
Woods SW. Chlorpromazine equivalent doses for the newer atypical antipsychotics. Journal of Clinical Psychiatry 2003;64:663–7.
Yang C, Winkelman JW. Clinical significance of sleep EEG abnormalities in chronic schizophrenia. Schizophrenia Research 2006;82:251–60.