The Impact of a 4-Hour Sleep Delay on Slow Wave Activity in Twins Discordant for Chronic Fatigue Syndrome

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Objectives: Chronic fatigue syndrome (CFS) has been associated with altered amounts of slow wave sleep, which could reflect reduced delta electroencephalograph (EEG) activity and impaired sleep regulation. To evaluate this hypothesis, we examined the response to a sleep regulatory challenge in CFS.

Design: The first of 3 consecutive nights of study served as laboratory adaptation. Baseline sleep was assessed on the second night. On the third night, bedtime was delayed by 4 hours, followed by recovery sleep. Total available sleep time was held constant on all nights.

Setting: A research sleep laboratory.

Participants: 13 pairs of monozygotic twins discordant for CFS.

Interventions: N/A

Measurements and Results: Power spectral analysis quantified slow wave activity (SWA) in the 0.5-3.9 Hz band in successive NREM periods (stage 2, 3, or 4) on each night. To ensure comparability, analyses were restricted to the first 4 NREM periods on each night. Data were coded for NREM period and twin pair. Repeated-measures analysis of variance (ANOVA) contrasted sleep delay effects across NREM periods between twin pairs. A second ANOVA calculated the SWA in each NREM period in recovery sleep relative to baseline SWA. The 2 groups of twins were similar on baseline SWA power. After sleep delay, CFS twins exhibited significantly less SWA power in the first NREM period of recovery sleep and accumulated a smaller percentage of SWA in the first NREM period than their co-twins.

Conclusions: CFS is associated with a blunted SWA response to sleep challenge, suggesting that the basic sleep drive and homeostatic response are impaired.

Keywords: Chronic fatigue syndrome, sleep, power spectral analysis, homeostasis, slow wave activity, twins

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INTRODUCTION

Chronic fatigue syndrome (CFS) is characterized by profound fatigue that lasts at least 6 months and is accompanied by disturbances of sleep, cognition, and mood; musculoskeletal pain; and other symptoms.1 Complaints of Insomnia and insufficient, nonrestorative sleep are among the most common and disabling subjective symptoms.2-4 Results from clinic-based studies have found that patients with CFS often have poor sleep efficiency5-8 as well as intrinsic sleep disorders such as obstructive sleep apnea.5,9 Longer sleep onset latency, sleep fragmentation, and decreased slow wave sleep (SWS) have been inconsistently reported.9 Previous studies of sleep in CFS have been limited by the absence of comparison groups,5,7,9 lack of objective measures of sleep6,8 and sleep architecture;2 use of in-home sleep studies;6 and performance of only one night of polysomnography.5,6,8,9 Rigorously designed studies have not provided strong evidence for abnormalities in sleep architecture in adults with CFS,10 even when sleep complaints were prevalent.11

Studies of sleep microarchitecture, based on quantitative EEG methods, have also examined adults with chronic pain.12-15 Increased alpha activity during NREM sleep has been observed in some,12,14 but not all,15 studies of fibromyalgia and related pain conditions.16,17 In one report, alpha (associated with a pattern of alpha/delta sleep) was significantly increased and SWS decreased among women with fibromyalgia compared to healthy controls.15 Increased alpha has also been linked both to reduced delta activity and to a pattern of alpha activity that is phase-locked with delta activity.12,13,18 Furthermore, experimental deprivation of SWS in healthy subjects leads to the emergence of lower pain thresholds, fatigue, and reduced vigor,12,19,20 and, in one study, alpha activity in NREM sleep.18 Nevertheless, reduced delta activity with or without an associated increase in alpha activity has been interpreted as evidence for impaired sleep homeostasis.21

Current theory suggests that 2 opposing processes, basic sleep homeostasis or Process S, and circadian drive or Process C, regulate sleep.22 Process S appears to accumulate during wakefulness, dissipates rapidly over NREM sleep time, and is reflected in the time course of slow wave activity (SWA) (i.e., delta power or amplitude in NREM sleep). In contrast, Process C reflects the circadian timing of REM sleep and the drive for wakefulness.23 The well-documented increase in SWA in response to acute total sleep deprivation strongly supports the homeostatic component of this theory.24 In addition, the amount of prior wakefulness appears to be monotonically related to an increase in SWA power after acute total sleep deprivation, that dissipates quickly over NREM sleep time.25,26 The time course of SWA and its response to a sleep regulatory challenge is crucial in understanding reduced delta and its potential role in nonrestorative sleep characteristic of CFS.

Over the last decade, our research group has used a co-twin con-
trol design to examine a cohort of monozygotic twins discordant for CFS. The research design accounts for genetic differences and numerous environmental factors not considered in typical studies of CFS and offers a powerful alternative to traditional approaches that compare CFS patients to healthy or depressed subjects. Sleep measures from the first study visit have been reported previously. In this paper, we report data collected approximately 5 years later on SWA and the response to a sleep regulatory challenge. We hypothesized that the twins with CFS would have a blunted SWA response to the sleep challenge.

METHODS

Twin Registry.

A detailed description of the CFS Twin Registry from which study participants were chosen has been published. Briefly, 600 twins were recruited through diverse means and mailed an intake questionnaire; 426 questionnaires (71%) were returned, and complete data were available for both members of 193 twin pairs (386 individuals). The questionnaire included data on demographics, zyosity, health conditions, fatigue level, and symptoms of CFS. For the non-fatigued twin, a control version of questions was used that did not reference fatigue. To determine psychiatric conditions, a structured interview, the Diagnostic Interview Schedule, Version III-A, was administered by telephone to Registry participants. CFS was diagnosed according to the Centers of Disease Control and Prevention criteria on the basis of responses to the questionnaire and the structured psychiatric interview.

Twenty-two sets of adult monozygotic twins, in which 1 twin met CFS criteria and the other was healthy, were chosen from the Registry for the initial 7-day in-person evaluation. Twins were required to have been reared together; to discontinue alcohol, caffeine, and all medications (except those deemed critical by a physician) known to affect sleep or cognition at least 2 weeks prior to evaluation; and to travel together to the sleep laboratory approximately 2 hours before their scheduled bedtime. Napping was proscribed. The bedtime was adjusted for twins who had traveled from Eastern, Central, and Mountain Time zones, as well as for daytime testing procedures that began at 08:00 on the mornings after night 1 and night 2 in the laboratory. Night 1 served as adaptation to the laboratory, night 2 was considered baseline, and on night 3 sleep onset was delayed by 4 hours. Polysomnographic recordings were obtained during the sleep delay. With the room lights on, the twins were positioned in bed with the head of the bed elevated 30º. They watched television while a technician monitored them to make sure they remained awake. The twins were awakened after 7 hours of sleep or by 09:00 on the morning after the sleep delay. All data scoring and processing was conducted blind to ill or well twin status.

Polysomnography

EEG electrodes were positioned at 2 frontal (F7, F8), 2 central (C3, C4), and 2 occipital (O1, O2) locations (International 10-20 system of measurement) and were referenced to the contralateral mastoids. Chin electromyogram electrodes and electrodes for right and left electro-oculogram also were attached. To monitor the twins for sleep apnea, airflow was measured using a nasal pressure cannula placed in the nose (Pro-Tech Services, Inc, Mukilteo, WA). Chest and abdominal respiratory effort was measured by piezoelectric respiratory effort bands (Pro-Tech Services, Inc, Mukilteo, WA) placed around the chest and abdomen. Oxygen saturation was measured from the left or right index finger by a pulse oximeter (MedCare, Reykjavic, Iceland). Snoring was measured by a small microphone sensor (Pro-Tech Services, Inc, Mukilteo, WA) placed on the throat, just lateral to the trachea. Electromyogram electrodes were placed on the anterior tibialis of each leg to document periodic leg movements during sleep. Two electrodes were placed on the chest to measure the electrocardiogram according to the modified Lead II configuration. Electrophysiological signals were recorded and digitized by the EMBLA Somnologica data acquisition recording system (A-AY101, MedCare, Reykjavic, Iceland) and displayed and stored on a desktop computer. The sampling rate was set at 200 Hz for electrodes for the electroencephalogram (EEG), electromyogram, leg leads, and electrocardiogram; 100 Hz for the electro-oculogram signal and snoring sensor; 20 Hz for the nasal airflow and respiratory effort; and 1 Hz for the oximeter. All digitized data were acquired and stored unfiltered. Before each recording session, a standard 50 microvolt, 10 Hz calibration signal was recorded for 5 minutes. Data were displayed in 30-second intervals during recording.

Sleep Stage Scoring

All channels of recorded data were displayed on a high-resolution 21-inch color monitor for visual sleep stage scoring. Filter settings for display were set at 0.3 Hz to 40 Hz. Sleep and wake stages were scored in 30-second epochs according to standard criteria. Given our focus on homeostatic challenge, we report only the following subset of key sleep architectural variables: total sleep time (total time in NREM stages 1-4 and REM); sleep efficiency (total sleep time/time in bed); sleep latency (time from lights out to first epoch of stage 2 sleep); REM latency (time from sleep latency to first epoch of REM); time spent awake; and minutes of NREM and REM time expressed as a percentage of total sleep time.

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Power Spectral Analysis

On-board power spectral software from the EMBLA Somnologica data acquisition system evaluated power in delta (0.5-3.9 Hz), theta (4.0-7.9 Hz), alpha (8.0-11.9 Hz), sigma (12.0-15.9 Hz), and beta (16.0-31.9 Hz) bands. The algorithm used a 512-point fast Fourier transform with Hamming windows (-53 dB stop band, filter degree 1068, transition bandwidth 0.622 Hz), in 6-second blocks. The delta power values, expressed in µV^2, were averaged in consecutive 30-second epochs in each frequency band to correspond to visual stage scoring to prepare for averaging data by NREM period. Only delta power values ascertained from C3 electrodes are reported here.

NREM Period Definition

The definition of NREM periods followed that of Dijk et al. and Feinberg and Floyd. NREM periods were defined as the succession of stages 2, 3, or 4, lasting at least 15 minutes, and terminated by more than 5 minutes of REM or wakefulness. No minimum REM period duration was required for the first or the last REM period. Power in the delta band was summed and averaged across successive NREM periods (excluding stage 1 epochs) on baseline and sleep delay nights for each subject. The %SWA power in each NREM period on the delay night was computed relative to SWA in each NREM period on the baseline night. This compensated for individual differences in raw power values. The latencies to the each NREM period and their durations were also computed for each twin.

Statistical Analysis

SWA data were coded for each CFS and healthy twin, for each NREM period, and for the baseline and sleep delay nights. Relative SWA was also coded for NREM periods and twin pairs. Repeated-measures MANOVA evaluated statistical differences separately for SWA and %SWA. Univariate analyses, which contrasted twin pairs within each NREM period, were computed only if a significant overall MANOVA effect was obtained. In addition, the latencies and durations of each NREM period for the CFS and healthy twins were compared on the baseline and sleep delay nights. The level of significance was set at P ≤0.05, corrected for the number of comparisons.

RESULTS

Participant Characteristics

One pair of twins was excluded because of technical problems that resulted in missing data. The remaining 13 pairs were all female, with an average age of 46.8 (± 10.3) years (range = 29–60 years). The twins’ demographic and clinical information is provided in Table 1. Lifetime major depression was diagnosed in 3 CFS twins and 2 healthy twins, but no twin in either group suffered from current major depression at the time of study. As expected, only the mean fatigue level during the study differed according to the health status of the twins (P <0.001).

Polysomnography Analyses

Table 2 presents the mean values of key polysomnographic (PSG) measures on the baseline and sleep delay nights. Sleep latency was shorter after sleep delay in the CFS but not the healthy twins (P = 0.001), but the sleep delay by twin pair interaction did not reach significance (F1,11 = 3.5, P <0.09). As shown in Table 2, the CFS and healthy twins slept approximately 1 hour less on the delay night, because they were awakened by 09:00 on the morning after sleep delay. A robust sleep delay main effect was obtained for total sleep time by ANOVA (F1,11 = 41.7, P <0.0001)

| Table 1—Demographic and clinical characteristic of CFS discordant twin pairs. |
| Characteristic | CFS Twins n = 13 | Healthy Twins n = 13 |
| Married, number | 10 | 9 |
| High school completion, number | 7 | 9 |
| Body mass index, mean (SD) | 30.1 (7.2) | 29.7 (6.1) |
| Lifetime major depression, number | 3 | 2 |
| Current major depression, number | 0 | 0 |
| Fatigue in years, mean* | 9.2 (3.7) | 0 |
| Fatigue score in mm, mean (SD)** | 55.4 (25.5) | 16.1 (8.3) |
| Myalgias, number* | 12 | 7 |

* P <0.001; SD = standard deviation; *mean Visual Analogue Scale fatigue rating across the 6-day evaluation period; ‘1 CFS twin had missing data

| Table 2—Selected sleep stage variables at baseline and after sleep delay in CFS discordant twin pairs. |
| Sleep Variable | CFS Twins n = 13 | Healthy Twins n = 13 |
| | Baseline Mean (SD) | Delay Mean (SD) | Baseline Mean (SD) | Delay Mean (SD) |
| Total sleep time, minutes* | 394.9 (53.4) | 344.5 (37.5) | 403.9 (35.8) | 335.8 (32.7) |
| Sleep latency, minutes* | 6.3 (5.8) | 4.3 (4.1) | 5.0 (4.7) | 4.7 (3.4) |
| REM latency, minutes* | 75.3 (51.5) | 58.3 (35.6) | 65.7 (27.0) | 46.8 (30.4) |
| Stage 1, % | 9.1 (2.2) | 9.0 (4.3) | 8.3 (3.7) | 8.7 (3.8) |
| Stage 2, % | 35.2 (8.9) | 32.2 (6.8) | 38.5 (8.1) | 35.6 (7.2) |
| Slow wave sleep, % | 18.4 (4.5) | 21.1 (6.2) | 20.0 (5.0) | 19.9 (3.8) |
| REM, % | 24.2 (5.5) | 27.7 (6.3) | 21.4 (5.8) | 23.9 (7.2) |
| Sleep efficiency* | 85.1 (8.4) | 87.5 (10.0) | 87.4 (8.0) | 85.2 (8.4) |
| Time awake, minutes | 56.3 (29.8) | 37.2 (25.8) | 52.4 (36.7) | 44.6 (31.7) |

* sleep delay main effect, P <0.03; * total sleep time / time in bed X 100%; SD = standard deviation
without a twin pair interaction \((F < 1.0)\). Sleep delay also shortened REM latency by about 20 minutes in both groups, with a sleep delay main effect \((F_{1,11} = 5.9, P < 0.03)\).

None of the remaining polysomnographic variables showed significant main effects or interactions.

NREM Period Analyses

Data on the latencies of each of the NREM periods are shown in Table 3 for the baseline and sleep delay nights. The CFS twins generally had a longer latency to each of the NREM periods than their co-twins during the baseline and sleep delay nights, but these differences were not significant. In addition, twin pair and sleep delay main effects and interactions were not detected for NREM period latency \((F < 1.0)\). However, the NREM period durations showed a sleep delay by NREM period interaction \((F_{2,10} = 4.13, P < 0.05)\); a tendency toward a delay main effect \((F_{1,11} = 3.71, P < 0.08)\); and a twin pair by delay interaction \((F_{1,10} = 3.89, P < 0.08)\). As shown in Table 3, the NREM periods were shorter on the delay night than on the baseline night among healthy twins, confirmed by univariate analysis \((P < 0.03)\). Likewise, the CFS twins had shorter durations of NREM periods 1, 3, and 4 after sleep delay, whereas they had longer durations of NREM period 2 after delay. Univariate analyses confirmed that NREM period 2 was longer \((P < 0.05)\) and NREM period 3 was shorter after sleep delay \((P < 0.02)\).

SWA Analyses

As shown in Table 3, raw or unadjusted SWA power increased on the sleep delay night in both CFS twins and healthy twins. The SWA power in NREM period 1 increased by less than 5% among CFS twins, compared to 10% among healthy twins \(46 \mu V^2 \text{ vs. } 141.5 \mu V^2\). MANOVA revealed NREM period main effects \((F_{2,10} = 32.8, P < 0.0002)\) and twin by NREM period interactions \((F_{2,10} = 4.8, P < 0.04)\). Although the SWA increase on the delay night was larger among healthy twins, the twin by sleep delay by NREM period interactions were not significant \((F_{2,10} = 2.2, P < 0.15)\).

Overall, %SWA provided a stronger discrimination between the CFS and healthy twins than did unadjusted SWA. The relative %SWA power measures (SWA on the delay night expressed relative to baseline SWA) in each NREM period are shown in Figure 1, by twin pair. In NREM period 1 during recovery sleep, the CFS twins accumulated less than 120% of baseline SWA, which dissipated very slowly over subsequent NREM sleep. In contrast, the healthy twins accumulated nearly 140% of baseline SWA, which dissipated rapidly over NREM sleep. MANOVA revealed a NREM period effect \((F_{2,10} = 27.9, P < 0.0001)\) and a NREM period by twin interaction.

**Table 3**—SWA and latency from sleep onset to, and durations of, each NREM period at baseline and after sleep delay in CFS discordant twin pairs.

<table>
<thead>
<tr>
<th>Sleep Variable</th>
<th>Baseline</th>
<th>CFS Twins</th>
<th>Healthy Twins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Delay Mean (SD)</td>
<td>Baseline Mean (SD)</td>
</tr>
<tr>
<td>NREM 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, minutes</td>
<td>17.5 (30.4)</td>
<td>18.5 (29.5)</td>
<td>8.1 (12.4)</td>
</tr>
<tr>
<td>Duration, minutes</td>
<td>54.4 (32.7)</td>
<td>52.3 (32.1)</td>
<td>63.2 (13.5)</td>
</tr>
<tr>
<td>Slow wave activity, (\mu V^2)</td>
<td>1180.8 (209.3)</td>
<td>1226.7 (229.5)</td>
<td>1237.3 (171.2)</td>
</tr>
<tr>
<td>NREM 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, minutes</td>
<td>111.8 (54.3)</td>
<td>126.7 (48.9)</td>
<td>101.9 (29.9)</td>
</tr>
<tr>
<td>Duration, (\mu V^2)</td>
<td>54.5 (25.6)</td>
<td>67.8 (31.6)</td>
<td>70.1 (29.9)</td>
</tr>
<tr>
<td>Slow wave activity, (\mu V^2)</td>
<td>1125.4 (298.2)</td>
<td>1108.4 (317.7)</td>
<td>1075.9 (218.5)</td>
</tr>
<tr>
<td>NREM 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, minutes</td>
<td>220.9 (80.1)</td>
<td>235.4 (49.6)</td>
<td>200.7 (50.2)</td>
</tr>
<tr>
<td>Duration, (\mu V^2)</td>
<td>65.3 (23.7)</td>
<td>46.2 (12.6)</td>
<td>68.5 (22.4)</td>
</tr>
<tr>
<td>Slow wave activity, (\mu V^2)</td>
<td>977.3 (257.6)</td>
<td>899.6 (259.9)</td>
<td>890.6 (269.4)</td>
</tr>
<tr>
<td>NREM 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, minutes</td>
<td>306.2 (84.2)</td>
<td>317.8 (54.5)</td>
<td>306.5 (58.0)</td>
</tr>
<tr>
<td>Duration, (\mu V^2)</td>
<td>39.0 (16.4)</td>
<td>32.1 (20.9)</td>
<td>44.0 (14.8)</td>
</tr>
<tr>
<td>Slow wave activity, (\mu V^2)</td>
<td>798.9 (197.8)</td>
<td>820.3 (201.4)</td>
<td>724.4 (170.0)</td>
</tr>
</tbody>
</table>

*NREM = Stages 2, 3, and 4; * sleep delay effect in both CFS and healthy twins, \(P < 0.05\); ** sleep delay effect in CFS twins, \(P < 0.05\); SD = standard deviation
period by twin interaction ($F_{1,0} = 4.3, P <0.05$). These analyses were based on %SWA relative to baseline, so the MANOVA model tested the sleep delay effect within the NREM period. The univariate analyses revealed a higher relative percentage of SWA in the healthy twins than in the CFS twins in NREM period 1 ($P <0.05$). Similarly, healthy twins accumulated higher %SWA than CFS twins, and it dissipated faster over NREM sleep time. By NREM period 4, SWA on the delay night was less than 50% of baseline in the healthy twin compared with 80% of baseline SWA in the CFS twin. The %SWA in NREM period 4 was higher in the CFS twins than in healthy twins ($P <0.05$), further supporting an abnormal time course of SWA in CFS.

**DISCUSSION**

This study demonstrated significant enhancement of SWA in the first NREM period in response to a 4-hour sleep delay in the healthy twins but not in the CFS twins. The effects were evident in relative %SWA measures adjusted for baseline but were not significant for unadjusted SWA. The higher initial accumulation of %SWA in the first NREM period was followed by a more rapid dissipation across NREM sleep time in the healthy twin. By contrast, the twins with CFS showed lower accumulation of %SWA with a blunted decline across NREM sleep episodes. By the end of the night, the %SWA was higher in the CFS twins. According to the 2-process model of sleep regulation$^{23}$ and approximations of SWA homeostasis,$^{24,25}$ one would expect the lowest amount of SWA in the latter NREM sleep episodes. Thus, the total amount of SWA in response to sleep delay does not differ between the CFS and healthy twin. Rather, it is the time course or distribution of SWA response across NREM sleep episodes that distinguishes those with CFS. This abnormal time course is consistent with a blunted homeostatic response in CFS sleep episodes. Thus, the total amount of SWA in response to sleep delay may be associated with a general adaptive failure that compromises recovery from pain and fatigue. Furthermore, SWS, and SWA in particular, may be important in regulating the immune system.$^{40}$ Our findings underscore the need to explore potential interactions between SWA, sleep homeostasis, and immune system activation in CFS.

Finally, an unexpected finding was significantly shorter REM latency after sleep delay in both twin groups. Because most current theories of sleep regulation, including the 2-process model, assume a reciprocal relationship between REM latency and SWA, we did not expect a shorter REM latency to accompany enhanced SWA in healthy twins. Previous studies have shown enhanced REM activity in response to sleep deprivation or restriction conditions, but we are unaware of any study that demonstrates concomitant shortened REM latency and enhanced SWA. Should this finding be replicated, some revision to theoretical models of sleep regulation may be necessary.

This study has several limitations. First, solicitation by advertisement resulted in a volunteer sample with potential ascertainment problems. However, the more desirable strategy of identifying twins from a population-based twin registry is impossible in the U.S. Second, chronic sleep habits or “jet lag” may also have affected our data in unknown ways. Third, although confounding due to genetic factors was ruled out by the study design, we could not establish the temporal relationship between physiological parameters of sleep and CFS. Fourth, our protocol included only a 4-hour sleep challenge, which may have been insufficient to provoke an SWA response in the CFS twins. Yet, even if a full night of sleep deprivation could elicit SWA rebound in CFS twins, a substantially greater challenge to the sleep regulatory system would be required to elicit a response in individuals with CFS than in healthy individuals.

In conclusion, our findings may help understand the sleep complaints so prevalent in CFS.$^{41}$ Challenging sleep regulation, however, is unlikely to improve subjective sleep or be clinically useful in reducing fatigue symptoms, because only minor increases in SWA were noted in the CFS twins. Although subjective ratings of sleep quality before and after sleep delay would be of interest, our objective data document that the CFS twins clearly under-responded to the sleep challenge.

**ABBREVIATIONS**

ANOVA = analysis of variance  
CFS = chronic fatigue syndrome  
EEG = electroencephalograph  
MANOVA = multivariate analysis of variance  
$\mu V^2$ = microvolts squared  
NREM = non-rapid eye movement  
REM = rapid eye movement  
SD = standard deviation  
SWA = slow wave activity  
%SWA = SWA after challenge expressed relative to baseline SWA  
SWS = slow wave sleep

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