Sleep depth and fatigue: Role of cellular inflammatory activation

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ABSTRACT

Individuals with underlying inflammation present with a high prevalence of non-specific co-morbid symptoms including sleep disturbance and fatigue. However, the association between cellular expression of proinflammatory cytokines, alterations of sleep depth and daytime fatigue has not been concurrently examined. In healthy adults (24–61 years old), evening levels of monocyte intracellular proinflammatory cytokine production were assessed prior to evaluation of polysomnographic sleep and measures of fatigue the following day. Stimulated monocyte production of interleukin-6 (IL-6), but not tumor necrosis factor α (TNF-α), was negatively associated with slow wave sleep ($\Delta R^2 = .17$, $p = .029$). In contrast, stimulated monocyte production of IL-6 was positively associated with rapid-eye movement (REM) sleep duration during the first sleep cycle ($\Delta R^2 = .26$, $p < .01$). Moreover, evening stimulated production of IL-6 was associated with fatigue the following day ($\Delta R^2 = .17$, $p = .05$). Mediation analyses showed that slow wave sleep, but not REM sleep duration, mediated the relationship between evening levels of IL-6 production and daytime fatigue. These results indicate that increases in stimulated monocyte production of IL-6 may be associated with decreases in slow wave sleep and increases in REM sleep duration. Relative loss of slow wave sleep may be one pathway through which cellular inflammation leads to daytime fatigue.

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1. Introduction

Sleep disturbance is a common problem, affecting approximately one third of Americans. Research suggests that individuals who report some form of sleep disturbance may be at greater risk for a number of medical problems, including obesity, diabetes, heart disease, and depression (LeBlanc et al., 2007; Ohayon, 2005, 2008; Spiegel, 2008; Van Cauter et al., 2008). In addition, a number of studies have demonstrated that sleep problems take a major toll on health related quality of life, in which inadequate sleep is associated with worse self-reported general health, fatigue, and less vitality (Lee et al., 2009; Winkelman et al., 2009). Likewise, population based studies have shown that individuals with sleep problems have more physical health complaints and are more likely to miss work in the past 30 days (Bolge et al., 2009; Stein et al., 2008). Further, sleep disturbance is related to reduced quality of life across several dimensions, including declines in mental health, fatigue, and impairments in both physical and social functioning (Bolge et al., 2009, 2003; LeBlanc et al., 2007).

Proinflammatory cytokines are associated with a cluster of behavioral symptoms known as sickness behaviors. These symptoms, which include fatigue, sleep disturbance, depression, loss of appetite, and inability to concentrate (Dantzer, 2001, 2009), are common in individuals with medical conditions involving underlying inflammation (Collado-Hidalgo et al., 2008a; Lorton et al., 2008; Myers, 2008; Wood et al., 2006). Additionally, recent findings from population based studies reveal that markers of inflammation are associated with fatigue or reductions in vitality (Cho et al., 2009; Raison et al., 2009), and poor health related quality of life (Raison et al., 2009). Moreover, increases of the inflammatory marker, C-reactive protein (CRP), prospectively predict fatigue in community-dwelling adults (Cho et al., 2009). Taken together, these findings suggest that inflammation and alterations in sleep may contribute to fatigue and/or reductions in vitality.

Inflammation is thought to induce disturbances of sleep via alterations in the sleep cycle as defined by changes in sleep architecture. Indeed, prior studies have found that increases in circulating levels of IL-6 correlate with decreases of slow wave sleep (SWS) (Burgos et al., 2006; Hong et al., 2005), as well as increases in the amount and percentage of rapid-eye movement (REM) sleep (Irwin et al., 2004) and REM sleep density (Motivala et al., 2005). In addition, stimulated production of IL-6 in mixed cell cultures is associated with increases in REM sleep amounts and percentage (Redwine et al., 2003). Moreover, in alcohol dependent persons who have high amounts of REM sleep, administration of a proinflammatory cytokine antagonist partially normalizes amounts of

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REM sleep, inducing lower levels of REM sleep comparable to levels found in controls (Irwin et al., 2009). Nevertheless, there are inconsistent results; one study found that IL-6 is associated with low amounts of REM sleep (Vgontzas et al., 2003).

In this study, we sought to clarify the functional basis for altered inflammation in association with sleep by measuring the production of proinflammatory cytokines by monocytes following ligation of the Toll-like receptor 4 with lipopolysaccharide (LPS). We have previously found that sleep loss induces marked increases in monocyte production of IL-6 and TNF-α (Irwin et al., 2010, 2006b), and such increases in the cellular expression of proinflammatory cytokines is associated with fatigue in breast cancer survivors (Collado-Hidalgo et al., 2008b). In addition, Toll-like receptors mediate innate immune responses to common pathogens (Cook et al., 2004), and aberrant increases of Toll-like receptor activity have been linked to inflammatory diseases such as rheumatoid arthritis (Andreakos, 2004), Crohn's disease (Andreakos, 2004), and heart failure (Satoh et al., 2006).

Given the associations between circulating levels of proinflammatory cytokines and measures of sleep architecture (i.e., SWS and REM sleep) (Burgos et al., 2006; Hong et al., 2005; Irwin et al., 2004; Motivala et al., 2005; Redwine et al., 2003) and evidence that such activation might drive increases in REM sleep (Irwin et al., 2009), we examined the impact of evening stimulated monocyte production of inflammatory cytokines on measures of sleep architecture, hypothesizing that greater evening expression of cellular markers of inflammation would be associated with less sleep depth (i.e., lower amounts of SWS) and greater amounts of REM sleep. Finally, given evidence that cellular markers of inflammation are associated with fatigue (Collado-Hidalgo et al., 2006, 2008b), as well as findings that decreases in sleep depth correlate with daytime fatigue (Irwin et al., 2006a), we evaluated the association between activation of cellular markers of inflammation and daytime fatigue, and explored whether alterations in sleep depth mediated this relationship.

2. Methods

2.1. Participants

Participants included 31 men ($N = 16$) and women ($N = 15$) who were recruited via referrals and advertisements seeking medically healthy adults to participate in a sleep study between October 2006 and June 2008. All gave consent to participate in this University of California, Los Angeles (UCLA) institutional review board approved research study. Participants were between the ages of 24 and 61 (Mean = 37.16, SEM = 1.59), had body mass indices (calculated as weight in kilograms divided by the square of height in meters) less than 30, were nonsmokers, and regularly slept between 10:30 PM and 7:30 AM as confirmed by 2-week sleep diaries. All participants were medically healthy, as determined by medical history, physical examination, and laboratory testing. None of them had a history of mental illness as determined by Structured Clinical Interview for Diagnostic and Statistical Manual-IV (SCID) and no participants had sleep disorders. None of the participants fulfilled criteria for primary substance dependence or had used substances including alcohol in the last 2 weeks as confirmed by random urine substance screenings. Further, no participants were taking medications known to alter sleep-wake activity (e.g., beta blockers, psychotropic medications) within 2 weeks of the sleep protocol.

2.2. Procedures

Participants spent three consecutive days (24-h periods) in the National Institutes of Health General Clinical Research Center,

which included an initial screening/adaptation night and two nights of sleep testing; only results from the first night of sleep testing are included as that night preceded the evaluation of fatigue. After adaptation to the sleep laboratory with screening for sleep apnea and nocturnal myoclonus, participants underwent testing with polysomnography, with ambient light dimmer than 50 lux. Participants adhered to their normal sleep schedules, with lights out between 11 PM and 7 AM. During the nocturnal period, a bedside urinal was used if participants needed to urinate during the night. Participants were awakened in the morning by turning on a dim light and calling the participant's name. All participants remained on the GCRC during with day until the sleep protocol was completed.

Sleep monitoring was conducted with placement of electrodes for EEG (C3 or C4), electrooculography, and submental electromyography recordings. EEG sleep records were visually scored according to the criteria of Rechtschaffen and Kales (Rechtschaffen and Kales, 1968) as previously described (Irwin et al., 2002). For purposes of this study, calculated sleep variables included total sleep time (TST), sleep efficiency (% of TST/time in bed), time to sleep onset, Stage 1 sleep, Stage 2 sleep, SWS (comprised of Stage 3 and Stage 4 sleep), REM latency, REM density, and REM duration during the first sleep cycle. TST was considered the total number of minutes from sleep onset to final wake-up time and included time spent in Stages 1 through 4 of sleep and REM sleep. There were no differences in the polysomnographic measures of sleep continuity and sleep architecture between the adaptation/screening night and the testing night.

Blood sampling was conducted at 11 PM (evening) prior to sleep testing and again at 8 AM (morning) after sleep, with collection of samples via an indwelling venous forearm. Samples were analyzed for expression of intracellular proinflammatory cytokines in monocyte populations.

Fatigue was measured in the evening following sleep testing using the vitality subscale of the *Short Form 36 Health Survey Questionnaire* (SF-36; Ware and Sherbourne, 1992). Items that make up the vitality subscale assess whether respondents are full of pep, tired, worn out, or have energy. High scores on this scale represent more vitality, whereas low scores represent fatigue.

2.3. Monocyte cellular expression of cytokines

Monocyte intracellular expression of IL-6 and TNF-α was assessed by flow cytometry (see Collado-Hidalgo et al., 2006 for a complete description of these procedures). Heparin treated blood was mixed with 100 pg/mL lipopolysaccharide (LPS; Sigma, St Louis, Mo) and 10 μg/mL brefeldin A (Sigma) and incubated for 4 h at 37 °C in a platform mixer followed by an overnight incubation at 4 °C. We have previously found in dose response profile studies that 100 pg/ml of LPS for 4 h yields a threshold response with detection of stimulated production of IL-6 and TNF-α combined. This is greater than levels found in unstimulated control samples, yet routinely less than the submaximal response, yielding combined levels of IL-6 and TNF-α response between 50% and 75% (Irwin et al., 2010, 2006b). Doses of LPS at 50 pg/ml fail to yield a threshold response in many persons, whereas doses in 200 pg/ml yield a clustering of responses in the maximal range between 80% and 90%. After cells were permeabilized in fluorescence-activated cell sorting permeabilizing buffer (BD Biosciences, San Jose, CA) and fluorescence-conjugated antibodies were added, about 12,000 CD14^{+} events were counted to determine the net stimulated percentage of cytokine secreting monocytes, with quadrant coordinates set based on unstimulated cells. To determine percentage of stimulated cells expressing TNF-α or IL-6, unstimulated cytokine-positive event percentages were subtracted from

stimulated percentages to obtain net stimulated cytokine-positive event percentages.

2.4. Statistical analyses

2.4.1. Monocyte cellular cytokine expression and polysomnographic sleep

Data were analyzed using SPSS 16.0 software. Prior to conducting the analyses, SWS and REM sleep measures were log transformed to control for skewness. Correlation analyses were conducted as an initial examination between evening- and morning stimulated proinflammatory cytokine expression and sleep variables. Based on these results, hierarchical linear regression analyses were then conducted to examine whether cellular expression of IL-6 and TNF-α in the evening were associated with SWS and REM sleep measures during the night after controlling for age and BMI. In these analyses, SWS and REM sleep measures were entered as outcome variables. Based on previous findings that age, BMI, gender, and ethnicity are associated with inflammation and sleep (Hong et al., 2005; Lim et al., 2005; O'Connor et al., 2009, 2007), these variables were entered as covariates on Block 1 of the model. Cellular expression of IL-6 and TNF-α were entered as predictor variables on Block 2 of the model.

2.4.2. Secondary mediation analyses: cytokine expression, sleep, and fatigue

For inflammatory markers that were significant predictors of REM sleep and SWS, we conducted mediation analyses to test whether the altered REM sleep and SWS observed in individuals with greater monocyte cellular cytokine production leads to fatigue the following day. These analyses were conducted in a series of hierarchical linear regression analyses using the method outlined by Baron and Kenny (Baron and Kenny, 1986), with REM sleep measures and SWS entered as mediators of relationships between monocyte cellular cytokine production and fatigue. To establish mediation, we then tested whether the following four conditions were met. First, the mediator (SWS, REM sleep measures) must be related to the outcome variable (fatigue). Second, the predictor variable (stimulated IL-6, TNF-α) must be related to the mediator (SWS, REM sleep measures). Third, the predictor variable must be related to the outcome variable (fatigue). Finally, when the outcome variable is regressed onto both the mediator and the predictor variables simultaneously, there should be a stronger association between the mediator and the outcome variables than between the predictor and the outcome variables. We tested these conditions in three steps using a series of hierarchical regression analyses. Age, BMI, gender, and ethnicity were entered as covariates on Block 1 of the analyses.

3. Results

3.1. Sample characteristics

Sample characteristics are listed in Table 1. The ethnic group breakdown of the sample was 34.3% Caucasian American, 31.4% African American, 14.3% Asian American, and 11.4% Hispanic. Approximately half of the sample had an annual income of $30,000 or less and the average years of education was 15.37 (SEM = .30). Immune and sleep data are included in Table 2.

Bivariate correlations examined relationships between stimulated monocyte production of IL-6 and TNF-α, sleep variables, and fatigue. Consistent with our hypotheses, evening stimulated monocyte production of IL-6 was associated with less SWS ($r = -.517$, $p = .01$) and a longer REM duration ($r = .553$, $p = .008$). Production of IL-6 was unrelated to stages 1 or 2 sleep, TST, sleep

Table 1
Sample characteristics (N = 31).

Variables	M ± SEM
Age	37.16 ± 1.59
BMI	24.60 ± .74
Years of education	15.55 ± .31
SF-36 vitality/fatigue	72.41 ± 2.423
Ethnicity	N (%)
African American	10 (32.3)
Asian American	3 (9.7)
Caucasian American	11 (35.5)
Hispanic	2 (6.5)
Income	N (%)
<$30,0000	15 (48.4)
$30,000–49,999	8 (25.8)
⩾$50,000	7 (22.6)

Table 2
Sleep characteristics and proinflammatory cytokine activity on both nights (N = 31).

Variables	Mean ± SEM	
	Night 1	Night 2
Evening IL-6 production (%)	10.21 ± 1.17	7.96 ± 1.14
Evening TNF-a production (%)	23.99 ± 2.39	20.28 ± 1.62
Total sleep time (TST; min)	391.12. ± 11.07	414.05 ± 14.46
Time to sleep onset (min)	32.13 ± 7.45	24.32 ± 5.14
Sleep efficiency (TST/time in Bed × 100; %)	80.89 ± 2.22	82.94 ± 2.30
Stage 1 (% TST)	4.34 ± 0.43	5.46 ± 0.56
Stage 2 (% TST)	65.04 ± 1.51	62.22 ± 2.06
SWS (% TST)	7.05 ± 1.21	9.29 ± 1.43
REM duration (min)	17.48 ± 2.29	16.22 ± 1.91
REM density (min)	1.37 ± 0.14	1.59 ± 0.18
REM latency (min)	64.25 ± 5.68	62.16 ± 4.61
REM (% TST)	22.63 ± 1.43	23.03 ± 1.29

efficiency, time to sleep onset, REM sleep, REM latency or REM density. Evening production of TNF-α was unrelated to sleep stages, total sleep time, sleep efficiency, or time to sleep onset. Neither morning production of IL-6 nor TNF-α was related to any of the sleep variables.

3.2. Monocyte cytokine production and sleep architecture

Given the bivariate associations between evening levels of monocyte cellular expression of IL-6 and measures of SWS and REM duration, hierarchical linear regression analyses were conducted with this predictor variable. Age, BMI, gender, and ethnicity explained 8.3% of the variance in SWS. After controlling for these covariates, greater evening stimulated monocyte production of IL-6 was associated with less SWS ($\Delta R^2 = .17$, $p = .029$) (see Fig. 1). As can be seen in Fig. 2, participants in the lowest quartile of IL-6 production spent 11.3% of TST in SWS, compared to 2.5% of TST among those in the highest quartile of IL-6 production.

The covariates explained 12.5% of the variance in REM duration. As can be seen in Fig. 1, greater evening stimulated monocyte production of IL-6 was associated with a longer REM duration ($\Delta R^2 = .18$, $p = .017$). Fig. 2 illustrates that REM duration was 10.6 min among participants in the lowest quartile of IL-6 production, compared to 22.5 min among those in the highest quartile of IL-6 production. TNF-α was unrelated to SWS or REM duration.

3.3. Mediation analyses: evening cytokine expression, sleep architecture, and fatigue

Mediation analyses were conducted to test our hypothesis that SWS and REM duration would mediate relationships between evening proinflammatory cytokine expression and fatigue. Age,
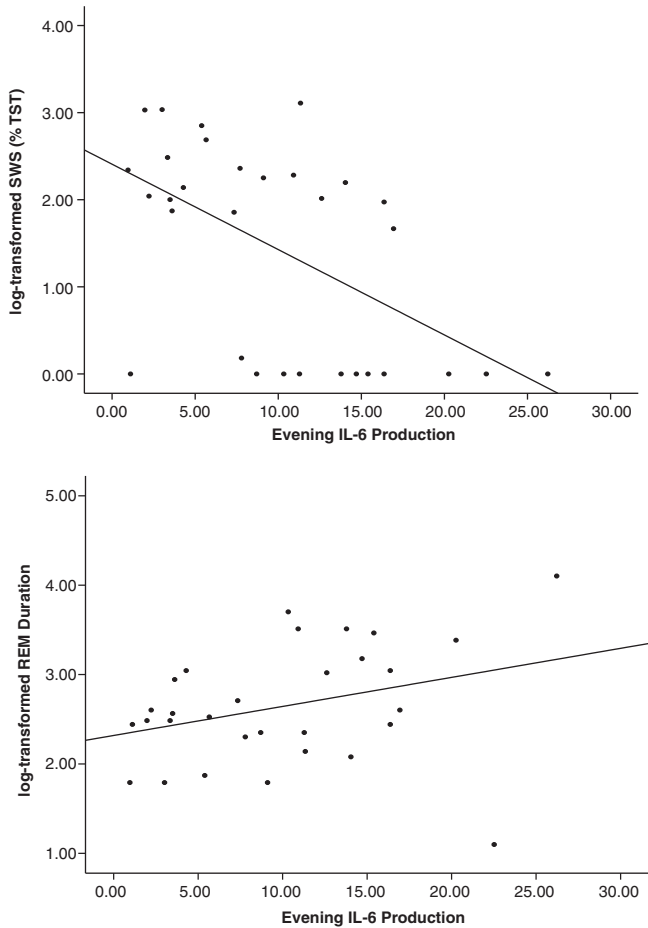
Fig. 1. Relationships between evening stimulated monocyte production of IL-6, log transformed SWS, and log transformed REM duration. Greater production of IL-6 was associated with shorter SWS and longer REM duration.

BMI, gender, and ethnicity accounted for 10% of the variance in fatigue. After controlling for these covariates, less SWS was associated with fatigue the subsequent day ($\Delta R^2 = .15$, $p = .03$; Condition 1 of the Mediation Model). Condition 2 of the Mediation
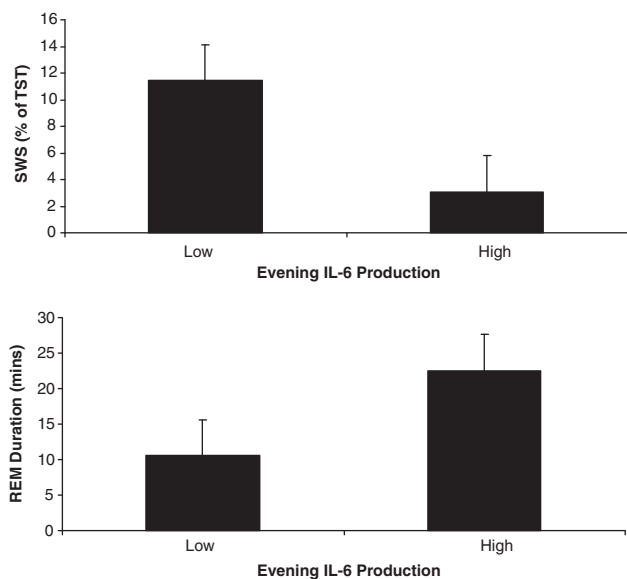
Fig. 2. Figure depicting SWS and REM duration in participants with the highest and lowest quartile evening stimulated monocyte production of IL-6.

Table 3
Final hierarchical regression analysis demonstrating that SWS mediates the relationship between evening IL-6 production and fatigue ($N = 31$).

| Variable	B	SEB	β	R^2	ΔR^2
Block 1				0.10	0.10
BMI	−0.32	0.57	−0.10		
Age	0.25	0.24	0.182		
Gender	−6.48	4.67	−0.25		
Ethnicity	0.24	1.39	0.03		
Block 2				0.32	0.22[*]
IL-6 production	−.59	0.45	−0.22		
SWS	6.38	2.44	0.55[*]		

[*] $p < .05$.

Model was satisfied by our finding that greater evening stimulated monocyte production of IL-6 was associated with less SWS (see previous section). Evening monocyte production of IL-6 was associated with fatigue ($\Delta R^2 = .17$, $p = .05$; Condition 3). Table 3 lists the final hierarchical linear regression analysis testing Condition 4 of the Mediation Model. As can be seen in Table 3, when both production of IL-6 and SWS were examined simultaneously as predictors of fatigue, only SWS was significantly associated with fatigue the subsequent day ($\beta = .55$, $p = .02$). In other words, increased evening production of IL-6 was associated with fatigue, and this relationship was mediated by shorter SWS (see Fig. 3). Thus, Condition 4 of the Mediation Model was satisfied and there was support for our hypothesis that associations between proinflammatory cytokine expression and fatigue would be mediated by altered SWS.

Mediation analyses were also conducted to determine whether alterations in REM duration mediated relationships between IL-6 and fatigue. After controlling for age and BMI, REM duration was unrelated to fatigue. Thus, there was no evidence that increased REM duration mediated relationships between production of IL-6 and fatigue.

4. Discussion

This study examined relationships between proinflammatory cytokine expression, sleep architecture, and fatigue. There was support for our hypothesis that increased proinflammatory cytokine expression would be associated with alterations in sleep architecture. Specifically, greater evening stimulated monocyte production of IL-6 was associated with less SWS. This supports the results of research demonstrating that individuals with high circulating IL-6 have less SWS (Burgos et al., 2006; Hong et al., 2005), and suggests that immune cells (i.e., monocytes) may be a source for changes in circulating levels of IL-6 in association with SWS.
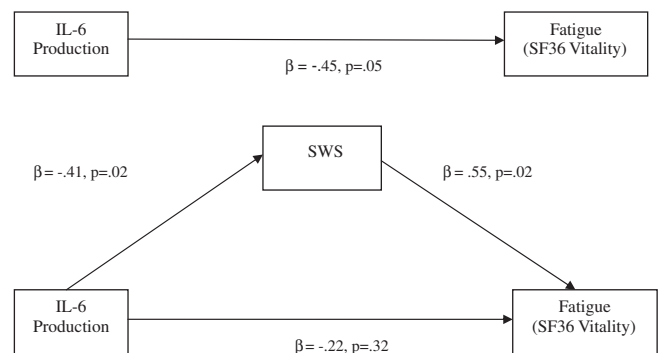
Fig. 3. Path analysis demonstrating that log transformed SWS mediates the relationship between evening stimulated monocyte production of IL-6 and fatigue. When both IL-6 and SWS are entered into the regression model, SWS, but not IL-6, remains a significant predictor of fatigue.

Increased evening production of IL-6 was also associated with a longer REM duration during the first sleep cycle. We have previously found that both circulating and stimulated production of IL-6 correlate with increases in REM sleep amounts (Irwin et al., 2004; Motivala et al., 2005; Redwine et al., 2003). However, in the present study, the strongest correlation between stimulated IL-6 and REM sleep was with REM duration, which is defined as time in REM sleep during the first sleep cycle. Given that the first sleep cycle is generally dominated by SWS in normal sleepers, our findings may reflect a shift away from SWS toward more REM sleep in participants who have high proinflammatory cytokine expression. Thus, as depicted in Fig. 2, this alteration in sleep stages significantly reduces the overall percentage of SWS that individuals obtain during their total sleep time.

It is interesting that TNF-α was unrelated to sleep architecture in the current study. Based on research finding that increased TNF-α is associated with longer REM sleep in alcoholics (Irwin and Rinetti, 2004), we expected that heightened production of TNF-α would have a deleterious effect on sleep architecture. However, these factors were unrelated in the current study. It is possible that IL-6, as opposed to TNF-α, may play a unique role in regulating SWS and REM sleep in healthy humans. Whereas we have found that a TNF antagonist partially normalizes REM sleep (Irwin et al., 2009), this medication has biologic effects on *in vivo* levels of both TNF-α and IL-6.

Psychological stress may be a key factor predicting increases in IL-6 production and alterations in sleep architecture. It has been well documented that chronic stress is associated with increased SNS activity (Thomas et al., 2004) as well as a greater production of proinflammatory cytokines (Kiecolt-Glaser et al., 2002, 2003). Additionally, studies have found that psychological stress is associated with a reduction in SWS and an increase in REM sleep (Cheeta et al., 1997; Kim and Dimsdale, 2007). Given that psychological stress triggers SNS activity (Grippo and Johnson, 2009), it is interesting that we observed a similar pattern concerning the relationship between IL-6 and sleep stages as has been shown between SNS and sleep stages. Specifically, increased nocturnal SNS activity is associated with a reduction in SWS and an increase in REM sleep (Plante, 2006; Rasch et al., 2007). Taken together, these findings suggest that heightened nocturnal SNS activity, possibly due to stress, may lead to increased production of proinflammatory cytokines, which may alter sleep stages. However, we did not examine whether psychological stress and SNS output are driving increased proinflammatory cytokine production in the current study. Future research should be conducted to examine relationships between proinflammatory cytokines, sleep architecture, and vitality in individuals who are experiencing stressful life events.

Our findings extend research on proinflammatory cytokines and sleep architecture beyond circulating cytokine levels to examine relationships between sleep architecture and cellular cytokine expression. Given that circulating cytokine levels can come from sources other than immune cells and may not necessarily be indicative of immune dysregulation, our findings provide further evidence that increased proinflammatory cytokine activity resulting from immune dysregulation plays an important role in regulating sleep stages.

Within the current study, increased production of IL-6 was associated with fatigue. It is noteworthy that SWS, but not REM sleep, mediated this relationship. Specifically, shorter SWS in individuals with increased proinflammatory cytokine activity explained reports of fatigue. Our previous research has shown that monocyte production of IL-6, as measured following ligation of the Toll-4 receptor, is increased following sleep deprivation (Irwin et al., 2010, 2006b), and is uniquely related to fatigue in breast cancer survivors (Collado-Hidalgo et al., 2006, 2008a). Moreover, a cytokine gene polymorphism that leads to the over-expression of IL-6 (Collado-Hidalgo et al., 2008b) correlates with increases in monocyte production of IL-6, which together are associated with fatigue in breast cancer survivors (Collado-Hidalgo et al., 2006).

These findings are also consistent with Cho and colleagues (Cho et al., 2009) in which circulating markers of inflammation (i.e., C-reactive protein) was linked to greater fatigue among healthy individuals who participated in the CARDIA population based study (Cho et al., 2009). Additionally, the current findings suggest that a reduction in SWS may be one pathway through which inflammation leads to a reduction in general health and fatigue in healthy individuals. It is interesting that in the current study as well as in the study conducted by Cho and colleagues (Cho et al., 2009), participants were generally young and had no major medical conditions. Given that inflammation was associated with fatigue in both studies, these findings highlight the deleterious effects of immune dysregulation on general health and well being in otherwise healthy individuals. It is unknown whether associations between proinflammatory cytokines, sleep architecture, and fatigue are the same in individuals who have already developed medical conditions. Future research should examine the extent to which these findings extend to these individuals.

There are several limitations in the current study that should be considered when interpreting these findings. First, this study was conducted on a small convenience sample of community-dwelling adults and should be replicated in larger samples. It should also be noted that screening for sleep apnea and myoclonus was conducted during the adaptation night. This could have impacted participants' ability to adapt to the sleep laboratory, resulting in "sleep rebound" during the first night of testing, although there were no differences in sleep parameters between the screening/adaption night and the testing night. Ideally, participants' sleep would be assessed in their home environment to obtain a more reliable measure of the relationship between proinflammatory cytokines and sleep parameters. However, this was not feasible in the current study.

This is the first published study to examine associations between stimulated IL-6 and sleep architecture. Additionally, given the correlational nature of this study, we cannot infer that a causal relationship exists between proinflammatory cytokines, sleep architecture, and fatigue. Thus, it should be cautioned that these findings are preliminary and need to be replicated in future research. Nonetheless, the current results have important implications on relationships between immune activity, sleep, and general health. Since it is believed that SWS serves a restorative function (Tasali et al., 2008), disruption of this sleep stage may lead to daytime fatigue.

Acknowledgments

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