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Publication Date

2015-07-01

DOI

10.1016/j.bbi.2014.09.017

Peer reviewed

HHS Public Access

Author manuscript

Brain Behav Immun. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Brain Behav Immun. 2015 July ; 47: 86–92. doi:10.1016/j.bbi.2014.09.017.

Sleep Loss Activates Cellular Inflammation and Signal Transducer and Activator of Transcription (STAT) Family Proteins in Humans

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Abstract

Sleep disturbance and short sleep duration are associated with inflammation and related disorders including cardiovascular disease, arthritis, diabetes mellitus, and certain cancers. This study was undertaken to test the effects of experimental sleep loss on spontaneous cellular inflammation and activation of signal transducer and activator of transcription (STAT) family proteins, which together promote an inflammatory microenvironment. In 24 healthy adults (16 females; 8 males), spontaneous production of IL-6 and TNF in monocytes and spontaneous intranuclear expression of activated STAT1, STAT3, and STAT5 in peripheral blood mononuclear cells (PBMC), monocyte-, and lymphocyte populations were measured in the morning after uninterrupted baseline sleep, partial sleep deprivation (PSD, sleep period from 3 a.m. to 7 a.m.), and recovery sleep. Relative to baseline, spontaneous monocytic expression of IL-6 and TNF-α was significantly greater after PSD ($P<0.02$) and after recovery sleep ($P<0.01$). Relative to baseline, spontaneous monocytic expression of activated STAT 1 and STAT 5 was significantly greater after recovery sleep ($P<0.007P<0.02$, respectively) but not STAT 3 ($P=0.09$). No changes in STAT1, STAT3, or STAT5 were found in lymphocyte populations. Sleep loss induces activation of spontaneous cellular innate immunity and of STAT family proteins, which together map the dynamics of sleep loss on the molecular signaling pathways that regulate inflammatory and other immune responses. Treatments that target short sleep duration have the potential to constrain inflammation and reduce the risk for inflammatory disorders and some cancers in humans.

Keywords

Sleep; Inflammation; Immunology; Sleep deprivation; Interleukin-6; Tumor necrosis factor-alpha; Signal transducer and activator of transcription; Flow cytometry; Humans; Monocytes

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The authors have no financial gain related to the outcome of this research, and there are no potential conflicts of interest.

Introduction

Insomnia complaints are associated with adverse health outcomes, including cardiovascular disease, certain cancers, and all cause mortality (Dew et al., 2003; Irwin, 2013; Irwin, 2015; Li et al., 2014; Vgontzas et al., 2010), and such health risks are further elevated among those who have both sleep disturbance and short sleep duration(Vgontzas et al., 2013). Epidemiologic evidence further shows that a decrease in sleep duration over six years prospectively predicts a two-fold increase in cardiovascular mortality 17 years later (Ferrie et al., 2007). Given that activation of cellular signals that initiate expression of inflammatory cytokines and systemic inflammation is associated with risk of a wide spectrum of medical conditions including cardiovascular disease, certain cancers, and mortality (Baune et al., 2011; DiDonato et al., 2012; Karin, 2006; Reuben et al., 2002; Ridker et al., 2003; Volpato et al., 2001), one of the most studied biological mechanisms underlying these observations is inflammation (Irwin, 2015).

Insomnia complaints, as well as nonrestorative sleep, have been associated with increases in CRP and other markers of inflammation in epidemiologic (Laugsand et al., 2012; Liukkonen et al., 2007; Zhang et al., 2013), naturalistic observational (Bornivelli et al., 2008; Chiu et al., 2009; Okun et al., 2007; Okun et al., 2009), and clinical studies of patients with insomnia disorder (Razeghi et al., 2012; Vgontzas et al., 2002, Burgos, 2006 #12189), as reviewed (Irwin, 2015). However poor sleep can also to be consequence of disease processes involving inflammation (Opp, 2009), and the causal role of sleep loss on inflammation is not known from these cross-sectional data. Two prospective epidemiologic studies suggest that disturbances of sleep, as well as decreases in sleep duration, uniquely contribute to cellular markers of inflammation with increases in interleukin-6 (IL-6), for example (Ferrie et al., 2013). Additionally, experimental sleep loss, such as partial night sleep deprivation (PSD) induces increases in C-reactive protein (CRP) (Meier-Ewert et al., 2004) and IL-6 (Haack et al., 2007; Vgontzas et al., 2004), as well as increases in inflammatory transcripts of IL-1β, IL-6, and IL-17 (van Leeuwen et al., 2009), although other studies have not consistently demonstrated such effects of sleep deprivation on inflammatory markers (Dimitrov et al., 2006; Frey et al., 2007; Haack et al., 2002).

Our prior work evaluated the cellular sources of proinflammatory cytokine activity by measuring the production of proinflammatory cytokines by monocytes following ligation of the Toll-like 4 receptor (TLR-4) with lipopolysaccharide (LPS) in monocytes; these cells make up about 5% of circulating leukocytes and are a major contributor to proinflammatory cytokine production in peripheral blood. Indeed, PSD induces an increase in TLR-4-stimulated production of IL-6 and tumor necrosis factor-α (TNF-α) (Irwin et al., 2010; Irwin et al., 2006). Sleep loss also induces an increase in transcription of IL-6 and TNF (Irwin et al., 2006) due an activation of NF-κB, the key transcription control pathway in the inflammatory signaling cascade (Irwin et al., 2008). However, it is not known whether sleep loss is associated with increases in the spontaneous production of IL-6 and TNF-α, which could determine whether PSD alters resting or constitutive expression of proinflammatory cytokines by monocytes.

The STAT protein family serves to transduce signals through the cytoplasm and to function as transcription factors in the nucleus. First discovered as cytoplasmic transcription factors that mediate cellular responses to cytokines and growth factors (Miklossy et al., 2013), STAT proteins act as a key signaling cascade mediating cytokine receptor-derived signals. There are six distinct STAT members (i.e., STAT1, STAT2, STAT3, STAT4, STAT5, STAT6) (Yu et al., 2009), of which STAT-3 is thought to be a mediator of inflammation (Levy et al., 2002). For example, IL-6 and TNF have been shown to activate STAT3, IL-6 mediates its effects through the STAT3 pathway, and STAT3 competes with the transcription factor NF-κB (Gao et al., 2012). Given that sleep-wake activity is also associated with the regulation of interferon-γ (IFN)(Petrovsky et al., 1997; Redwine et al., 2003) and with IL-2 (Irwin et al., 1996), it is further possible that sleep loss might alter activation of STAT1 that is linked to IFN signaling, and of STAT5 that is linked to IL-2 (Shuai et al., 2003). In addition, STAT family members show cross-regulation, and STAT1 and STAT3 are both activated by IL-6 (Shuai et al., 2003) in which the signaling specificity of IL-6 is modulated by activation of STAT3 such that removal of STAT3 results in prolonged STAT1 activation by IL-6. Other cytokines downstream of STATs can also cause synergistic or antagonistic effects. For example, IFN-γ pretreatment sensitizes cells to IFN-α due in part to increased expression of STAT1 that is induced by IFN-γ. Moreover, there is cross-talk between the two main cytokine signaling pathways, STATs and NF-κB (Shuai et al., 2003) in which activation of one STAT pathway can have opposing roles in the regulation of the immune response. Activation of STAT3, for example, promotes NF-κB and IL-6 pathways, but opposes STAT1 and NF-κB mediated Th1 immune response (Yu et al., 2009). Hence, evaluating the effect of sleep loss on STAT activation could provide understanding about the broader role of sleep in the regulation of immune responses, in addition to the effects of sleep on inflammatory mechanisms. In this study, we used an experimental model of PSD to determine whether this sleep disruption pattern, ubiquitous in the general population and often found in persons with chronic medical disorders, might activate spontaneous monocytic production of proinflammatory cytokines, and activate STAT family proteins, namely STAT1, STAT3, and STAT5 in monocyte, lymphocyte, and peripheral blood mononuclear cell populations.

Methods

Subjects

The subjects included 24 healthy volunteers (16 women and 8 men) between the ages of 36 and 76 years (mean ± SD, 59.5 ± 5.7 years), who gave informed consent; the Institutional Review Board of UCLA approved the protocol. Inclusion criteria required that subjects be healthy as assessed by medical interview and physical examination, with normal screening laboratory tests; none had history of an inflammatory disorder, cancer, or chronic or active infections. Subjects had a body mass index < 30 kg/m^2 (25.3 ± 5.3 kg/m^2), were non-smokers, fulfilled DSM-IV criteria for Never Mentally Ill (American Psychiatric Association, 2000) and regularly slept between 10:30 p.m. and 7:30 a.m. as confirmed by 2-week sleep diaries. Additionally, none of the subjects reported symptoms of sleep disturbance or depression with scores on the Pittsburgh Sleep Quality Index <5 (2.1 ± 2.1) and scores on the Beck Depression Inventory <5 (1.0 ± 2.0).

Protocol

Subjects spent four nights in the UCLA Clinical Translational Research Center (CTRC). We have previously shown that this period of assessment in a sleep laboratory setting does not significantly increase feelings of depression or anxiety in healthy volunteers (Irwin et al., 2012). After adaptation to the sleep laboratory with screening for sleep apnea and nocturnal myoclonus, subjects underwent three nights of testing (baseline; partial sleep deprivation, PSD; recovery). During each night, subjects underwent polysomnography, with ambient light <50 lux. During baseline and recovery nights, uninterrupted sleep occurred between 11 p.m. and 7 a.m.; whereas during PSD, sleep was restricted to the period between 3 a.m. and 7 a.m. Subjects were prohibited from exercise and behaviorally monitored to ensure awake during the day and PSD night period.

Peripheral blood samples were obtained via an indwelling venous forearm catheter at 8 a.m., after baseline, after PSD, and after recovery nights. The indwelling catheter was placed at 7 a.m., or one hour before the blood sample. Samples were analyzed for spontaneous production of intracellular proinflammatory cytokines in monocyte populations, and for spontaneous expression of activated STAT 1, STAT 3, and STAT 5 in PBMC, and lymphocyte- and monocyte populations.

Assays

Spontaneous monocyte intracellular production of IL-6 and TNF-α was assessed by flow cytometry; we have previously reported on the effects of PSD to increase stimulated monocytic production of IL-6 and TNF-α (Irwin et al., 2006). Briefly, heparinized whole blood was treated with 10 μg/mL brefeldin A (Sigma) and incubated for 4 hours with mixing at 37 °C. After lysis of red blood cells and washing, white cells were incubated with fluorescence-conjugated cell surface antibody for CD14, then permeabilized (FACS Permeabilizing 2 Buffer, BD Biosciences) and incubated with conjugated antibodies for intracellular cytokine detection. Approximately 5,000 CD14+ events were acquired by three-color flow cytometry (FACScan, BD Immunocytometry) to determine the percentage of cytokine-secreting monocytes with spontaneous production of IL-6 only, TNF-α only, or the co-expression of IL-6 and TNF-α. Quadrant coordinates were set at baseline, and maintained for samples at PSD and recovery.

Spontaneous PBMC, monocyte, and lymphocyte intranuclear levels of activated transcription factors STAT1, STAT3, and STAT5 were also assessed by flow cytometry. PBMC were isolated from heparinized whole blood using ficoll hypaque density gradient separation, and resuspended in phosphate-buffered saline (PBS) at 1×10^6 cells/mL. Intranuclear levels of activated (phosphorylated) STAT1 (pTyr-701), STAT3 (pTyr-705), and STAT5 (pTyr-694) were determined by single-color flow cytometry using phycoerythrin-labeled antibodies (BD Immunocytometry) specific for the phosphorylated forms of each transcription factor. Aliquots of 1×10^6 PBMC were fixed in a final concentration of 2% methanol-free formaldehyde solution, then washed twice with PBS solution containing 0.5% BSA and 0.1% sodium azide. 5×10^5 cells/well were incubated in sterile round-bottom 96 well plates for 30 minutes on ice with 90% methanol for permeablization of the nuclear membrane. PBMC were washed two times, then incubated

with 5 μL (0.015 μg STAT1 or STAT5, 0.031 μg STAT3) of one antibody per well for 1 hour at room temperature. After incubation, PBMCs were washed and 25,000 events were acquired by flow cytometry (FACScan, BD Immunocytometry). Cell Quest software (BD Biosciences) was used to gate on total PBMC, monocytes only, or lymphocytes only; since methanol fixation necessary to permeabilize the nuclear membrane rendered cell surface molecules unrecognizable for routine phenotyping, monocytes and lymphocytes were gated based on forward versus side scatter. A histogram analysis plot was then used to determine the levels of each activated transcription factor within each cell population based on the mean fluorescence intensity (MFI).

Statistical Analysis

Data were available on >95% of the subjects at all time points. Analyses were carried out with IBM SPSS for Windows, version 22. To determine the effects of PSD on spontaneous monocytic levels of IL-6 and TNF-α, repeated measures mixed-model analyses of variance were performed across the three nights (baseline night, PSD, recovery night). *A priori* contrasts (LSD) tested differences between baseline night and PSD, and between baseline night and recovery night. Significance level was set at $p < 0.05$. To determine the effects of PSD on spontaneous monocytic, as well as PBMC and lymphocytic expression of activated STAT1, STAT3, and STAT5, repeated measures mixed-model analyses of variance were also performed across the three conditions (baseline night, PSD, and recovery night), in which *a priori* contrasts (LSD) tested differences between baseline night and PSD, and between baseline night and recovery night for each STAT in each cell population.

Results

Polysomnographic sleep

Consistent with the experimental manipulation, total sleep time (TST) was reduced during the PSD relative to baseline- and recovery nights, with evidence of shorter time to sleep onset during PSD. (Table 1) There were no significant changes in the relative amounts of sleep stages from baseline to PSD, or from baseline to recovery night, except for a decrease in the 10percentage of Stage 2 sleep during PSD relative to baseline, and relative to recovery nights; and an increase in Stage 3 during PSD relative to baseline. REM density showed an increase in PSD relative to baseline, and relative to recovery night consistent with the sleep period during PSD as being restricted to the second half of the night when REM sleep is more prominent.

Cellular inflammation

Spontaneous intracellular production of proinflammatory cytokines were examined in three unique monocyte populations: cells that simultaneously produced both IL-6 and TNF-α together; cells that produced only IL-6; and those that produced only TNF-α.

In the monocyte population that produced both IL-6 and TNF-α together, PSD induced an increase in the spontaneous expression of these proinflammatory cytokines, with a night effect (F (2,41.8)= 4.18, P = 0.02; Figure 1). As compared to baseline levels, percentage of

monocytes spontaneously producing IL-6 and TNF-α was significantly increased after PSD ($P < 0.02$) and after recovery night ($P < 0.01$).

In the monocyte population that produced IL-6 only, PSD did not induce an increase in the spontaneous production of IL-6, with a night effect (F (2,41.3)= 2.59, $P = 0.08$). As compared to baseline levels, percentage of monocytes spontaneously producing IL-6 was significantly increased after PSD ($P < 0.03$) and but not after recovery night ($P = 0.39$). (Data not shown)

In the monocyte population that produced TNF-α only, PSD did not induce an increase in the spontaneous production of TNF-α, with a night effect (F (2,42.4)= 2.92, $P = 0.06$). As compared to baseline levels, percentage of monocytes spontaneously producing TNF-α was significantly increased after PSD ($P < 0.03$), but not after recovery night ($P = 0.08$). (Data not shown)

Sex was not related to any of the changes in spontaneous monocytic expression of proinflammatory cytokines, and covarying for sex in the analyses did not alter the effects.

STAT family proteins

Spontaneous levels of intranuclear expression of activated STAT1, STAT3, and STAT5 were examined in PBMC, monocyte populations, and lymphocytes populations, with the primary hypotheses focused on PSD changes in monocyte populations consistent the measures obtained for spontaneous production of proinflammatory cytokines.

PSD induced an increase in the spontaneous expression of activated STAT1 in monocyte populations, with a night effect (F (2,45.1)= 4.04, $P = 0.02$; Figure 2). As compared to baseline levels, spontaneous expression of STAT1 in monocytes was not increased immediately after PSD ($P = 0.26$), but was significantly increased after recovery night ($P < 0.007$). Similar results were found in PBMC populations. PSD induced increases in the spontaneous expression of STAT1 in PBMC, (F (2,45.0)= 3.57, $P < 0.04$) with increases at recovery night ($P < 0.01$), but not immediately after PSD ($P = 0.08$). No changes in STAT1 were found in lymphocyte populations (all P's > 0.2).

For STAT3, PSD did not induce an increase in the spontaneous expression of this transcription factor in monocyte populations (F (2,45.4)= 2.21, $P = 0.12$; Figure 3). As compared to baseline levels, spontaneous expression of activated STAT3 in monocytes did not increase immediately after PSD ($P = 0.87$) or after recovery night (P =0.09). Similar results were found in PBMC populations. PSD did not increase the spontaneous expression of STAT3 in PBMC (F (2,45.2)= 2.14, P =0.12), with no increase after PSD ($P = 0.80$) or after recovery night ($P = 0.06$). No changes in STAT3 were found in lymphocyte populations (all P's > 0.2).

Finally, PSD induced an increase in the spontaneous expression of STAT5 in monocyte populations (F (2,45.0)= 3.26, $P = 0.05$; Figure 4). As compared to baseline levels, spontaneous expression of activated STAT5 in monocytes did not increase immediately after PSD ($P = 0.31$), but significantly increased after recovery night ($P < 0.02$). Similar results

were found in PBMC populations. PSD did not induce increases in the spontaneous expression of STAT5 after PSD ($P < 0.31$) but significant increases were found after recovery night ($P < 0.02$). No changes were found in STAT 5 in lymphocyte populations (all $P's > 0.2$).

Changes in the spontaneous monocytic production of proinflammatory cytokines (IL-6 and TNF-α together, or either one by itself) were not correlated with changes in spontaneous levels of STAT1, STAT3, or STAT5 (data not shown). In addition, sex was not related to any of the changes in STAT1, STAT3, or STAT5, and covarying for sex in the analyses did not alter the effects. Finally, the increase in Stage 3 sleep found during PSD relative to baseline was not correlated with spontaneous monocytic production of proinflammatory cytokines or with STAT levels at either PSD or recovery.

Discussion

This study provides the first evidence of activation of spontaneous cellular innate immunity and of STAT family proteins in association with sleep loss. In the morning after a night of sleep loss, there was greater spontaneous production of IL-6 and TNF-α in peripheral blood monocyte populations relative to morning levels following uninterrupted sleep at baseline, and this activation persisted in monocytes that were co-producing IL-6 and TNF-α even after a night of recovery sleep. In addition, sleep loss activated STAT1 and STAT5, but not STAT3. Relative to morning levels following uninterrupted sleep at baseline, activation of the STAT family proteins in monocyte populations in response to sleep loss was temporally delayed, as increases did not become evident until recovery sleep. Because cytokines serve as activators of STATs, it is possible that increases in proinflammatory and other cytokines must occur first with later effects on STAT family proteins. No changes in STAT1, STAT3, or STAT5 were found in lymphocyte populations. These observations that sleep loss results in activation of spontaneous monocytic production of proinflammatory cytokines with potential downstream effects on STAT family proteins in monocyte populations provide further understanding of the cellular and molecular mechanisms by which sleep loss enhances inflammatory biology and regulates immune responses in humans.

Sleep loss has previously been found to induce increases in TLR-4 signaling and monocytic production of proinflammatory cytokines (Irwin et al., 2006), but it was not known whether such aberrant increases in TLR activity might be associated with a priming of monocyte populations as indexed by increases in the spontaneous levels of IL-6 and TNF-α. This study suggests that even in the absence of an exogenous activator of innate immunity, such as LPS, the baseline production of IL-6 and TNF-α by monocytes is increased by sleep restriction. Given that monocytes are a primary source for inflammatory cytokines in the peripheral blood, these increases in the spontaneous production of proinflammatory cytokines may in turn induce increases in circulating inflammatory markers with consequent systemic inflammation and metabolic changes. Small elevations in circulating inflammatory mediators, for example, have been associated with type II diabetes mellitus, independent of adiposity (Festa et al., 2000) as well with cardiovascular disease (Ridker et al., 2003; Ridker et al., 2004; Ridker et al., 2000). The causal role of sleep in inducing activation of cellular inflammation is further confirmed by findings that insomnia treatment reduces TLR-4

stimulated monocytic production of proinflammatory cytokines (Irwin et al., In press), and also reduces the likelihood of high risk CRP levels (>3 pg/ml)(Irwin, 2014). The persistent increase of spontaneous monocytic production of inflammatory cytokines contrasts with prior findings on TLR-4 stimulated monocytic production of IL-6 and TNF, which return to baseline levels after a night of recovery sleep (Irwin et al., 2010; Irwin et al., 2006).

The STAT protein family cross-talks with the other major cytokine signaling pathway, NF-κB (Shuai et al., 2003). Given that PSD results in nuclear translocation of NF-κB that signals increases in inflammation (Irwin et al., 2008), it is surprising that STAT3 was only modestly activated. However, because activation of the STAT3 pathway also competes with NF-κB (Gao et al., 2012), PSD activation of NF-κB may have attenuated increases of STAT3. Although STAT1 is predominantly activated by IFN (Gao et al., 2012), increases in IL-6 also signal STAT1 activation, which may have contributed to the robust activation of STAT1 (Gao et al., 2012). Moreover, STAT1 activation is known to antagonize activation of STAT3 (Gao et al., 2012; Shuai et al., 2003).

The clinical implications of transient changes in activation of monocyte STAT family proteins might not translate into increased risk for disease. However, aberrant increases in STAT3 and STAT5 signaling are thought to dysregulate genes that control cell growth, which is implicated in tumorigenesis (Yu et al., 2009). For example, constitutively active STAT3 and STAT5 are detected in numerous malignancies (Miklossy et al., 2013). STAT1, in contrast to contributing to anti-viral immunity, also has a proinflmmatory role in liver disease (Gao et al., 2012).

The mechanisms by which sleep disruption activates cellular inflammation and STAT family proteins were not evaluated in this study. However, we have previously found that PSD induces increases in sympathoadrenal activity on awakening (Irwin et al., 2005), and such adrenergic signaling is known to increase the expression of inflammatory response genes (Irwin et al., 2011), possibly by activation of NF-κB (Bierhaus et al., 2003; Pace et al., 2006). Additionally, we and others have found that PSD induces increases in growth hormone and prolactin and decrease in cortisol in the second half of the night (Parry et al., 1996; Redwine et al., 2000); as reviewed previously, these neuroendocrine changes could account for the increases in spontaneous monocytic production of proinflammatory cytokines (Besedovsky et al., 2012). It is thought that increases in slow wave sleep following PSD drive increases in growth hormone, for example. Indeed, we did find an increase in relative amounts of Stage 3 sleep, but not Stage 4, during the second half of PSD consistent with our prior findings (Redwine et al. 2000), but this increase was not maintained into the recovery night as previously reported (Irwin et al. 2002), possibly because this sample is older and there may be less homeostatic drive for slow wave sleep in older persons. Additionally, increases in Stage 3 sleep were not correlated with any of the immune measures at PSD or recovery. Less is known about the neural and neuroendocrine mediators of STAT family protein activation, although one study found that increasing concentrations of norepinephrine or epinephrine both independently increased levels of phosphorylated STAT3 in a dose-dependent fashion, and that activation of STAT3 was inhibited by blockade of adrenergic receptor signaling (Landen et al., 2007). Research using

signal antagonists is needed to test these potential mechanisms in humans experiencing sleep loss.

One of the most common sleep complaints, loss of sleep during part of the night, has robust effects of cellular- and innate immune responses. The results presented here further define the effects of sleep loss on inflammatory and molecular mechanisms that regulate the immune response, with implications for cardiovascular and chronic inflammatory disorders, as well as cancer in humans. Targeting sleep disturbance shows promise as a new strategy to constrain inflammation and to promote health (Irwin et al., In press; Irwin, 2015; Irwin, 2014).

Acknowledgments

This work was supported in part by grants T32-MH18399, T32-MH073517, HL 079955, HL095799-01, AG 026364, AG034588, CA160245, DA032922, P30-AG028748, 1UL1RR033176-0, the UCLA Cousins Center at the Semel Institute for Neurosciences.

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Highlights

- Sleep loss activates spontaneous cellular innate immunity.
- Sleep loss activates spontaneous monocytic production of inflammatory cytokines.
- Sleep loss activates signal transducer and activator of transcription family proteins.
- Sleep loss promotes an inflammatory microenvironment that increases risk for disease.

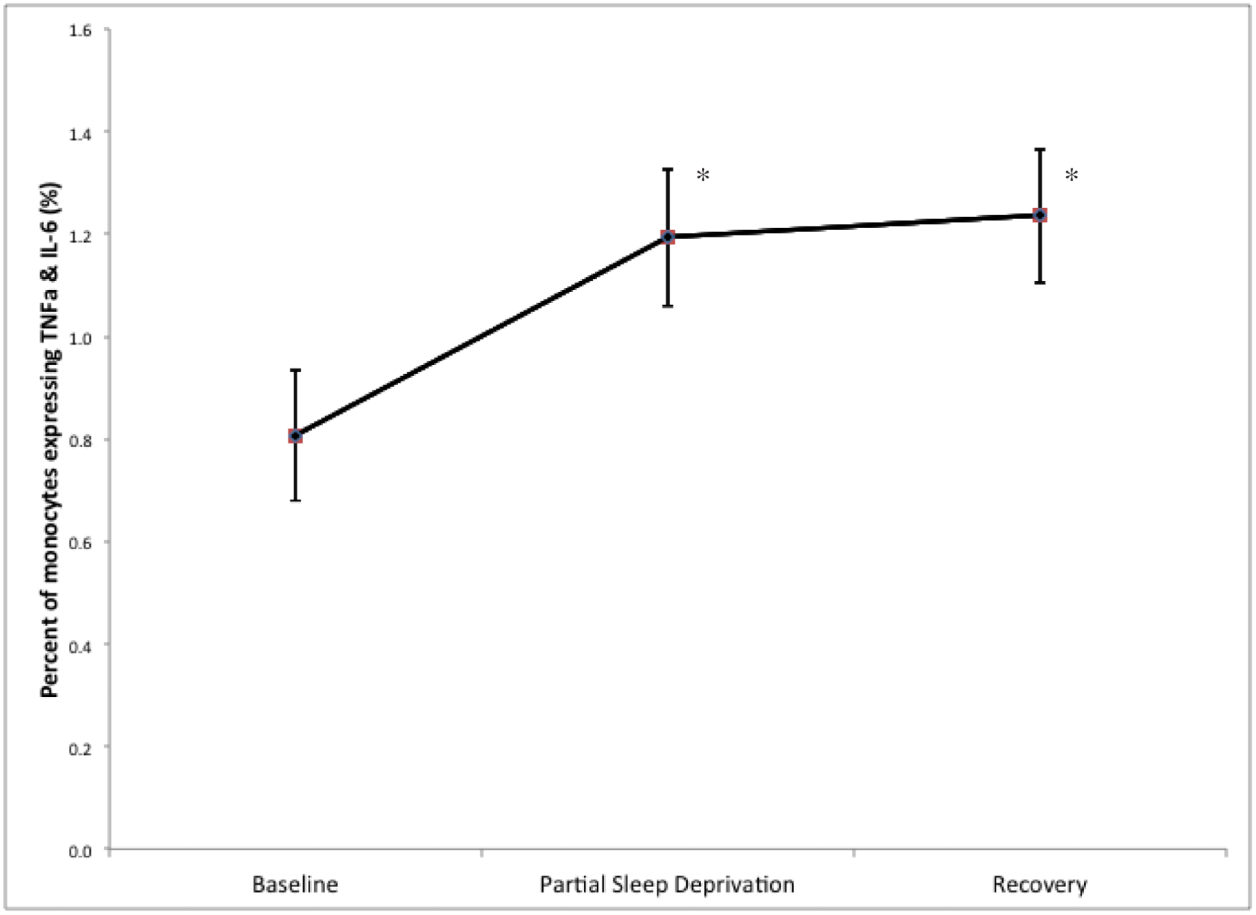

Figure 1.

Spontaneous production of both interleukin (IL) 6 and tumor necrosis factor-α (TNF) in monocyte, CD14+ cells, after baseline uninterrupted sleep, partial sleep deprivation (sleep period: 3 a.m. to 7 a.m.), and recovery sleep. Data are represented as mean percentage ± SEM. * $P<0.05$ as compared to baseline

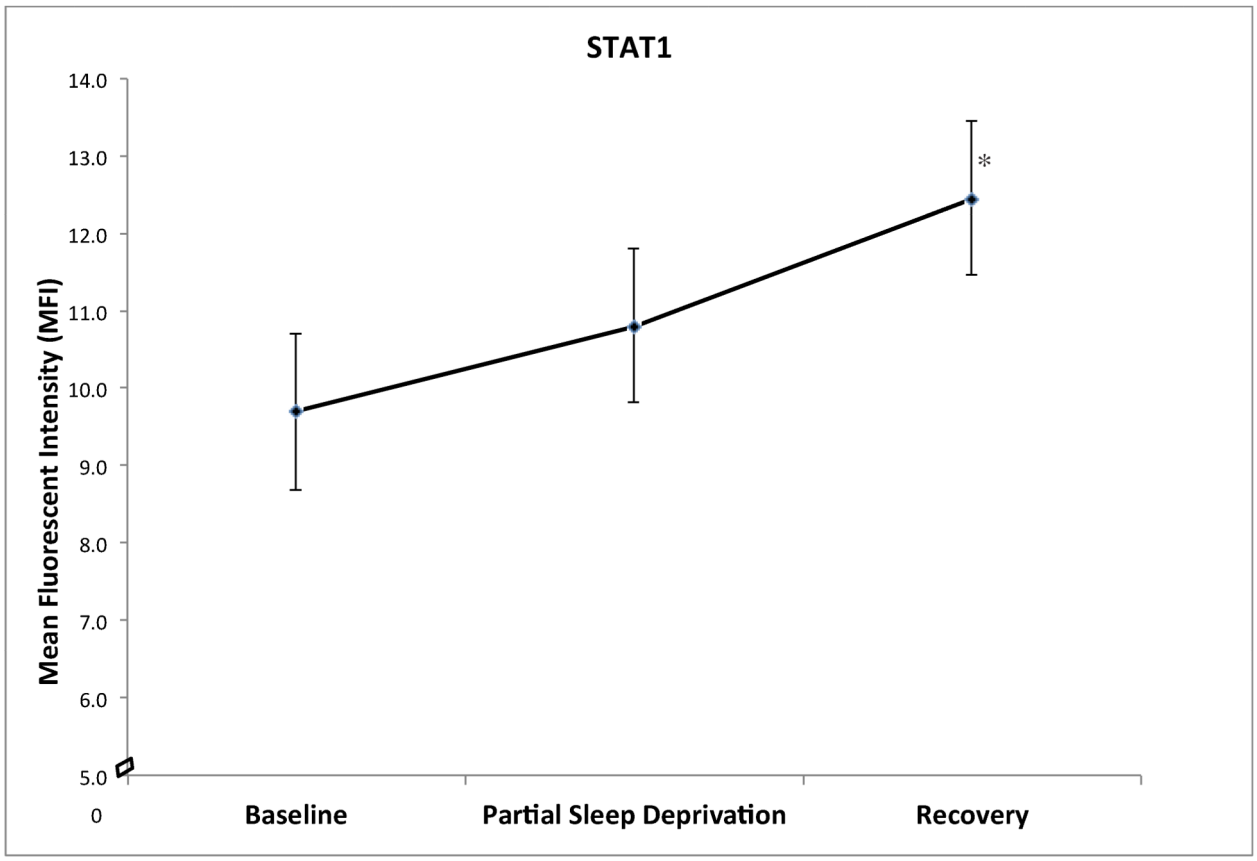

Figure 2.

Spontaneous intranuclear expression of STAT1 in monocyte, CD14+ cells, after baseline uninterrupted sleep, partial sleep deprivation (sleep period: 3 a.m. to 7 a.m.), and recovery sleep. Data are represented as mean fluorescent intensity ± SEM. * $P<0.05$ as compared to baseline

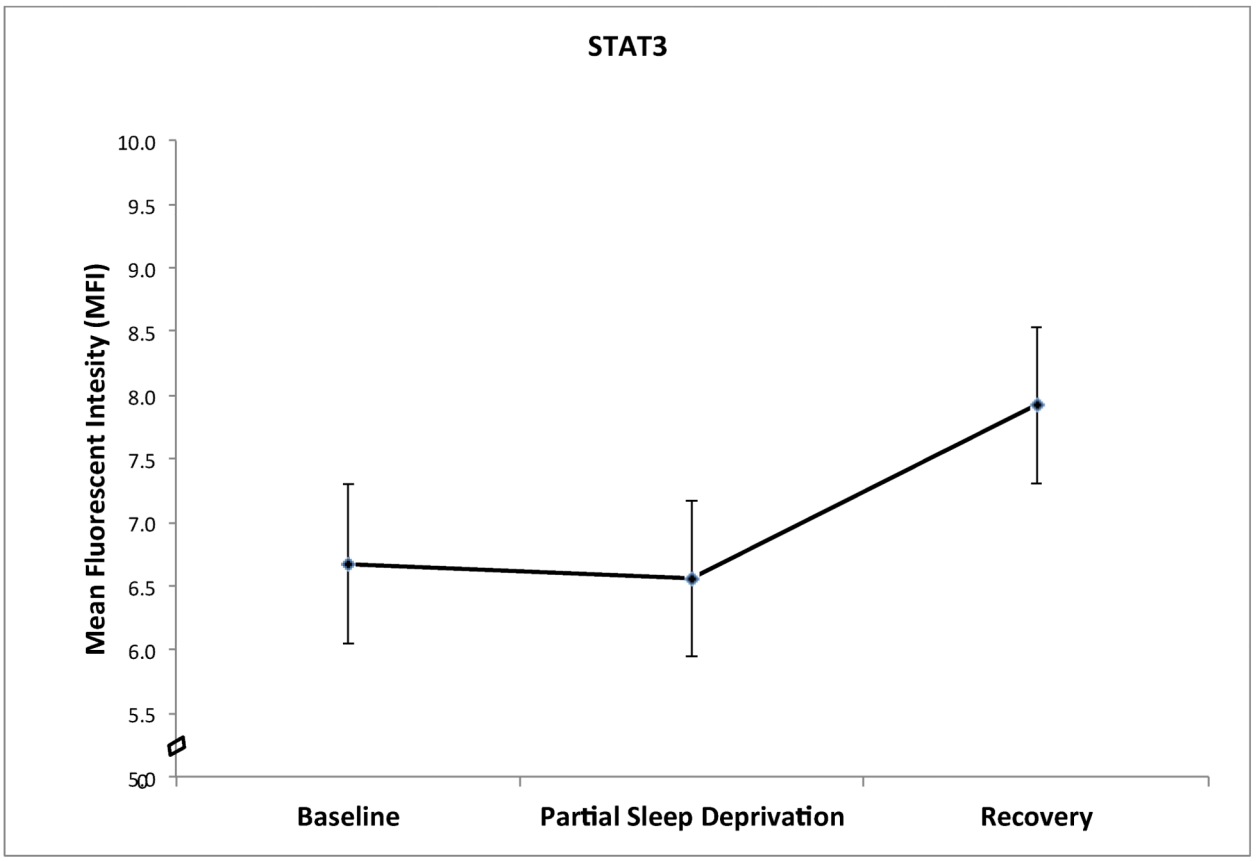

Figure 3.

Spontaneous intranuclear expression of STAT3 in monocyte, CD14+ cells, after baseline uninterrupted sleep, partial sleep deprivation (sleep period: 3 a.m. to 7 a.m.), and recovery sleep. Data are represented as mean fluorescent intensity ± SEM.

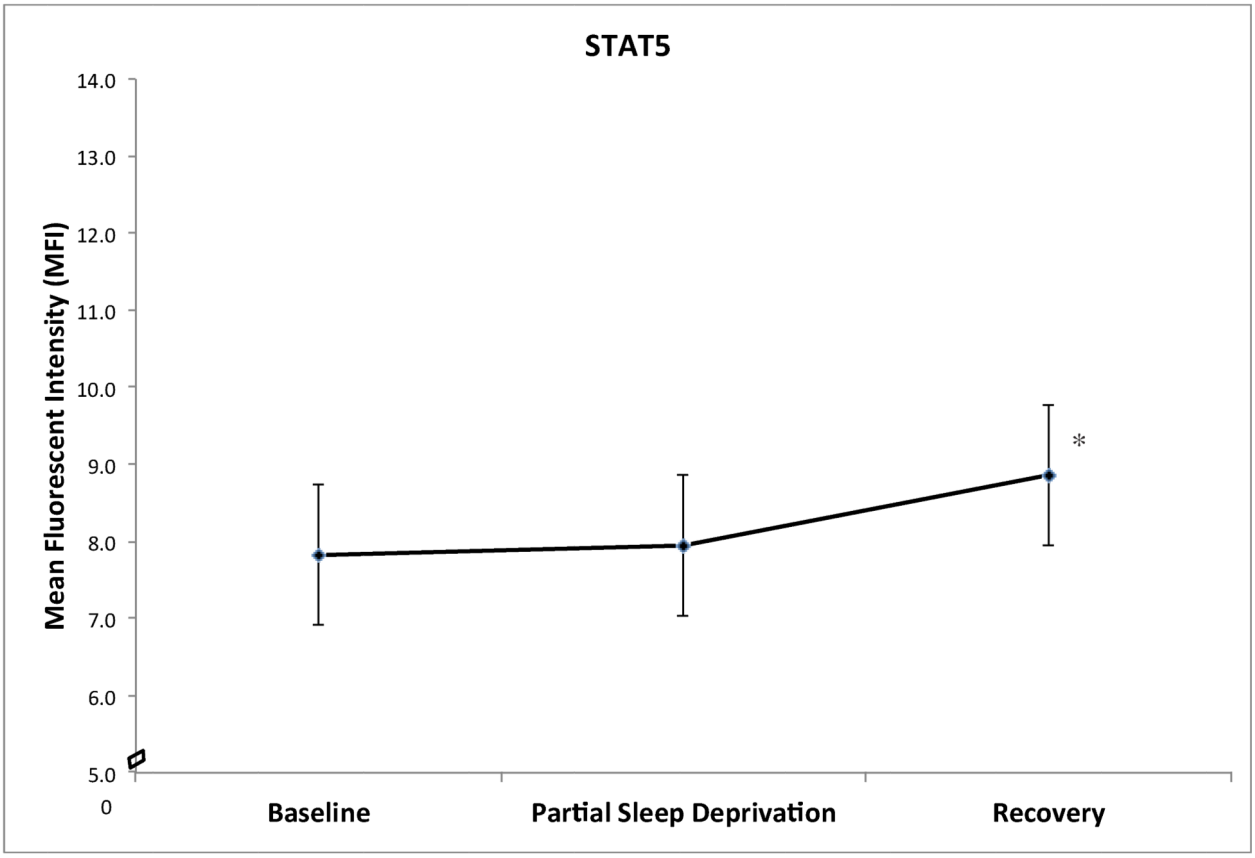

Figure 4.

Spontaneous intranuclear expression of STAT5 in monocyte, CD14+ cells, after baseline uninterrupted sleep, partial sleep deprivation (sleep period: 3 a.m. to 7 a.m.), and recovery sleep. Data are represented as mean fluorescent intensity ± SEM. * $P<0.05$ as compared to baseline

Table 1

Polysomnographic Sleep Variables during Baseline, Partial Night Sleep Deprivation, and Recovery Sleep Nights

Variable	Baseline Night Mean (SD)	PSD Night Mean (SD)	Recovery Night Mean (SD)	F statistic; P*
Sleep onset latency (min)	25.6 (23.0)	7.8 (8.4)	22.6 (22.8)	7.25; < 0.01[1,2]
Sleep efficiency (%)	89.6 (5.7)	88.8 (12.2)	91.0 (5.4)	0.49; 0.62
Total sleep time (min)	397.7 (30.9)	201.4 (31.5)	409.6 (42.2)	278.9; <0.001[1,2]
Stage 1 (%)	5.6 (5.1)	5.4 (8.5)	3.5 (2.1)	2.32; 0.11
Stage 2 (%)	54.2 (12.1)	43.9 (15.4)	53.1 (8.2)	10.5; <0.001[1,2]
Stage 3 (%)	11.8 6.4)	17.3 (9.6)	11.3 (5.8)	12.2; <0.001[1,2]
Stage 4 (%)	3.5 (4.2)	4.2 (5.6)	4.2 (5.3)	0.12; 0.89
REM (%)	24.8 (7.7)	29.1 (9.9)	27.8 (5.4)	2.44; 0.10[1]
REM Duration	21.7 (13.7)	27.3 (18.0)	21.7 (11.2)	1.72; 0.20
REM Density	1.4 (0.7)	1.8 (0.9)	1.5 (0.7)	4.68; <0.051

*Between-condition differences were tested with one-way analysis of variance Pairwise differences (P<0.05) are indicated by superscripts:

[1]Baseline vs. PSD;

[2]PSD vs. Recovery;

[3]Baseline vs. Recovery