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Chronic sleep restriction elevates brain interleukin-1 beta and tumor necrosis factor-alpha and attenuates brain-derived neurotrophic factor expression

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HIGHLIGHTS

- Chronic sleep restriction enhances cortical IL-1β and TNF-α mRNAs.
- Chronic sleep restriction attenuates acute sleep loss elevations in BDNF mRNA.
- Chronic sleep restriction reduces hippocampal BDNF mRNA levels below baseline levels.

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ABSTRACT

Acute sleep loss increases pro-inflammatory and synaptic plasticity-related molecules in the brain, including interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and brain-derived neurotrophic factor (BDNF). These molecules enhance non-rapid eye movement sleep slow wave activity (SWA), also known as electroencephalogram delta power, and modulate neurocognitive performance. Evidence suggests that chronic sleep restriction (CSR), a condition prevalent in today's society, does not elicit the enhanced SWA that is seen after acute sleep loss, although it cumulatively impairs neurocognitive functioning. Rats were continuously sleep deprived for 18 h per day and allowed 6 h of ad libitum sleep opportunity for 1 (SR1), 3 (SR3), or 5 (SR5) successive days (i.e., CSR). IL-1β, TNF-α, and BDNF mRNA levels were determined in the somatosensory cortex, frontal cortex, hippocampus, and basal forebrain. Largely, brain IL-1β and TNF-α expression were significantly enhanced throughout CSR. In contrast, BDNF mRNA levels were similar to baseline values in the cortex after 1 day of SR and significantly lower than baseline values in the hippocampus after 5 days of SR. In the basal forebrain, BDNF expression remained elevated throughout the 5 days of CSR, although IL-1β expression was significantly reduced. The chronic elevations of IL-1β and TNF-α and inhibition of BDNF might contribute to the reported lack of SWA responses reported after CSR. Further, the CSR-induced enhancements in brain inflammatory molecules and attenuations in hippocampal BDNF might contribute to neurocognitive and vigilance detriments that occur from CSR.

1. Introduction

Accumulating evidence indicates that chronic sleep restriction (CSR), a condition prevalent in today's societies, impairs health, immune functioning [1], and cognition [2,3]. These functions are regulated by inflammatory and synaptic plasticity-related molecules [4]. In addition, individuals with various pathologies that are associated with enhanced inflammation, such as insomnia, cancer, and major depression, exhibit disturbed and/or reduced sleep [1,5,6]. Elevated pro-inflammatory molecules, including the cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), and reduced synaptic plasticity-related molecule brain-derived neurotrophic factor (BDNF) are associated with impairments in performance and cognition, sleepiness, and altered sleep [1,4]. IL-1β, TNF-α, and BDNF enhance NREM sleep electroencephalogram (EEG) delta power [also known as slow-wave activity

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(SWA); an indicator of sleep intensity]. Unlike SWA after acute sleep loss, SWA is not enhanced after several days of CSR and the exact mechanisms by which this occurs through are currently unknown [7,8].

IL-1β and TNF-α enhance sleep when injected centrally or peripherally [1]. Further, IL-1β and TNF-α injected intracerebroventricularly or locally to the cortex enhance SWA. When these molecules are inhibited via knockout mice or pharmacologically, homeostatic sleep responses to sleep loss are attenuated. In rats, mice, or rabbits, IL-1β and TNF-α messenger ribonucleic acids (mRNAs) and proteins are enhanced in sleep-related brain areas, including the cortex and hippocampus, after acute sleep loss [1]. In humans, acute sleep loss and 5 days of CSR enhance circulating pro-inflammatory cytokines including IL-6 and TNF-α [9]. Nevertheless, the effect of CSR on brain inflammatory molecules has received little attention.

BDNF is a neurotrophin and growth factor found centrally and peripherally that has many functions including activity-dependent synaptic plasticity, cognition, modulating local inflammation, and sleep regulation [4,10]. BDNF enhances spontaneous sleep in rats and rabbits [10] and enhances SWA in the cortical hemisphere where injected relative to the contralateral hemisphere [11]. Acute sleep deprivation enhances BDNF mRNA expression in sleep-related brain areas, such as the cortex [12]. However, reductions in BDNF mRNA expression within the hippocampus after acute sleep deprivation have been reported [13]. Herein, we determined the effects of CSR on cortical and subcortical brain area IL-1β, TNF-α and BDNF mRNA levels in rats.

2. Methods

2.1. Animals

Twenty-eight three-month-old male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were housed individually and provided water and food ad libitum throughout the experiments. Rats were maintained on a 12:12 h light/dark cycle at 22 ± 3 °C. All experimental protocols were approved by Veteran Affairs Boston Healthcare system Institutional Animal Care and Use Committee and were in compliance with the National Institutes of Health guidelines.

2.2. Experimental treatment groups and sleep restriction

Rats were randomly placed into 4 experimental treatment groups ($N = 7$ per group) that included: 18 h of acute sleep restriction (SR1), 3 consecutive days of CSR (SR3), 5 consecutive days of CSR (SR5), and an ad libitum sleep baseline control (BL) group. Rats were deprived of sleep for 18 h [zeitgeber time (ZT) 6–24] by being placed in a periodically rotating wheel (35.5 cm in diameter × 11 cm in width) (Lafayette Instrument Company, Lafayette, IN, USA), which continuously revolved for 3 m/min for 4 s followed by 12 s of immobility as previously described [14]. Immediately after the sleep deprivation periods, rats in the SR3 and SR5 treatment groups returned to their home cages allowing 6 h of ad libitum sleep opportunity. This CSR protocol induces at least 93% wakefulness during the SR period and little fragmented sleep during the 12 s of wheel immobility [7].

2.3. Tissue collection

At ZT 0 (i.e., immediately after the end of the sleep deprivation), rats were anesthetized with isoflurane, decapitated, and brains were dissected as previously described [15]. Briefly, rat brains were placed on a frozen petri dish, and using a brain punch technique, 4 brain areas were collected: somatosensory cortex, frontal cortex, hippocampus and basal forebrain. The brain tissues were flash frozen in liquid nitrogen, and stored at −80 °C until further analysis.

Table 1
Primers and probes used for RT-PCR analysis.

Gene	Sequence	Bases
IL-1β		
Primer-f	CACCTCTCAAGCAGAGCACAG	21
Primer-r	GGGTTCCATGGTGAAGTCAAC	21
Probe-FAM/TAMRA	TGTCCCGACCATTGCTGTTTCCTAGG	26
TNF-α		
Primer-f	CCAGGTTCTCTTCAAGGGACAA	22
Primer-r	CTCCTGGTATGAAATGGCAAATC	23
Probe-FAM/TAMRA	CCCGACTATGTGCTCCTCACCCACA	25
BDNF		
Primer-f	CCATAAGGACGCGGACTTGTAC	22
Primer-r	GAGGAGGCTCCAAAGGCACTT	21
Probe-FAM/TAMRA	CTTCCCGGGTGATGCTCAGCAGT	23
GAPDH		
Primer-f	CAATGTGTCCGTCGTGGATCT	21
Primer-r	GTCCTCAGTGTAGCCCAAGATG	22
Probe-FAM/TAMRA	CGTGCCGCCTGGAGAAACCTGCC	23

2.4. Real-time polymerase chain reaction analysis (RT-PCR)

Brain tissues were homogenized and RNA extracted with Trizol reagent as previously described [16]. A TaqMan Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) was used to prepare cDNA from each RNA sample as described by the manufacturer. Primer Express Software (Applied Biosystems, Foster City, CA, USA) was used to choose the primers and probes for our genes of interest (IL-1β, TNF-α, BDNF) (Table 1). RT-PCR was used to analyze the mRNA levels of the genes of interest in the selected brain areas. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene to compare expression levels of mRNAs.

The delta threshold cycle value method was used to quantify the experimental treatment effects on gene expression as previously described [16,17]. Briefly, mean cycle threshold values for each rat undergoing baseline experimental treatments were computed for each gene of interest within each particular brain area separately. The change in cycle threshold values were evaluated by subtracting the mean GAPDH cycle threshold value from the baseline experimental treatment cycle threshold value and served as the baseline control mean measure. The gene expression of SR1, SR3, and SR5 experimental treatments was determined using the formula 2^−(change in cycle threshold for experimental treatment from the baseline control mean) − (change in cycle threshold for the baseline experimental treatment from the baseline control mean).

2.5. Statistical analysis

Two-way analysis of variance was used to determine differences in gene expression between the number of SR days and brain areas. Independent *t*-tests were used for post hoc analysis of gene expression. Data are presented as means ± SEM. Significance differences were set at $p < 0.05$.

3. Results

3.1. IL-1β

As shown in Fig. 1A, a main effect was found for the duration of SR enhancing IL-1β mRNA expression [$F(3,96) = 8.257$, $p < 0.001$], although this effect differed depending upon the brain area [number of days of SR x brain area interaction: $F(3,96) = 2.938$, $p = 0.037$].