Brain, Behavior, and Immunity xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Environmental disruption of the circadian clock leads to altered sleep and immune responses in mouse

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ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 26 November 2014
Accepted 5 December 2014
Available online xxxx

Keywords:
Allostatic load
Biological rhythms
Neuroimmune
Lipopolysaccharide

ABSTRACT

In mammals, one of the most salient outputs of the circadian (daily) clock is the timing of the sleep–wake cycle. Modern industrialized society has led to a fundamental breakdown in the relationship between our endogenous timekeeping systems and the solar day, disrupting normal circadian rhythms. We have argued that disrupted circadian rhythms could lead to changes in allostatic load, and the capacity of organisms to respond to other environmental challenges. In this set of studies, we apply a model of circadian disruption characterized in our lab in which mice are housed in a 20 h long day, with 10 h of light and 10 h of darkness. We explored the effects of this environmental disruption on sleep patterns, to establish if this model results in marked sleep deprivation. Given the interaction between circadian, sleep, and immune systems, we further probed if our model of circadian disruption also alters the innate immune response to peripheral bacterial endotoxin challenge. Our results demonstrate that this model of circadian disruption does not lead to marked sleep deprivation, but instead affects the timing and quality of sleep. We also show that while circadian disruption does not lead to basal changes in the immune markers we explored, the immune response is affected, both in the brain and the periphery. Together, our findings further strengthen the important role of the circadian timing system in sleep regulation and immune responses, and provide evidence that disrupting the circadian clock increases vulnerability to further environmental stressors, including immunological challenges.

1. Introduction

Circadian (daily) rhythms are oscillations in physiology and behavior that take approximately 24 h to complete (Moore-Ede et al., 1984). In humans, perhaps the most salient circadian rhythm is that of the sleep-wake cycle, but circadian oscillations occur at even the most basic cellular level, and are likely present in almost every cell in the body (Hastings et al., 2003). The master circadian clock is located in the suprachiasmatic nucleus (SCN) of the hypothalamus, a small (approximately 10,000 cells in each hemisphere) bilateral structure at the base of the anterior hypothalamus, with a remarkable phenotypic and functional heterogeneity (Antle and Silver, 2005). The SCN controls various other rhythms throughout the brain and body by synchronizing "peripheral" clocks through a variety of behavioral, humoral and other mechanisms (Buhr et al., 2010; Buijs and Kalsbeek, 2001; Buijs et al., 2003; Kalsbeek and Buijs, 2002; Kalsbeek et al., 2006). This wide-ranging control of all body rhythms by the SCN is remarkable, considering we currently do not know all of the mechanisms by which the SCN entrains these peripheral oscillators, nor do we understand the myriad pathways that can feedback to alter SCN function. This is a significant gap in our knowledge considering that disruption of the circadian clock is nearly ubiquitous in our modern society, through nighttime lighting, industrial schedules requiring shift work, changes in social norms (i.e. "social jet lag", (Roenneberg et al., 2012; Roenneberg and Merrow, 2005)) and jet lag caused by transmeridian air travel. The health costs of this disruption is now acknowledged by the American Medical Association (Stevens et al., 2013), and determining how circadian disruption alters multiple physiologic processes is key to understanding how we may be able to combat the effects of circadian disruption.

One of the most significant effects of circadian disruption, and perhaps the most obvious, is a disruption of the sleep–wake cycle. Two main processes regulate sleep: a circadian process and a homeostatic process (Borbely, 1982; Dijk and Czeisler, 1995). The circadian process is generated in the SCN and is entrained by the natural light/dark (LD) cycle. The homeostatic process tracks sleep need, and results in "sleep pressure" that must eventually be dissipated. Sleep loss and sleep misalignment are significant problems

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http://dx.doi.org/10.1016/j.bbi.2014.12.008
0889-1591/

in and of themselves, but in some instances studying these processes by using sleep deprivation models can lead to confounds such as physical or psychological stress. Since circadian rhythms in sleep are regulated by the SCN, we posit that using a non-invasive environmental circadian disruption could alter sleep–wake patterns without stress-like changes, thus providing a novel model to explore how environmental circadian disruption affects sleep.

Sleep and circadian disruption can affect many aspects physiology, including significant effects on immune function. For instance, even modest sleep restriction (6 h/night) can lead to elevated pro-inflammatory cytokine levels and deterioration in cognitive performance as measured by tests of psychomotor vigilance (Vgontzas et al., 2004). In addition, poor sleep and sleep deprivation in humans is associated with elevated plasma levels of IL-6 (Vgontzas et al., 1999, 2002). In non-human animal models, weekly phase-shifts (6 h/week for 4 weeks) result in altered immune responses to high-dose (12.5 mg/kg) lipopolysaccharide (LPS) challenge, and increased mortality (Castanon-Cervantes et al., 2010). Using a different model, of dim nighttime light exposure, similar effects have been demonstrated, showing that inappropriately timed light exposure can lead to immune dysregulation (Fonken et al., 2013a,b). Thus, evidence in human research as well as from some animal studies indicate there is a link between disrupted sleep and circadian clocks on immune function. The bidirectional relationship between the immune and sleep systems is also well known. Sleep processes are exquisitely sensitive to the effects of cytokines, while altered antigen meditated immune responses are sensitive to alterations in sleep and/or circadian rhythms (reviewed in: Scheiermann et al., 2013). Thus, the consequences of our modern industrialized society, with near constant electric lighting, shifting work schedules, and a breakdown of the linkage between the solar day and the endogenous circadian clock, are significant, and represent a growing health concern.

The purpose of this set of experiments was twofold. The first aim was to ascertain how disrupting the circadian clock by altered light–dark cycles (a shortened 20 h day, 10 h light, 10 h dark; LD10:10) affects sleep. We have previously characterized this model and demonstrated that it disrupts body temperature rhythms, results in metabolic dysregulation and weight gain, affects cognitive flexibility, and results in atrophy of pyramidal neurons in the medial prefrontal cortex (Karatsoreos et al., 2011), all without stress-like changes (no change in adrenal, spleen or thymus weight, nor elevated circulating glucocorticoids, unpublished observation). The second aim was to investigate how this model of chronic circadian disruption (CD) affects the innate immune response to LPS, both peripherally and in the hypothalamus and hippocampus. By using this approach we hoped to determine if our model of CD leads to reorganization of sleep or sleep deprivation, and if CD leads to changes in peripheral and/or central responses to an innate immune challenge.

2. Methods

2.1. Animals

For immune experiments, adult male C57 Bl6 mice (45–52 d old on arrival) were acquired from Charles River Laboratories, and for sleep experiments adult WT C57 Bl6 mice from our own breeding colony were used. In both cases, mice were group housed ($n = 3$–4) per cage with food and water available ad libitum in sound attenuated and ventilated isolation cabinets (Phenome Technologies, Chicago, IL). Throughout the experiments, light at cage level was maintained at ~200lux using white LEDs. Room temperature was maintained between 21–23 °C on a 12 h light:12 h dark (LD12:12) cycle for at least 1 week prior to experiment start. All experimental procedures were approved by the Washington State University Animal Care and Use Committee.

2.2. Surgical procedure

For circadian and sleep measurement (EEG and EMG) implants, mice ($n = 6$) were anesthetized using 5% isoflurane in oxygen, and then maintained at 2–2.5% during the surgical procedure. Body temperature and activity telemeters (VitalView/E-Mitter, Philips Respironics, Bend, OR) were implanted into the peritoneal cavity. Next, each mouse was prepared for electrophysiology measurements as previously described (Clegern et al., 2012). Briefly, the skull surface was exposed and four stainless steel screw electrodes (Antrin Miniature Specialties, Inc. Fallbrook, CA) were implanted, two over the frontal lobe (0.5 mm lateral to the midline, 1 mm anterior to bregma), and two over the parietal lobe (1.5 mm lateral to the midline, 1.5 mm anterior to lambda). Two additional screws were implanted in the parietal lobe to anchor the head stage. The two frontal electrodes were used to measure electroencephalogram (EEG), while the two parietal electrodes were used as a reference and ground. These electrodes were soldered to a printed circuit board (PCB) with a plastic 6-pin connector (Pinnacle Technology, Inc., Lawrence, KS). Two stainless steel wires (1.5 cm in length with ~2 mm insulating material removed at the terminating end) attached to the PCB were inserted into the neck muscle to record electromyogram (EMG). The electrodes and PCB board were enclosed with a light activated flowable composite resin (Prime-Dent). Mice were then allowed at least 1 week recovery following surgery before experimental manipulations.

2.3. Sleep and circadian measures

Following surgery, mice were single housed in either their home cage, or a circular 10-inch diameter sleep chamber (during sleep recording). Food (Purina LabDiet 5001) and water were available ad libitum. Home cages were placed on telemeter pads, and body temperature and activity were recorded in 5-min bins (VitalView/E-Mitter) for 7 d. Mice were then transferred to the sleep recording chambers where they were tethered through a commutator to the sleep recording system (Pinnacle Technology, Inc.) for a 5 d baseline sleep recording. Sleep data was acquired and archived using Sirenia Acquisition (Pinnacle Technology, Inc.) with a sample rate of 2 kHz, amplified 100×, and filtered with a high pass of 0.5 Hz and low pass of 120 Hz. During baseline experiments, mice were housed on a 12-h on, 12-h off light–dark cycle (LD12:12). Following baseline sleep, mice were returned to their home cages in the environmental boxes and the LD cycle was altered to a 10 h light:10 h dark cycle (LD10:10) to induce circadian disruption (CD), as we have previously published (Karatsoreos et al., 2011). Following 4 weeks of CD, mice transferred back into the sleep recording chambers for 5 d under LD10:10 to determine changes in sleep patterns due to circadian disruption.

2.4. Sleep scoring

Archived data was loaded into Sirenia Sleep Pro (Pinnacle Technology, INC. Lawrence, KS) for analysis. Data was parsed into 10-s epochs, and the power spectrum was calculated for EEG and EMG signals. Sleep scoring was done using a cluster cutting technique based on the density clusters of EEG delta and total EMG power (Rector et al., 2009). Briefly, scatter plots were generated with EEG delta power on the y-axis and total EMG power plotted on the x-axis. Clusters associated with low EEG delta power and high amplitude EMG were considered wake, high EEG delta power and low amplitude EMG were assigned to NREM sleep, and low EEG delta power and low amplitude EMG represented REM sleep. After

Please cite this article in press as: Phillips, D.J., et al. Environmental disruption of the circadian clock leads to altered sleep and immune responses in mouse. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.12.008