Brain, Behavior, and Immunity 38 (2014) 53–58

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Short Communication

Increased sleep fragmentation in experimental autoimmune encephalomyelitis

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ARTICLE INFO

Article history:
Received 6 January 2014
Received in revised form 9 February 2014
Accepted 9 February 2014
Available online 22 February 2014

Keywords:
Sleep efficiency
Sleep fragmentation
Sleep architecture
EAE

ABSTRACT

Sleep disturbance in patients with multiple sclerosis is prevalent and has multifactorial causes. In mice with experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis, we determined the dynamic changes of sleep architecture and the interactions between sleep changes and EAE symptoms. The changes of sleep patterns were mainly reflected by altered sleep stage distribution and increased sleep fragmentation. Increased waking and decreased non-rapid eye movement sleep occurred after EAE onset and persisted through the symptomatic phase. There also was increased sleep state transition, indicating a reduction of sleep cohesiveness. Furthermore, the extent of sleep fragmentation correlated with the severity of disease. This is the first study of sleep characteristics in EAE mice demarcating specific changes related to the autoimmune disorder without confounding factors such as psychosocial impact and treatment effects. The reduction of sleep efficiency and cohesiveness supports the notion that enhancing sleep might facilitate the recovery of mice from EAE, pertinent to the multimodality treatment of multiple sclerosis.

1. Introduction

Patients with multiple sclerosis (MS) frequently have multiple sleep complaints, such as insomnia, hypersomnolence, sleep apnea, parasomnia, and secondary narcolepsy. Sleep disorders occur in more than 50% of MS patients and contribute to fatigue. Poor sleep may be manifested by a decrease in total sleep time, rapid eye movement (REM) sleep, or sleep efficiency. This is associated with an increase in Stage 1 non-REM (NREM) sleep, as well as changes in sleep stage and arousals (Lunde et al., 2012, 2013; Neau et al., 2012). Sleep disturbance is influenced by MS-related symptoms and adverse effects from immunotherapy and symptomatic medications (Pokryszko-Dragan et al., 2013). Therefore, experimental autoimmune encephalomyelitis (EAE), a widely used animal model of MS, provides a useful tool to determine changes in sleep architecture in the absence of the psychosocial interactions and treatment effects seen in human beings.

In animal studies, cytokines and other inflammatory challenges have been associated with hypersomnolence as well as increased sleep fragmentation (Krueger et al., 2007; Kaushal et al., 2012). However, altered sleep patterns in EAE have not been characterized. We hypothesize that the development of EAE is associated with disrupted sleep, shown by decreased sleep efficiency and increased sleep fragmentation. This was tested by simultaneous monitoring of disease severity and sleep–wake activities. The results provide a basis for further studies to determine whether sleep treatment helps resolve EAE and the human disease MS.

2. Materials and methods

2.1. Mice and surgery

The animal studies were approved by the Institutional Animal Care and Use Committee. Female FVB mice (Jackson Laboratory, Bar Harbor, ME) were group-housed, lights on at 6 am (Zeitgeber time ZT = 0) and lights off at 6 pm (ZT12), and fed *ad lib*. Headmounts for sleep recordings were placed on mice 7–8 weeks old as described previously (Wang et al., 2013). Mice were allowed to recover for 2 weeks before baseline sleep recording and subsequent EAE induction.

2.2. EAE induction and scoring

Two groups of mice were studied: EAE or vehicle control (n = 9–10/group). EAE was induced in 9–10 week old mice on day 0 following an established protocol (Li et al., 2011; Mishra et al.,

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http://dx.doi.org/10.1016/j.bbi.2014.02.005
0889-1591/

2013). Each mouse received subcutaneous injection of 100 μg of myelin oligodendrocyte glycoprotein (MOG) fragment 79–96. MOG_{79-96} was emulsified in 100 μl of complete Freund's adjuvant (CFA) containing 500 μg of heat-killed Mycobacterium tuberculosis H37RA (DIFCO Laboratories, Detroit, MI). The injection was performed at 3–5 pm (ZT 9–11 h). Pertussis toxin (PT, Sigma, St. Louis, MO) was injected intraperitoneally immediately after induction and again 48 h later. The mice in the vehicle control group underwent the same procedure except that they received CFA + PT only without MOG_{79-96}. Symptoms were monitored at ZT 5–6 h daily by use of a standard EAE scoring system (Pan et al., 1996; Wu et al., 2010, 2013; Mishra et al., 2013), with 0 being symptom-free and 5 being the worst (moribund or dead).

2.3. Sleep recording and scoring

Mice with headmounts were individually housed in cylindrical sleep recoding cages, and acclimatized for 3 days before the initiation of recording by connection of the headmount to a preamplifier. Data acquisition and processing were achieved by use of the Pinnacle Serenia System (Pinnacle Technology, Lawrence, KS), as described previously (Wang et al., 2013). Sleep data were acquired for at least 24 h continuously from ZT0, first at baseline before EAE induction, and then again at days 7, 14, 21, and 28 after EAE induction. Manual scoring was performed on 10 s epochs of EEG (0.5–40 Hz) and EMG (10–100 Hz) signals by an experienced sleep researcher, following standard scoring criteria (Veasey et al., 2000; Louis et al., 2004). Hypnograms, the percentage of each sleep stage (wake, NREM, or REM), and sleep bouts were analyzed with Sleep Pro software (Pinnacle Technology). A bout was defined as a minimum of 3 consecutive epochs at a length of 10 s/epoch for a given state.

2.4. Statistical analysis

Repeated measures two-way analysis of variance was used to determine the changes of EAE scores over time and the difference between EAE and CFA + PT control groups. Randomized block analysis was performed to determine the effects of EAE and time after induction on sleep architecture, followed by the Bonferroni post hoc test when significant overall changes were identified. Linear regression was used to analyze the correlation between EAE score and sleep fragmentation. Prism GraphPad 5 statistical and graphic program (GraphPad, San Diego, CA) was used to conduct statistical analyses and generate graphs.

3. Results

3.1. EAE mice have decreased sleep efficiency after the onset of disease

Both EAE induction and disease duration affected the EAE scores significantly. EAE symptoms occurred around day 12 and persisted throughout the course of the study. There was an initial peak at day 13–14, followed by incomplete recovery, and further worsening of the ascending weakness that reached a plateau on days 25–34 (Fig. 1E and F, inset).

The total amount as well as the percentage of wake time during 24 h, representative of sleep efficiency, showed significant changes as a result of EAE and time after induction as shown by two-way ANOVA. During the 24 h baseline recording, there was no difference between the EAE and control groups in the % wake. In the subsequent month, the CFA + PT control mice ($n = 9$) did not show a significant increase of % wake when sampled weekly. The EAE group ($n = 10$), however, showed an increase of % wake at day 14, 21, and 28 after induction. The difference between the two groups was most apparent during the light phase; there was an effect of EAE [$F(1,59) = 14.1$, $p < 0.0005$] as well as time [$F(4,59) = 4.4$, $p < 0.005$], and there was also a significant interaction [$F(4,59) = 3.2$, $p < 0.05$]. Post-hoc analysis confirmed significant increases on day 14, 21, and 28 after induction of EAE, though the CFA + PT group did not show changes (Fig. 1A). In the dark phase, the % wake only showed a significant overall effect of time ($p < 0.05$). There was no compensatory recovery sleep that would be reflected by decreased wake percentage (Fig. 1B).

The %NREM was also similar at baseline and a week after EAE induction in comparison with the CFA + PT group. While the CFA + PT mice did not show subsequent reduction of NREM sleep, the EAE mice had a significant decrease of %NREM on day 14 ($p < 0.05$), day 21 ($p < 0.005$), and day 28 ($p < 0.01$) in comparison with their own baseline. The decrease was most pronounced during the light phase (Fig. 1C) but not the dark phase (Fig. 1D). By contrast, the %REM did not show significant change, possibly related to large intra-group variation, in either the light phase (Fig. 1E) or dark phase (Fig. 1F).

3.2. EAE is associated with increased sleep fragmentation, the extent of which correlates with symptom severity

The number of wake bouts reflects sleep state transition as well as prolonged arousal. It did not show a significant change in the CFA + PT control group. The EAE group had a similar baseline and day 7 waking bout counts as the control group, but there was an increase on day 14 ($p < 0.01$), day 21 ($p < 0.05$), and day 28 ($p < 0.01$) when compared with its own baseline before EAE induction. There was also an increase on day 14 ($p < 0.05$) and day 28 ($p < 0.01$) when the EAE group was compared with the same-date CFA + PT controls. When the 24 h interval was analyzed separately in the light and dark phases, wake bouts showed significant changes as a result of EAE and time after induction in the light phase, without significant interactions of the two factors or post hoc difference overtime (Fig. 2A). In the dark phase, there was a significant overall effect of EAE (Fig. 2B). Representative hypnograms of an EAE mouse at baseline, day 14, day 21, and day 28 are shown in Fig. 2C.

Whereas CFA + PT alone did not change NREM bouts, EAE mice had more NREM bouts on day 14 and day 28 ($p < 0.01$) when compared with either baseline or the CFA + PT controls on the same date after induction. NREM bout counts were not different on day 21, a time point corresponding to partial resolution of EAE scores that is shown in the insets of Fig. 1E and F. The significant overall effect of EAE to increase NREM bouts was present in both the light phase (Fig. 2D) and dark phase (Fig. 2E).

The frequency of sleep state transition correlated with the severity of EAE. When the number of wake/sleep bouts was plotted against EAE score at 14, 21, and 28 days, there was a linear correlation between EAE score and wake bouts ($p < 0.01$), as well as between EAE score or NREM bouts ($p < 0.01$). Increased severity of EAE (higher scores) coincided with an increased number of wake bouts and sleep bouts (Fig. 2F).

4. Discussion

We found that sleep disturbance occurred after the onset of EAE and mainly presented with decreased sleep efficiency and increased sleep fragmentation. The extent of sleep fragmentation was positively correlated with the severity of the disease. This is the first study involving sleep and EAE of which we are aware. It addresses the biologically important question how a CNS autoimmune disorder induces dynamic changes of sleep quality. The findings differ from previous reports on sleep architecture in rodents