Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Impact of traumatic brain injury on sleep structure, electrocorticographic activity and transcriptome in mice

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ARTICLE INFO

Article history:
Received 31 October 2014
Received in revised form 22 December 2014
Accepted 22 December 2014
Available online xxxx

Keywords:
Closed-head injury
Sleep architecture
Recovery sleep
EEG spectral analysis
Transcriptome
Cerebral cortex
Hippocampus
Plasticity genes
Clock genes
Chemokines

ABSTRACT

Traumatic brain injury (TBI), including mild TBI (mTBI), is importantly associated with vigilance and sleep complaints. Because sleep is required for learning, plasticity and recovery, we here evaluated the bidirectional relationship between mTBI and sleep with two specific objectives: (1) Test that mTBI rapidly impairs sleep–wake architecture and the dynamics of the electrophysiological marker of sleep homeostasis (i.e., non-rapid eye movement sleep delta (1–4 Hz) activity); (2) evaluate the impact of sleep loss following mTBI on the expression of plasticity markers that have been linked to sleep homeostasis and on genome-wide gene expression. A closed-head injury model was used to perform a 48 h electrocorticographic (ECoG) recording in mice submitted to mTBI or Sham surgery. mTBI was found to immediately decrease the capacity to sustain long bouts of wakefulness as well as the amplitude of the time course of ECoG delta activity during wakefulness. Significant changes in ECoG spectral activity during wakefulness, non-rapid eye movement and rapid eye movement sleep were observed mainly on the second recorded day. A second experiment was performed to measure gene expression in the cerebral cortex and hippocampus after a mTBI followed either by two consecutive days of 6 h sleep deprivation (SD) or of undisturbed behavior (quantitative PCR and next-generation sequencing). mTBI modified the expression of genes involved in immunity, inflammation and glial function (e.g., chemokines, glial markers) and SD changed that of genes linked to circadian rhythms, synaptic activity/neuronal plasticity, neuroprotection and cell death and survival. SD appeared to affect gene expression in the cerebral cortex more importantly after mTBI than Sham surgery including that of the astrocytic marker *Gfap*, which was proposed as a marker of clinical outcome after TBI. Interestingly, SD impacted the hippocampal expression of the plasticity elements *Arc* and *EfnA3* only after mTBI. Overall, our findings reveal alterations in spectral signature across all vigilance states in the first days after mTBI, and show that sleep loss post-mTBI reprograms the transcriptome in a brain area-specific manner and in a way that could be deleterious to brain recovery.

1. Introduction

Traumatic brain injury (TBI) is a public health concern that causes important short- and long-term physical, cognitive and neurobehavioral impairments (McAllister, 2011). Up to 70% of TBI survivors suffer from sleep disturbances, including insomnia, fatigue and somnolence (Cohen et al., 1992; Duclos et al., 2014; Orff et al., 2009), which are among the most common and persistent symptoms after both moderate/severe and mild TBI (mTBI) (Ayalon et al., 2007; Mahmood et al., 2004; Pillar et al., 2003). Sleep alterations have been found to be associated with several comorbidities and with impaired quality of life (Chaput et al., 2009; Fichtenberg et al., 2000; Hou et al., 2013; Ouellet et al., 2004; Schiehser et al., 2014). Importantly, these disturbances could

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http://dx.doi.org/10.1016/j.bbi.2014.12.023
0889-1591/

interfere with brain recovery (Meerlo et al., 2009; Ouellet et al., 2004), which likely originates from the key roles of sleep in brain plasticity, learning and memory consolidation (Diekelmann and Born, 2010; Ohlmann and O'Sullivan, 2009).

Animal models are required to unravel the pathophysiology of sleep disturbances after mTBI and to understand the role of sleep in brain recovery in this context. An initial step is to describe how these disturbances develop. Despite the abundant literature on sleep–wake disturbances weeks to months following TBI of all severities in humans, less is known about sleep characteristics in the immediate days following injury, even when it comes to studies in rodents. Recent studies using a piezoelectric cage system showed that brain-injured mice had an increased percentage of rest and an increased duration of rest bouts in the first few hours or the first week after a mild or moderate TBI (Rowe et al., 2013, 2014). Another group showed a decreased ability to maintain prolonged wakefulness during the active (dark) period in the first three days after a moderate-severe TBI using EEG recording in mice, which was indexed by more bouts of wakefulness of shorter duration (Willie et al., 2012). Similar findings were reported one week post-mTBI (Lim et al., 2013). Importantly, this last study also reported alterations in the mean EEG power spectra measured during wakefulness, non-rapid eye movement sleep (NREMS) as well as rapid eye movement sleep (REMS) one week post-mTBI (Lim et al., 2013). However, to our knowledge, the progression of electroencephalographic (EEG) activity during vigilance states remains unexplored in the first days after mTBI. This includes the dynamics of EEG delta activity (1–4 Hz) during NREMS, which index a sleep homeostatic process (Borbély, 1982) thought to underlie the recovery function of sleep.

Alterations in neuronal network connectivity and the inflammatory response are among potential mechanisms responsible for sleep–wake disturbances after mTBI. Indeed, imaging and histology studies presented evidence of subtle brain injuries in mTBI such as diffuse axonal injuries (Bazarian et al., 2007; Blumbergs et al., 1995; Browne et al., 2011; Inglese et al., 2005). Also, extensive axonal and dendritic degeneration and decreased synaptic density were reported after mTBI (Gao and Chen, 2011). Given that sleep depends on the activity of neuronal networks (Krueger et al., 2008), these alterations could contribute to sleep alterations post-mTBI. In parallel, secondary injury processes are immediately activated following brain injury. Glial cells release inflammatory mediators, such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNFα) (Bachstetter et al., 2013; Helmy et al., 2011; Rowe et al., 2013). These cytokines have specifically been shown to promote sleep and to alter the sleep EEG (Krueger et al., 2011).

Changes in the expression of genes involved in neuronal plasticity and regrowth after mTBI may also directly modulate sleep structure and EEG. Neuronal growth and survival is associated with high expression of neurotrophic factors and plasticity genes (Huang and Reichardt, 2001). Research has shown that some of these elements may guide regrowth of damaged neurons in developed organisms (Deister and Schmidt, 2006). One such element is brain-derived neurotrophic factor (BDNF) that seems to profit brain recovery after TBI (McAllister et al., 2012; Rostami et al., 2011). The expression of plasticity genes such as *Bdnf, Homer1a* and *Fos* or of their protein product is increased in the hippocampus and cortex during the first hours after mTBI (Abrous et al., 1999; Colak et al., 2012; Crack et al., 2009; Hicks et al., 1999). On the other hand, the expression of several of these plasticity elements is decreased a few days after injury (Colak et al., 2012; Hou et al., 2012; Wu et al., 2010). Importantly, most of these plasticity markers were proposed to have a role in sleep regulation and were shown to respond to sleep deprivation (SD) (El Helou et al., 2013; Huber et al., 2007; Maret et al., 2007; Mongrain et al., 2010). In addition to a probable implication of changes in plasticity genes in sleep-wake disturbances post-TBI, this points to an impact of sleep loss on brain recovery after TBI.

In the present study, we used a closed-head injury model of mTBI to test the hypothesis that mTBI disturbs sleep–wake architecture and the dynamics of low frequency EEG activity in the first two days following injury. In parallel, we assessed if sleep loss is detrimental to brain gene expression following mTBI by measuring the effect of two consecutive days of enforced wakefulness post-mTBI on several plasticity markers, with focus on those linked to sleep homeostasis, and on the transcriptome in both the cerebral cortex and the hippocampus. The cerebral cortex, a direct target of the injury, was chosen because of its predominant role in the generation of delta activity (Amzica and Steriade, 2000). The hippocampus was targeted because of its important plasticity properties (Meerlo et al., 2009) and its vulnerability to TBI (Kernie and Parent, 2010). Closed-head injury was performed since it does not involve a craniotomy or a craniectomy allowing for both a more stable electrocorticographic (ECoG) recording montage and for a closer parallel to injuries observed in humans. Using this model, our results support that mTBI acutely alters the capacity to sustain wakefulness and reveal that it impacts ECoG activity in all vigilance states as well as the dynamics of slow ECoG activity during wakefulness. Moreover, our findings show that sleep loss following mTBI affects the brain transcriptome in a manner that depends on the targeted areas and that likely modulates brain recovery after injury.

2. Material and methods

2.1. Animals and protocols

Male C57BL/6 J mice purchased from Jackson Laboratories were used for two different experiments ($n = 51$). Animals were housed in individual cages in a 12 h light/12 h dark cycle at a temperature between 23 and 25 °C with food and water available *ad libitum*, and were acclimated to these conditions for at least 6 weeks before experiments. Mice were studied between 10 and 21 weeks of age, when their weight was around 30 g to reduce mortality due to TBI (see Flierl et al., 2009; Stahel et al., 2009). Mice were either subjected to a mTBI or a control Sham surgery as detailed below, which was preceded by 5 days of animal handling (5 min/day) to decrease stress due to manipulation by experimenters. All experimental procedures were approved by the Ethical Committee for Animal Experimentation of the Research Center of the Hôpital du Sacré-Coeur de Montréal in accordance with Guidelines from the Canadian Council on Animal Care.

2.1.1. Experiment 1 (ECoG measurement, Fig. 1A)

Fourteen mice (19 ± 0.5 weeks, 29.7 ± 1.0 g) were subjected to mTBI or Sham surgery ($n = 7$ per group, matched for weight) in the late afternoon (between ZT8 and ZT11 with ZT0 referring to Zeitgeber time 0, the onset of the light period, and ZT12 to the onset of the dark period). Surgeries were performed at this time, the latest possible in the light period, to ensure the shortest interval between surgery and the start of the recording, and thus to capture the earliest effect of mTBI. During that same surgical procedure, implantation of electrodes for ECoG and electromyographic (EMG) recording was performed as detailed in Section 2.3. Mice were cabled the next morning before light onset and recordings started at light onset (ZT0) for both mTBI and Sham animals, thus 13 to 16 h post-mTBI. ECoG/EMG was continuously recorded for 48 h (Day 1 and Day 2; Fig. 1A).

2.1.2. Experiment 2 (gene expression measurement, Fig. 1B)

Thirty-seven mice (14 ± 0.2 weeks, 27.2 ± 0.4 g) were used and separated into two surgical conditions (mTBI vs. Sham) and two

Please cite this article in press as: Sabir, M., et al. Impact of traumatic brain injury on sleep structure, electrocorticographic activity and transcriptome in mice. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2014.12.023