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Diffuse brain injury does not affect chronic sleep patterns in the mouse

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Abstract

Primary objective: To test if the current model of diffuse brain injury produces chronic sleep disturbances similar to those reported by TBI patients.

Methods and procedures: Adult male C57BL/6 mice were subjected to moderate midline fluid percussion injury ($n = 7$; 1.4 atm; 6–10 minutes righting reflex time) or sham injury ($n = 5$). Sleep–wake activity was measured post-injury using a non-invasive, piezoelectric cage system. Chronic sleep patterns were analysed weekly for increases or decreases in percentage sleep (hypersomnia or insomnia) and changes in bout length (fragmentation).

Main outcomes and results: During the first week after diffuse TBI, brain-injured mice exhibited increased mean percentage sleep and mean bout length compared to sham-injured mice. Further analysis indicated the increase in mean percentage sleep occurred during the dark cycle. Injury-induced changes in sleep, however, did not extend beyond the first week post-injury and were not present in weeks 2–5 post-injury.

Conclusions: Previously, it has been shown that the midline fluid percussion model used in this study immediately increased post-traumatic sleep. The current study extended the timeline of investigation to show that sleep disturbances extended into the first week post-injury, but did not develop into chronic sleep disturbances. However, the clinical prevalence of TBI-related sleep–wake disturbances warrants further experimental investigation.

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Keywords

Chronic; concussion; diffuse; mouse; sleep; TBI

Introduction

Sleep disturbances are commonly reported neurological impairments in the acute phase of traumatic brain injury (TBI), some of which persist through more chronic periods [1–3]. Pathological processes initiated at the time of injury develop into neurological impairments, with chronic sleep disturbances among the somatic, cognitive and emotional neurological impairments [1, 2]. According to the literature, an incidence as high as 70% of TBI survivors suffer from sleep–wake disturbances [4, 5]. Similar sleep disorders develop across the spectrum of TBI, including children and adolescents [6]. The high prevalence of sleep disorders and impact on quality-of-life reported in both the adult and paediatric population of TBI survivors warrants investigation of this injury-induced neurological impairment.

Excessive daytime sleepiness is a common sleep–wake disturbance reported among TBI patients [1, 3, 7] and is characterized primarily by an increase in sleep propensity. Post-traumatic hypersomnia, an increased need in sleep over a 24-hour period, is reported equally frequently following TBI [7, 8]. These disturbances of increased sleepiness and fatigue can remain several years after injury, becoming chronic impairments [6, 8, 9] and overall lowering the quality-of-life of TBI patients. Injury-induced sleep disturbances also potentially affect the course of recovery [6, 10] and hinder rehabilitation [11]. Chronic sleep disturbances not only compromise recovery, but can intensify co-morbidities including anxiety, depression, cognitive deficits and pain [11–14]. For these reasons, exploring animal models that recapitulate aspects of injury-induced sleep problems is timely.

With compromised sleep affecting patient outcome and quality-of-life, understanding the role of sleep in recovery from brain injury is an important health concern. Focusing on pre-clinical experimentation can potentially accelerate the understanding of the relationship between brain injury and sleep. Experimental models of TBI can be used to evaluate secondary injury mechanisms underlying the pathophysiology of the injury and may be a useful model to investigate chronic post-traumatic sleep. The lab has recently shown diffuse brain injury in mice increases sleep during the first 6 hours post-injury [15]. This study investigates whether the same experimental model shows chronic sleep disturbances associated with TBI. This study examines the relationship between TBI and chronic sleep using non-invasive piezoelectric sleep cages to measure sleep for 5 weeks after midline fluid percussion injury (mFPI) [16]. It is hypothesized that this model of diffuse brain injury will produce chronic sleep deficits relevant to those reported by TBI patients, thereby permitting further exploration of the role of sleep in recovery from brain injury.

Methods

Animals

Male C57BL/6 mice (Harlan Laboratories, Inc., Indianapolis, IN) were used for all experiments (n = 12). The animals were housed in a 12 hour light/12 hour dark cycle at a constant temperature (23 °C±2°C) with food and water available *ad libitum* according to the Association for Assessment and Accreditation of Laboratory Animal Care International. Animals were acclimated to their environment following shipment for at least 3 days prior to any experiments. After surgery, daily post-operative care was provided, including physical examination and documentation of each animal's condition. Animal use was approved by the Institutional Animal Care and Use Committee at St. Joseph's Hospital and Medical Center (Phoenix, AZ). All animals used in this study were singly housed in the non-invasive sleep-monitoring cage system (Signal Solutions, Lexington, KY).

Midline fluid percussion injury (mFPI)

Mice (20–24 grams) were subjected to midline fluid percussion injury (mFPI) consistent with methods previously described [17]. Briefly, mice were anaesthetized for 5 minutes using 5% isoflurane in 100% oxygen. The head of the animal was then placed in a stereotaxic frame and isoflurane anaesthesia was maintained at 2.5% via nosecone. A 3 millimetre craniotomy was centred on the sagittal suture midway between bregma and lambda without disruption of the underlying dura. An injury cap prepared from the female portion of a Luer-Loc needle hub was fixed over the craniotomy using cyanoacrylate gel and methyl-methacrylate (Hygenic Corp., Akron, OH) and mice were placed in a heated recovery cage until ambulatory before being returned to their sleep cages. For injury induction 24 hours postsurgery, animals were re-anaesthetized with 5% isoflurane for 5 minutes. The dura was visually inspected through the hub to confirm it was intact with no debris. The hub was then filled with normal saline and attached to a tube connected to the male end of the fluid percussion device (Custom Design and Fabrication, Virginia Commonwealth University, Richmond, VA). An injury of moderate severity (1.4 atm) was administered by releasing the pendulum onto the fluid-filled cylinder. Sham-injured animals underwent the same procedure except the pendulum was not released. Animals were monitored for the presence of a forearm fencing response and righting reflex times were recorded for the injured animals as indicators of injury severity [18]. The injury hub was removed and the brain was inspected for uniform herniation and integrity of the dura. Moderate brain-injured animals had righting reflex recovery times greater than 6 minutes and a positive fencing response. Sham injured animals recovered from anaesthesia within 20 seconds. After spontaneously righting, animals were placed in a heated recovery cage and monitored until ambulatory (~5–15 minutes) before being returned to their sleep cages.

Sleep recordings

The non-invasive sleep cage system (Signal Solutions, Lexington, KY) used in this study consisted of 16 separate units which simultaneously monitor sleep and wake states, as previously published [15, 19]. Briefly, sleep was characterized primarily by periodic (3 Hz) and regular amplitude signals recorded from the PVDF sensors, typical of respiration from a sleeping mouse. In contrast, signals characteristic of wake were both the absence of

characteristic sleep signals and higher amplitude, irregular spiking associated with volitional movements. The piezoelectric signals in 2-second epochs were classified by a linear discriminant classifier algorithm based on frequency and amplitude to assign a binary label of ‘sleep’ or ‘wake’ [20]. Mice sleep in a polycyclic manner (more than 40 sleep episodes per hour) [21] and so mouse sleep was quantified as the minutes spent sleeping per hour, presented as a percentage for each hour. Data collected from the cage system were binned over specified time periods (e.g. 1 hour) using the average of percentage sleep, as well as binned by length of individual bouts of sleep and the median bout lengths were calculated.

Sleep data were collected continuously for 5 weeks and organized into week-long intervals for analysis. Daily percentage sleep was calculated by averaging the percentage sleep at each of 24 hours for all days of a given week post-injury.

Statistical analysis

Data are shown as mean ± SEM and analysed using statistical software (GraphPad-Prism 6). Differences in mean percentage sleep and mean bout length were determined with a repeated measures two-way analysis of variance (ANOVA) followed by Sidak’s multiple comparison test. Statistical significance was assigned when $p < 0.05$.

Results

Diffuse TBI impacted percentage sleep and mean bout length during the first week post-injury

Significant increases in mean percentage sleep have been previously reported in brain-injured mice compared to uninjured shams over the first 6 hours post-injury [15]. The present study repeated this observation and brain-injured mice slept significantly more over the first 6 hours compared to uninjured shams ($F(1,10) = 7.209$, $p = 0.0229$; sham $n = 5$, injury $n = 7$; data not shown). Overall, brain-injured mice slept significantly more than sham during the first week post-injury ($F(1,10) = 17.61$, $p = 0.0018$; sham $n = 5$, injury $n = 7$; Figure 1a). To investigate ‘excessive daytime sleepiness’, sleep propensity was evaluated during the dark phase. Mice are nocturnal, sleeping more during the day with prolonged wakefulness at night and, therefore, the mouse equivalent of ‘excessive daytime sleepiness’ in humans would most likely occur at night. Mean percentage sleep was evaluated over the dark cycle (the time the lights went off at night until they turned on again the following morning). The data indicate a significant increase in mean percentage sleep in brain-injured mice compared to uninjured shams ($F(1, 10) = 11.29$, $p = 0.0072$; sham $n = 5$, injury $n = 7$; Figure 1b) during the dark cycle. Brain-injured mice had the typical initial bout of high wakefulness at dark onset (low percentage sleep), but did not sustain this wakefulness to the same degree as uninjured mice (Figures 1a and b). By 01:00 in the dark cycle, brain-injured mice were sleeping significantly more than uninjured sham mice. Further, mean bout length was significantly increased in brain-injured mice compared to uninjured shams during the first week post-injury ($F(1, 10) = 5.186$, $p = 0.0460$; sham $n = 5$, injury $n = 7$; Figure 1c), suggesting that the increase in mean percentage sleep observed was due to mice sleeping for longer durations each bout, as opposed to sleeping more bouts, during the first week post-injury (Figures 1a and c).

Diffuse TBI did not induce sleep disturbances between weeks 2 and 5 post-injury

Sleep recordings were extended beyond week 1 for the same mice to investigate chronic sleep patterns post-injury. Identical percentage sleep, dark cycle sleep and mean bout length analyses were also conducted for weeks 2–5 post-injury. No significant injury-dependent effect on daily percentage sleep was detected in brain-injured mice ($n = 7$) compared to sham mice ($n = 5$) during post-injury week 2 ($F(1, 10) = 2.206$, $p = 0.1683$; Figure 2a), week 3 ($F(1, 10) = 0.4557$, $p = 0.5150$; Figure 2b), week 4 ($F(1, 10) = 0.7659$, $p = 0.4020$; Figure 2c) or week 5 ($F(1, 10) = 0.1282$, $p = 0.7277$; Figure 2d).

Further analysis of mean percentage sleep focused on sleep only during the dark cycle, placing emphasis on the nocturnality of mice. No injury-dependent effect during the dark cycle on percentage sleep was detected in brain-injured mice compared to sham mice during post-injury week 2 ($F(1, 10) = 0.074\ 26$, $p = 0.7908$; Figure 3a), week 3 ($F(1, 10) = 0.2760$, $p = 0.6108$; Figure 3b), week 4 ($F(1, 10) = 0.018\ 92$, $p = 0.8933$; Figure 3c) or week 5 ($F(1, 10) = 0.4322$, $p = 0.8395$; Figure 3d).

No significant injury-dependent effect on daily mean bout length was detected in brain-injured mice compared to sham mice during post-injury week 2 ($F(1, 10) = 0.3694$, $p = 0.5569$; Figure 4a), week 4 ($F(1, 10) = 0.8686$, $p = 0.3733$; Figure 4c) or week 5 ($F(1, 10) = 0.2344$, $p = 0.6387$; Figure 4d). During week 3, brain-injured mice slept significantly longer average bouts than shams ($F(1, 10) = 8.437$, $p = 0.0157$; Figure 4b), without specific post-hoc differences at particular hours. Overall, brain-injury did not result in consistent chronic changes in mean percentage sleep or mean bout lengths of sleep.

Discussion

Sleep research associated with TBI has focused on sleep disturbances in human injury [3, 4, 7]. These chronic disturbances are present in both paediatric and adult cases of TBI extending up to 3 years after injury [2, 22]. Commonly reported sleep–wake disturbances after TBI include excessive daytime sleepiness, fatigue, hypersomnia and insomnia [2, 3, 7, 23]. The high prevalence and subjective nature of clinically reported chronic sleep disturbances warrant the investigation of chronic sleep following experimental TBI. Previous studies showed a significant increase in percentage sleep of brain-injured mice compared to uninjured shams over the first 6 hours following diffuse TBI [15], termed post-traumatic sleep. Injury-induced changes in sleep patterns were limited to the first week after diffuse brain injury in the mouse, not extending into chronic time points. Only in the first week post-injury did the total percentage sleep, sleep during the dark cycle and sleep bout length increase in brain-injured compared to uninjured sham mice.

Excessive daytime sleepiness and post-traumatic hypersomnia, characterized primarily by an increase in sleep propensity, have been reported to be among the most common sleep–wake disturbances following TBI [7]. Hypersomnia, an increased need for sleep over a 24-hour period, differs from excessive daytime sleepiness in which the increased need for sleep is exclusively in the daytime [7]. In the current study, mean percentage sleep was evaluated to determine if experimental brain injury resulted in hypersomnia. After diffuse brain injury, an increase in overall mean percentage of sleep, hypersomnia, was observed only in the first

week post-injury in brain-injured mice compared to uninjured shams. This increase did not extend into the chronic period 2–5 weeks post-injury. To investigate excessive daytime sleepiness, sleep propensity was analysed during the dark phase, as mice typically have prolonged wakefulness at night. Brain-injured mice slept significantly more during the dark phase compared to uninjured shams only within the first week post-injury. Taken together, injury-induced increases in sleep are restricted to the first week post-injury. The first week becomes a critical window for future investigations of brain-injury induced sleep. Sleep may contribute to the natural recovery process following injury, which may be most salient in the first week post-injury. Further investigation of the increase in sleep observed over the first week may continue to inform clinical decisions and improve treatment of TBI survivors.

In the current study, mean bout length was analysed as an indicator of sleep fragmentation. Sleep fragmentation, an increase in awakenings during sleep, leads to excessive daytime sleepiness and can cause changes in daytime function similar to those found following sleep deprivation [24, 25]. Interruptions in sleep may prevent the benefit of the period of sleep prior to the arousal [24, 26, 27]. Short sleep bout lengths are indicative of arousals during the sleep cycle and could potentially confound the reparative processes following brain injury. In this study, sleep bout lengths in diffuse brain-injured mice were significantly longer than sham mice during the first week post-injury, but not beyond. Hence, brain-injured mice had longer periods of uninterrupted sleep compared to uninjured shams; whether this is beneficial to outcome remains unknown. After controlled cortical impact in mice, electroencephalography (EEG) recordings showed in the first 3 days post-injury, brain-injured mice exhibit reduced ability to maintain prolonged wakefulness [28]. Together, these data indicate experimental TBI increases sleep bout length and decreases prolonged wakefulness, which could contribute to recovery from brain injury. It is possible sleep bout length is only increased during the first week post-injury, because the bulk of cellular recovery occurs during this period; an increase in sleep beyond this time point may not be necessary. Clinically, these data suggest that allowing patients to sleep uninterrupted during the first week post-injury may potentially improve long-term outcome.

The exact pathophysiology of post-traumatic sleep–wake disturbances remains elusive. Excessive daytime sleepiness has been correlated to the injury itself, concomitant with other pathophysiology associated with TBI, including damage to the hippocampus [7, 29]. A prospective patient study showed as many as 43% of patients have sleep wake disturbances directly related to the injury itself [29]. The secondary injury processes which affect sleep following diffuse TBI likely include processes such as ATP depletion, an increased reactive oxygen species (ROS), higher intracellular concentrations of free radicals and elevated inflammation mediating cytokines [30, 31]. Impaired signalling of sleep–wake modulating systems such as the hypocretin (orexin) neuropeptide system may also contribute to both acute and chronic sleepiness following TBI [28, 29, 32]. Future studies can focus on the relationship between these signalling processes and sleep in the first week post-injury.

The reparative function of sleep is associated with increased brain ATP levels [33, 34]. Blocking ATP depletes energy and increases sleep [35], suggesting that increases in sleep following TBI may result from depletions in ATP. Decreases in ATP alter cellular function contributing to cell death, as demonstrated in experimental TBI [36–39]. Fluid percussion

injury decreases ATP levels in both the cortex and hippocampus of rats starting as early as 2 hours post-injury with declines remaining up to 24 hours post-injury [36, 38]. Similarly, an impact acceleration model of TBI decreases ATP levels in rats 2 hours following a moderate injury and as early as 10 minutes following a severe injury [39]. If a decrease in ATP is an immediate response to brain injury, post-traumatic sleep may increase brain ATP levels. Similar secondary injury mechanisms after brain injury increase oxidative stress through enhanced production of ROS, reactive radicals and lipid peroxidation [40], which could increase sleep. Sleep subsequently could remove accumulated free radicals [41, 42].

In diffuse brain injury, cortical levels of interleukin 1-beta (IL-1β) immediately increased and then normalized by 12 hours post-injury through 1 week [15]. In controlled cortical impact in the rat, interleukins (IL-4, IL-5, IL-13) and tumour necrosis factor alpha (TNF-α) levels increased acutely and recovered to baseline levels by 3 days post-injury [43]. A temporal profile of inflammatory cytokines following human TBI confirms the translational relevance of these findings with peak levels of IL-1β, IL-6 and TNF occurring within the first 3 days post-injury [44]. The pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF, have dual roles as sleep regulatory substances [45–47], which may contribute to the acute increase in sleep post-injury.

A limitation of the present study is the inability to distinguish between REM (rapid eye movement) and non-REM sleep. While overall percentage sleep and bout length were not impacted at chronic time points, injury-induced alterations in sleep architecture could occur, but not be measured by the non-invasive monitoring system used in this study. Clinical studies show brain injury contributes to changes in both types of sleep [48, 49]. Increases in REM sleep in the second sleep cycle have been reported close to a year after TBI [48], while data suggest TBI patients have less stage one non-REM sleep [49]. Further analysis of REM and non-REM sleep may reveal more subtle injury-induced chronic sleep disturbances.

The literature search showed numerous clinical reports of chronic sleep–wake disturbances associated with TBI [2, 3, 7, 23]; however, experimental studies to date have been terminated too early to evaluate chronic sleep disturbances [15, 28, 44]. To more completely understand chronic sleep disorders in TBI survivors, future studies could investigate secondary injury processes at chronic time points, focusing on processes that may confound sleep physiology (e.g. ATP, free radicals, cytokines). A secondary insult, such as a second brain injury, may be necessary to induce chronic sleep disturbance in the mouse. Moreover, the present study disregarded the contraindications of psychiatric sequelae, such as depression and anxiety, which contribute to the development, if not maintenance, of sleep–wake disturbances in TBI patients [7].

Conclusion

In conclusion, sleep was increased following diffuse TBI during the first week post-injury. These injury-dependent changes in sleep were not maintained thereafter. Further studies are needed to understand the contribution of sleep on recovery following TBI, as well as other neurological conditions.

Acknowledgments

Declaration of interest

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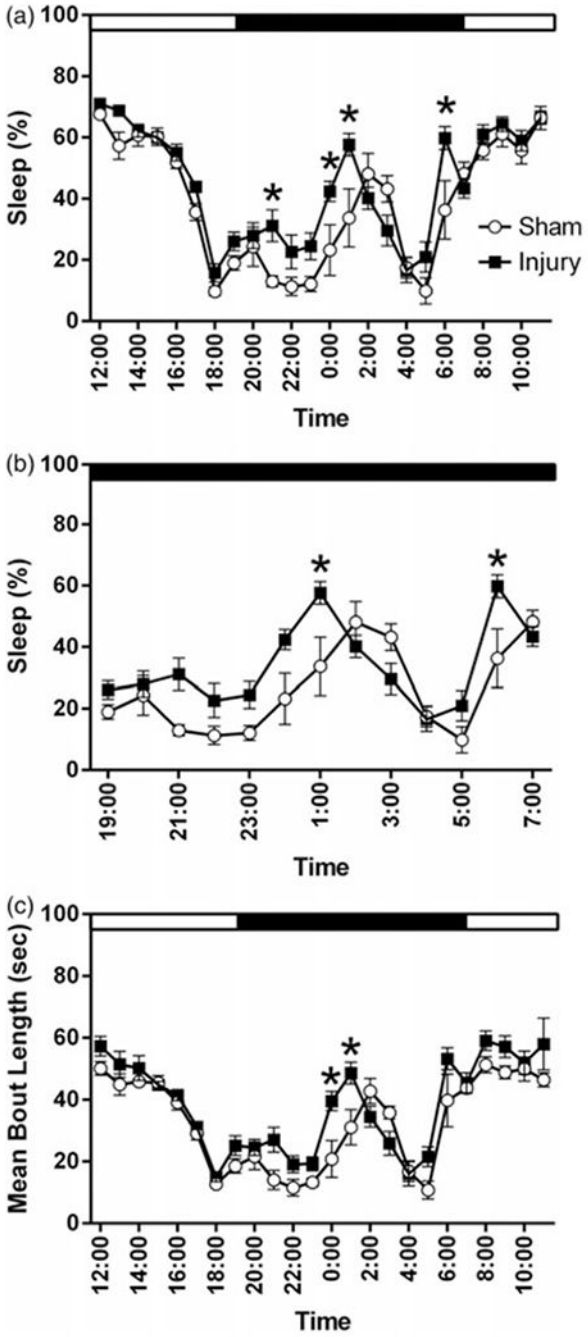

Figure 1.

Diffuse TBI impacted percentage sleep and mean bout length in the first week post-injury. (a) Overall, brain-injured mice slept significantly more than shams during the first week post-injury (mean±SEM; $F(1,10) = 17.61$, $p = 0.0018$; sham $n = 5$, injury $n = 7$). Differences between brain-injured mice and uninjured shams as indicated (Sidak's multiple comparison test, $*p<0.05$). (b) Percentage sleep in brain-injured mice increased significantly compared to uninjured shams (two-way ANOVA, mean±SEM; $F(1,10) = 11.29$, $p = 0.0072$; sham $n = 5$, injury $n = 7$) during the dark cycle, with significant differences at 01:00 and 06:00

(Sidak's multiple comparison test; *p<0.05). (c) Overall, the average bout length of sleep was significantly longer in brain-injured mice compared to uninjured shams during the first week post-injury (mean±SEM; $F(1,10) = 5.186$, $p = 0.0460$; sham $n = 5$, injury $n = 7$), with significantly longer sleep bouts in the middle of the dark cycle for brain-injured compared to uninjured mice (Sidak's multiple comparison test; *p<0.05).

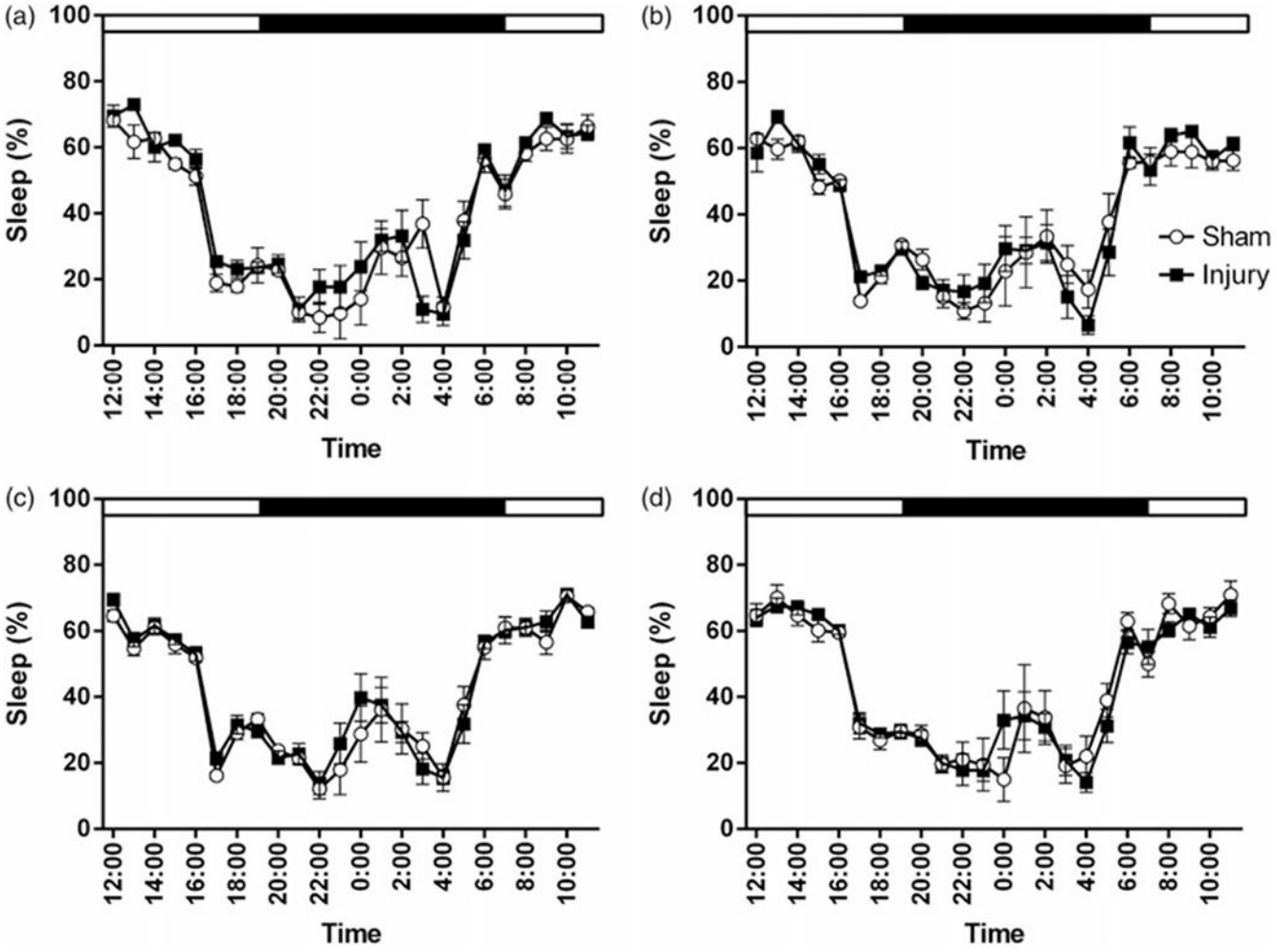

Figure 2.

Diffuse TBI did not chronically impact percentage sleep. Daily percentage sleep was calculated by averaging the percentage sleep at each hour for all days of weeks 2 (a), 3 (b), 4 (c) and 5 (d) post-injury. No significant differences in daily percentage sleep were found between brain-injured and sham mice during weeks 2 ($F(1, 10) = 2.206$, $p = 0.1683$), 3 ($F(1, 10) = 0.4557$, $p = 0.5150$), 4 ($F(1, 10) = 0.7659$, $p = 0.4020$) or 5 ($F(1, 10) = 0.1282$, $p = 0.7277$).

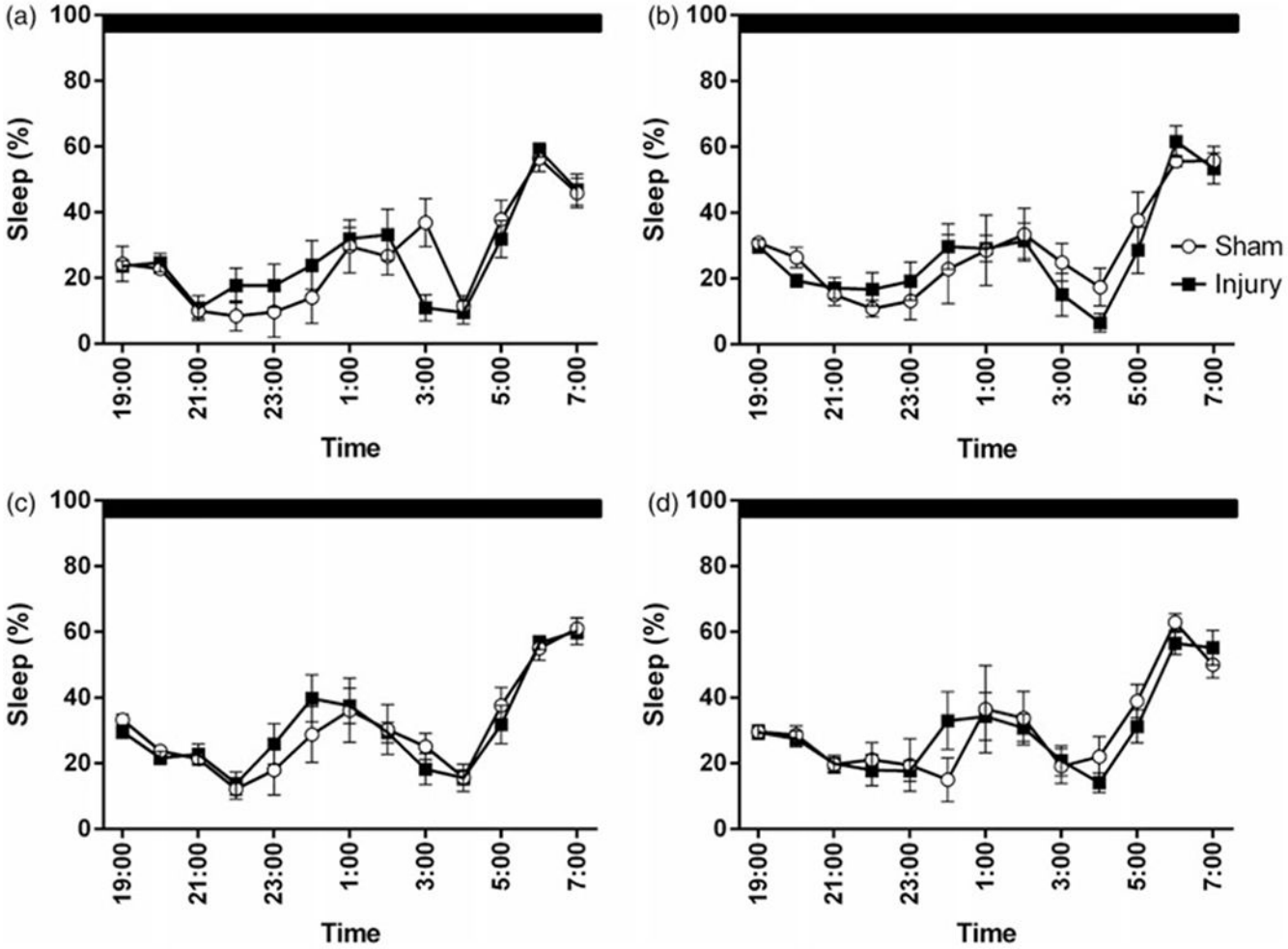

Figure 3.

Diffuse TBI did not chronically impact percentage sleep during the dark cycle. The mean percentage sleep was evaluated during the dark cycle by calculating the average percentage sleep at each hour of the dark cycle for all days of weeks 2 (a), 3 (b), 4 (c) and 5 (d). No injury-dependent effect during the dark cycle on percentage sleep was detected in brain-injured mice compared to sham mice during post-injury weeks 2 ($F(1, 10) = 0.074\,26$, $p = 0.7908$), 3 ($F(1, 10) = 0.2760$, $p = 0.6108$), 4 ($F(1, 10) = 0.018\,92$, $p = 0.8933$) or 5 ($F(1, 10) = 0.4322$, $p = 0.8395$).

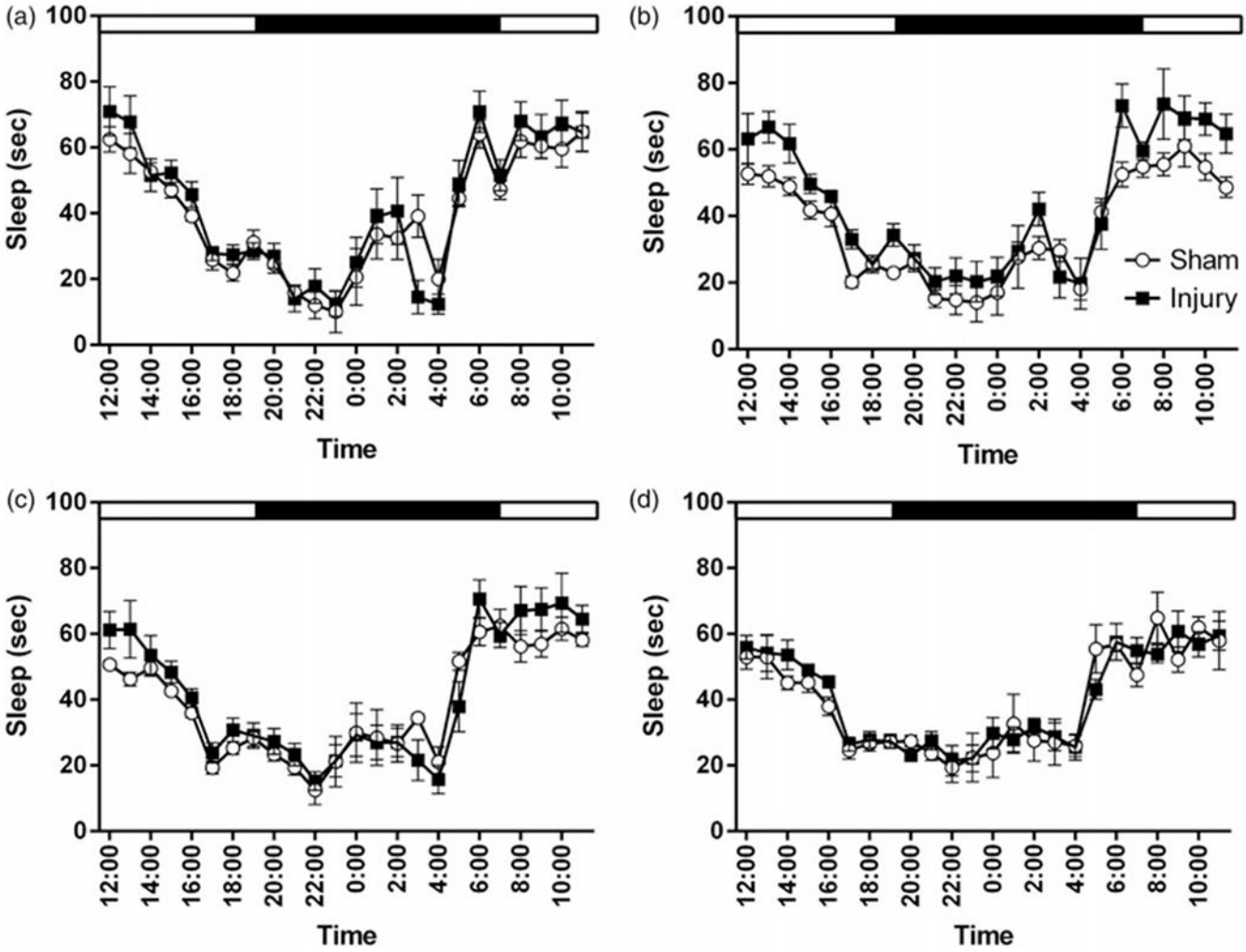

Figure 4.

Diffuse TBI did not chronically impact mean sleep bout length. Daily mean bout length was calculated by averaging the mean bout length at each hour for all days of weeks 2 (a), 3 (b), 4 (c) and 5 (d). No significant differences in average bout length slept were found between brain-injured and sham mice during weeks 2 ($F(1, 10) = 0.3694$, $p = 0.5569$), 4 ($F(1, 10) = 0.8686$, $p = 0.3733$) or 5 ($F(1, 10) = 0.2344$, $p = 0.6387$). Overall, during week 3, brain-injured mice slept significantly longer average bouts than shams ($F(1, 10) = 8.437$, $p = 0.0157$), without specific effects.