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Environmental Circadian Disruption Worsens Neurologic Impairment and Inhibits Hippocampal Neurogenesis in Adult Rats After Traumatic Brain Injury

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Abstract

Circadian rhythms modulate many physiologic processes and behaviors. Therefore, their disruption causes a variety of potential adverse effects in humans and animals. Circadian disruption induced by constant light exposure has been discovered to produce pathophysiologic consequences after brain injury. However, the underlying mechanisms that lead to more severe impairment and disruption of neurophysiologic processes are not well understood. Here, we evaluated the effect of constant light exposure on the neurobehavioral impairment and survival of neurons in rats after traumatic brain injury (TBI). Sixty adult male Sprague–Dawley rats were subjected to a weight-drop model of TBI and then exposed to either a standard 12-/12-h light/dark cycle or a constant 24-h light/light cycle for 14 days. Our results showed that 14 days of constant light exposure after TBI significantly worsened the sensorimotor and cognitive deficits, which were associated with decreased body weight, impaired water and food intake, increased cortical lesion volume, and decreased neuronal survival. Furthermore, environmental circadian disruption inhibited cell proliferation and newborn cell survival and decreased immature cell production in rats subjected to the TBI model. We conclude that circadian disruption induced by constant light exposure worsens histologic and neurobehavioral impairment and inhibits neurogenesis in adult TBI rats. Our novel findings suggest that light exposure should be decreased and circadian rhythm reestablished in hospitalized TBI patients and that drugs and strategies that maintain circadian rhythm would offer a novel therapeutic option.

Dongpeng Li and Shanshan Ma have contributed equally to this work.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Keywords

Circadian rhythms; Traumatic brain injury; Constant light; Hippocampus; Neurogenesis

Introduction

Most organisms on earth exhibit a 24-h circadian rhythm cycle that modulates many physiologic processes and behaviors, such as sleep–wake cycles (Miyamoto et al. 2012), blood pressure (Alibhai et al. 2015), hormone secretion (Chakir et al. 2015), body temperature (Mohawk et al. 2012), and organ activity (Bailey et al. 2014). Studies in rodents and humans have demonstrated that circadian rhythm plays an important role in the brain, such as in neurotransmitter activity (Colwell 2011), aging (Fonseca Costa and Ripperger 2015; Giebultowicz and Long 2015), learning and memory (Castro et al. 2005), and mood regulation (Bedrosian et al. 2011).

Recently, increasing evidence has shown that circadian rhythms modulate the process of neuronal maturation and survival and the functional network of hippocampal neurogenesis (Bouchard-Cannon et al. 2013). Furthermore, studies have shown that levels of hippocampal cell proliferation are higher at night than in the day (Bouchard-Cannon et al. 2013). Moreover, circadian rhythm disruption may be involved in the pathophysiology of brain injury (Boone et al. 2012; Zuurbier et al. 2015). In a rat model of traumatic brain injury (TBI), the expression of key circadian genes, such as *Bmal1* and *Cry1*, were altered in both the suprachiasmatic nucleus and the hippocampus concurrently with disruption of daily rhythms of locomotor activity (Boone et al. 2012).

The environmental light/dark cycle plays a pivotal role in modulating circadian rhythm in mammals (Antle et al. 2009; Zelinski et al. 2014). Thus, disruption of cyclic light conditions has a variety of effects on physiology and behavior (Panda et al. 2002; Roenneberg et al. 2013; Stevens et al. 2013; Tataroglu et al. 2004). Abundant evidence has indicated that constant light conditions disrupt circadian rhythm in human and animal models (Stevens et al. 2007). Exposure to dim light at night is sufficient to provoke a depression-like phenotype and to impair hippocampal long-term potentiation and learning in female hamsters (Bedrosian et al. 2011). Thus, constant light exposure in hospitals can disturb the sleep–wake cycle of patients with TBI and may be associated with greater severity and longer duration of stay in the intensive care unit (ICU) and hospital (Duclos et al. 2014; Hardin 2009). However, the underlying mechanisms that lead to worsened physical and mental recovery and disruption of neurophysiologic processes remain unclear.

In this study, we investigated whether circadian disruption by constant light exposure exacerbates functional and histologic outcomes of TBI in a rat weight-drop model. We examined the sensorimotor and cognitive function, histology (lesion volume and neuronal survival) as well as hippocampal neurogenesis of rats exposed to constant light during the post-TBI period. Our studies suggest that constant light exposure after TBI might impair cell proliferation in hippocampus, an effect that could be deleterious to histologic and neurobehavioral recovery after TBI.

Materials and Methods

Animals

This study was approved by the Institutional Animal Care and Use Committee of Zhengzhou University, China. All procedures were performed in strict accordance with the NIH guidelines for the Care and Use of Laboratory Animals (1996). Adult male Sprague–Dawley rats (230–250 g) were obtained from the Experimental Animal Centre of Henan Province, Zhengzhou, China. The animals underwent surgery in a random order, and outcome assessments were made by investigators blinded to the experimental groups. All efforts were made to minimize suffering and reduce the number of rats used. The rats were housed under controlled laboratory conditions with a 12-h light/dark cycle, a temperature of 22 ± 2 °C, and a humidity of 60–70 % for at least 1 week before injury.

TBI Model

TBI was induced in rats with a modified weight-drop device developed in our laboratory (Cheng et al. 2015). Rats were first anesthetized with 10 % chloral hydrate (3 ml/kg) and then fixed in a stereotactic frame. A rectal temperature probe was placed to maintain body temperature at 37 °C with a feedback-controlled heating pad. A midline longitudinal incision was made, and the skull was exposed. A dental drill was used to create a right parietal bone window (5 mm in diameter) 3 mm behind the anterior fontanel and 2 mm anterior to lambda, adjacent to the central suture. A 50-g steel rod with a flat end (4 mm in diameter) was allowed to fall from a height of 30 cm onto a piston resting on the dura. The piston was allowed to compress the tissue a maximum of 5 mm, causing focal injury to the right hemisphere. The cut was then sutured. After this procedure, the skin was placed back into position and cemented, and the rat was placed on a feedback-controlled heating pad until it regained consciousness (return of the righting reflex and mobility).

Environmental Circadian Disruption Model

Among the circadian disruption models that have been reported (Bedrosian et al. 2011; Coomans et al. 2013; Fonken et al. 2013b; Ma et al. 2007; Park et al. 2013; Roenneberg et al. 2013), we used constant light exposure. The primary advantage of using this model is that the paradigm is simple and constant light is often present in the hospital ICU setting (Ma et al. 2007). Immediately after TBI, the rats were divided randomly into four groups and maintained under a standard 12-h/12-h light/dark (LD) cycle, with lights on at 08:00 and lights off at 20:00, or a constant 24-h light/light (LL) cycle (>180 lux). The four groups consisted of the following: (1) Sham/LD group, (2) Sham/LL group, (3) TBI/LD group, and (4) TBI/LL group.

BrdU Injections

5-Bromo-2'-deoxyuridine (BrdU) is a substance that incorporates into the DNA during S phase of the cell cycle, thus allowing the immunohistochemical identification of new cells. To identify the degree of newborn cell survival in the dentate gyrus (DG), we injected animals intraperitoneally with BrdU (50 mg/kg; Sigma-Aldrich, St. Louis, MO) once daily on days 1 through 7 after TBI.

Neurobehavioral Evaluation

Neurologic function was graded in all rats on a scale of 0–21 (normal score = 21; maximal deficit score = 0) on days 1, 3, 7, 11, and 14 after TBI (Hunter et al. 2000; Martinez-Vargas et al. 2012).

Motor Performance and Cognitive Function Assessment

We evaluated motor coordination of the rats with the beam balance and beam walking tests as described previously (Bales et al. 2012). Briefly, in the beam balance test, rats are placed on a suspended wooden beam (length: 35 cm; width: 2.0 cm; height: 2.5 cm), and their latency to stay on the beam is recorded up to maximum of 60 s. Rats were assessed prior to injury and again on days 1, 3, 7, 11, and 14 after injury. In the beam walking test, we measured the latency of the rats to travel across a narrow wooden beam (length: 122.5 cm, width: 2.5 cm) away from loud white noise and a bright environment into a darkened goal box at the end of the beam. The test contains four obstacles (diameter: 1 cm, height: 3 cm) to challenge the coordination and agility of rats during the travel. Three days before injury, rats were trained to traverse the beam in 5 s for three consecutive trials. At 1, 3, 7, 11, and 14 days after TBI, latency of travel was recorded up to a maximum of 60 s. The average latency of the three trials each day was used for statistical analysis.

Cognitive functions were evaluated with the Morris water maze (MWM) test as described previously (Vorhees and Williams 2006; Wang et al. 2015). Briefly, a rat was placed in a large circular pool (130 cm in diameter and 60 cm high) filled with warm (19–22 °C) water to a depth of 30 cm. A target platform was hidden 2 cm below the surface of the water in the first quadrant. On days 10–14, we measured acquisition of memory retention and cognitive functions. In four trials per day, each rat was randomly released in the pool facing a wall at one of four points (east, west, south, or north) and was allowed up to 90 s to find the invisible platform. Upon locating the platform (or being guided to the platform after 90 s), the animal was permitted to remain on the platform for 10 s before the next trial. The average latency to find the platform and the percentage of time spent in the correct quadrant were recorded. On day 14, a probe trial was performed 4 h after the goal latency test. Detailed procedures have been described previously (Matulka et al. 2013; Vorhees and Williams 2006). Using a video tracking system, we measured the frequency with which the rats were able to find the platform and the average percentage of time spent in the goal platform quadrant.

Lesion Volume

On day 14 after TBI, rats were deeply anesthetized and then transcardially perfused with saline followed by 4 % paraformaldehyde. The brains were post-fixed in the same perfusate at 4 °C overnight and then transferred to 30 % sucrose for 24 h. Each brain was cut with a rat brain matrix (RWD Life Science Co, Ltd. Shenzhen, China) into 2-mm-thick coronal blocks for a total of 7 blocks from 1 mm anterior to the lesion site to occipital lobe (from bregma +4 to –10 mm) and embedded in paraffin for sectioning. One 6-μm-thick section from each of the seven coronal blocks was collected and stained with Cresyl Violet. Image J software (NIH, Bethesda, MD, USA) was used to quantify lesion volumes as previously described (Cheng et al. 2015). The lesion volume in cubic millimeters was calculated as the

sum of the damaged areas of each block multiplied by the interslice distance (2 mm) (Chang et al. 2014).

Nissl Staining

We performed Nissl staining on day 14 post-injury in separate groups to evaluate the effect of circadian disruption on neuronal loss after TBI. Six-micrometer-thick sections from bregma −1.5 mm to bregma −4.5 mm at intervals of 240 µm were collected and stained with Cresyl Violet. Only cells with a clear cell body and conspicuous nucleus were counted as intact surviving neurons; pyknotic or shrunken cells were excluded. For each animal, cells were counted in six fields of view (×200) from different areas near the injury core in each slice. The average number from all fields is presented as the neuronal count for cortex according to previously described methods (Wang et al. 2014). Cell numbers in the hippocampal DG and CA1 area were calculated in the identical position at a length of 1 mm as described previously (Wang et al. 2002). Briefly, every sixth section (spaced 240 µm apart) throughout the entire hippocampus was selected for quantification in each rat. Ten sections were manually quantified with a semi-automatic stereology system (Zeiss, AxioCam-MRc, Oberkochen, Germany). The averages of all fields are presented as the neuronal counts for the DG and CA1 subregions.

Immunohistochemistry and Immunofluorescence

After the rats were exposed to constant light for 14 days, we used immunohistochemistry and immunofluorescence to determine the percentage of surviving newborn and immature cells as described previously (Mueller et al. 2011). Five coronal sections collected from −1.5 to −4.5 mm of bregma were analyzed from each animal. The reagents were as follows: mouse anti-BrdU (recognizing newborn cells, Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-Ki-67 (recognizing proliferative cells; Santa Cruz Biotechnology) and diaminobenzidine (DAB) Kit (Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd., China). The sections were incubated in 2 N HCl at room temperature for 30 min, washed with borate buffer for 10 min, and then washed three times with phosphate-buffered saline for 10 min each. The brain sections were blocked with 5 % goat serum for 30 min and then incubated with primary antibody (mouse anti-BrdU or rabbit anti-Ki-67) at 4 °C for 24 h. After being washed with PBS, sections were incubated with secondary antibody (HRP-conjugated anti-mouse-IgG) in blocking buffer for 1 h at room temperature. Finally, sections were incubated with DAB and mounted on glass slides. All positive cells in the subgranular zone of the contralateral DG were counted and averaged.

For immunofluorescence of DCX, the sections were rinsed with PBS, blocked with blocking buffer (1 % bovine serum albumin, 5 % normal goat serum in PBS) for 2 h at 4 °C, and then incubated with primary rabbit anti-doublecortin (DCX) (recognizing immature neurons; Santa Cruz Biotechnology, Dallas, TX, USA; diluted in 1:500) for 24 h at 4 °C. After they were rinsed with PBS, the sections were incubated with the appropriate secondary antibody (Goat Anti-Rabbit Alexa Fluor 594-labeled IgG, 1:500, Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd., China) for 1 h at 4 °C. After rinsing with PBS, the sections were counterstained with DAPI for 2 min, washed with PBS and then briefly with water. They

were then mounted on slides with the mounting medium. Images were taken by using a Zeiss microscope (Zeiss, AxioCam-MRc, Oberkochen, Germany).

Statistical Analysis

Quantitative data were analyzed by investigators blinded to the groups and are presented as mean $\pm$ standard error of the mean (SEM). For changes in food intake, water intake, body weight, neurologic score, performance in the beam walking and MWM tests; and changes in the numbers of the Ki-67-, BrdU-, and DCX-positive cells, we applied two-way repeated measures ANOVA followed by Bonferroni post hoc test. All remaining data were analyzed by two-tailed unpaired Student's *t* test or one-way ANOVA followed by Bonferroni post hoc test. Statistical analysis and data graphing were carried out with SPSS (17.0, Chicago, USA) and Graph Pad Prism5 (San Diego, CA, USA). Differences were considered significant at $P < 0.05$.

Results

Environmental Circadian Disruption Reduces Body Weight, Food Intake, and Neurologic Scores after TBI

As shown in Fig. 1a, body weight was highest in the Sham/LL group, but least in the TBI/LL group compared with the other groups on day 14. There was a significant TBI effect ($F(1,3) = 16.38, P < 0.0001$) and LL effect ($F(1,4) = 23.56, P < 0.0001$) as well as a significant interaction effect between TBI and LL ($F(1,12) = 4.492, P < 0.0001$).

On day 1 after TBI, water consumption was substantially lower in TBI/LD and TBI/LL rats than in the Sham groups ($F(3, 30) = 36.57, P < 0.0001$; Fig. 1b). However, no significant difference was observed at any other time point (day 3: $F(3, 30) = 1.073, P = 0.152$; day 7: $F(3, 30) = 1.073, P = 0.375$; day 14: $F(3, 30) = 1.833, P = 0.162$).

Food intake was significantly less in TBI rats than in Sham rats on days 1 and 3 but began to increase on day 7 (Fig. 1c). Rats in the Sham/LL group had the highest food intake, whereas food intake in the TBI/LL group was lower than that in all other groups on day 7 ($F(3,30) = 19.28, P < 0.0001$) and day 14 ($F(3,30) = 12.68, P = 0.0002$).

We found no significant difference in neurologic function score between the Sham/LD and Sham/LL groups at any time point. The main effect of TBI on neurologic scores was significant ($F(3,30) = 30.49, P < 0.0001$) when compared to the Sham groups. In addition, neurologic scores were significantly lower in TBI/LL rats than in TBI/LD rats on day 14 ($F(3,30) = 15.58, P < 0.0001$; Fig. 1d).

Environmental Circadian Disruption Delays Motor Function Recovery and Accelerates Memory Impairment after TBI

Vestibulomotor function and motor performance were assessed by the beam balance and beam walking tasks, respectively. As shown in Fig. 2a, all rats in the Sham/LD and Sham/LL groups were capable of maintaining the beam balancing task for 60 s, but TBI followed by environmental circadian disruption significantly reduced the performance on the beam balance test. Analysis of the data revealed significant interaction of TBI and LL ($F(1,12) =$

4.492; $P < 0.0001$) as well as significant differences of TBI ($F(3,30) = 165.30$, $P < 0.0001$) and LL ($F(1,4) = 29.55$, $P < 0.0001$) effect. Balance was significantly reduced in TBI/LD ($P < 0.05$) and TBI/LL ($P < 0.05$) groups compared to that in the Sham/LD and Sham/LL groups, respectively. Additionally, rats in the TBI/LL group spent significantly less time on the beam than did rats in the TBI/LD group ($P < 0.05$). In the beam walking task, latency to cross the beam was significantly increased in both TBI/LD and TBI/LL groups (Fig. 2b), and rats in the TBI/LL group spent significantly more time to cross the beam than did rats in the TBI/LD group ($P < 0.05$). There were significant differences in TBI ($F(3, 47) = 78.404$, $P < 0.001$) and LL ($F(3, 47) = 70.900$, $P < 0.001$) effect and significant interaction between TBI and LL ($F(15, 235) = 9.479$, $P < 0.001$) effect.

We assessed spatial learning capacity based on acquisition time in the MWM test. Analysis of the data showed a significant difference in TBI ($F(3, 180) = 63.38$, $P < 0.001$) and LL ($F(4, 180) = 60.46$, $P < 0.001$) effect. However, there was no TBI and LL interaction ($F(12, 180) = 0.973$, $P = 0.477$). The latency to find the platform was significantly longer in the TBI/LD and TBI/LL groups than in the two Sham groups from day 10 to day 14 (Fig. 2c, $P < 0.05$), but no significant difference was observed between Sham/LD and Sham/LL animals ($P = 0.538$). Rats in the TBI/LL group required more time to find the platform than did rats in the TBI/LD group ($P < 0.001$). In addition, TBI/LD and TBI/LL groups spent less time in the correct quadrant than did the Sham/LD group; the TBI/LL group spent the least amount of time in the correct quadrant on days 13 and 14 ($P < 0.001$, Fig. 2d). In the probe trial of the MWM test, TBI/LL rats showed significant impairment in spatial memory compared with that of TBI/LD rats, as reflected by a reduced frequency of finding the platform and less time spent in the platform quadrant (Fig. 2e, f, $P < 0.05$).

Environmental Circadian Disruption Exacerbates Lesion Volume After TBI

Lesion volume was quantified by measuring the tissue loss on day 14 after TBI. Histologic assessment indicated that TBI resulted in extensive damage to the brain tissue in rats subjected to TBI/LD ($23.97 \pm 2.94 \text{ mm}^3$, Fig. 3a). Constant light exposure significantly increased the lesion volume ($34.33 \pm 1.58 \text{ mm}^3$, Fig. 3b, c) compared to that in the TBI/LD group ($t = 3.369$; $P = 0.007$). No obvious lesions were observed in the Sham/LD or Sham/LL rats (data not shown). Regression analysis showed a correlation between lesion volume and impaired sensorimotor function as measured by the beam walking task (Fig. 3d; $R^2 = 0.76$; $P < 0.05$).

Environmental Circadian Disruption Reduces Neuronal Survival in the Cortex and Hippocampus After TBI

To evaluate the effect of circadian disruption on neuronal loss, we used Nissl staining to identify surviving neurons in hippocampus on day 14 after TBI or Sham surgery. In Sham/LD and Sham/LL groups, the visual field was full of clear and intact neurons. However, neurons in TBI/LD and TBI/LL groups were significantly damaged, exhibiting extensive degenerative changes, including sparse cell arrangement and loss of integrity. TBI significantly decreased the number of surviving neurons in cortex, DG, and CA1 compared to that in Sham groups. Moreover, the number of surviving neurons in the cortex and CA1 area was significantly less in the TBI/LL group than in the TBI/LD group ($P < 0.001$). There

was a significant TBI effect in the cortex ($F(1,28) = 22.242$, $P < 0.001$), DG area ($F(1,28) = 41.022$; $P < 0.001$), and CA1 area ($F(1,28) = 89.780$, $P < 0.001$) as well as an LL effect in the cortex ($F(1,28) = 53.968$, $P < 0.001$) and CA1 area ($F(1,12) = 12.340$; $P = 0.002$). However, we found no significant LL effect in the DG area ($F(1,28) = 1.697$; $P = 0.060$) or interaction effect between TBI and LL in the cortex ($F(1,28) = 9.26$, $P = 0.195$), DG area ($F(1,28) = 0.203$; $P = 0.954$), and CA1 area ($F(1,28) = 0.262$, $P = 0.595$) (Fig. 4).

Environmental Circadian Disruption Inhibits Cell Proliferation and Newborn Cell Survival

To measure the effects of circadian disruption on hippocampal cell proliferation on day 14 after TBI, we quantified cell proliferation and newborn cell survival by Ki-67 and BrdU immunohistochemistry (Fig. 5). We found that the average number of Ki-67-positive or BrdU-positive cells in DG of the TBI/LD group was elevated significantly as compared to that in the Sham/LD group ($P < 0.001$). After constant light exposure, Sham animals did not show any changes; however, rats in the TBI/LL group had fewer Ki-67- and BrdU-labeled cells in DG than did rats in the TBI/LD group ($P < 0.001$). Additionally, we found a significant effect of TBI on Ki-67-positive cells ($F(1,24) = 11.733$, $P < 0.001$) and BrdU-positive cells ($F(1,24) = 70.055$, $P < 0.001$) as well as an effect of LL on Ki-67-positive cells ($F(1,24) = 68.251$, $P < 0.001$) and BrdU-positive cells ($F(1,24) = 13.577$; $P < 0.001$). We detected a significant interaction effect between TBI and LL in Ki-67-positive cells ($F(1,24) = 22.395$, $P < 0.001$) but not in BrdU-labeled cells ($F(1,24) = 0.295$; $P = 0.559$).

Environmental Circadian Disruption Decreases Immature Neuron Production After TBI

The total number of DCX-positive cells was significantly greater in the subgranular zone of DG in the TBI/LD group than in that of the Sham/LD group on day 14 after TBI ($P < 0.001$), but the number in the Sham/LL, Sham/LD, and TBI/LL groups did not differ significantly ($P > 0.05$). These data indicate that the production of immature neurons was inhibited in the TBI/LL group compared to that in the TBI/LD group ($P < 0.001$). There was a significant TBI effect ($F(1, 24) = 30.222$, $P < 0.001$) and LL effect ($F(1,24) = 18.766$; $P < 0.001$) as well as an interaction effect between TBI and LL ($F(1,24) = 5.521$; $P < 0.001$) (Fig. 6).

Discussion

Our results provide novel findings that 14 days of constant light exposure after TBI can significantly worsen sensorimotor and cognitive deficits, decrease body weight, food and water intake, and reduce neuronal survival while increasing lesion volume and inhibiting neurogenesis.

Studies have shown that dim light at night can increase body weight in mice (Aubrecht et al. 2014; Fonken et al. 2013a; Kott et al. 2012). In this study, rats that underwent TBI and received constant light exposure had a lower body weight than rats that underwent TBI with a normal LD cycle, suggesting that circadian disruption, which is associated with temporal alterations in feeding behavior, might inhibit post-TBI functional recovery.

Previous studies reported that rats exposed to constant light for 3–8 weeks exhibited shorter escape latency during the initial phase of spatial learning and less time in the target quadrant compared with control rats (Ma et al. 2007). In the present study, 14 days of constant light

exposure had no significant effect on the latency of Sham/LD and Sham/LL rats to find the platform in the MWM, but rats in the TBI/LL group required more time to find the platform than did rats in the TBI/LD group. Our study had a shorter light-exposure time than the previous study (Ma et al. 2007), and it is possible that constant light exposure and TBI may have a synergistic influence on cognitive function. In the probe trial, constant light exposure accelerated memory impairment after TBI, causing TBI/LL rats to find the platform less frequently and spend less time in the platform quadrant than TBI/LD rats. In addition, TBI caused cell/tissue loss and sensorimotor deficits which was exacerbated by constant light exposure. Regression analysis indicated that increased lesion volume is strongly correlated with impaired sensorimotor function.

An increased number of degenerating neurons in the hippocampus is often correlated with impaired learning and memory functions after TBI (Sun et al. 2015). In our study, Nissl staining revealed that constant light exposure after TBI exacerbates loss of surviving neurons in the cortex and CA1 area, but not in the DG area. Furthermore, it has been reported that TBI can induce hippocampal neurogenesis in DG (Sun et al. 2015), but not in the cortex or hippocampal CA1 area. The increase in DG neurogenesis may be a reason that no significant neuronal loss was observed in that region of the TBI/LL group. Neuronal cell death after TBI is a major cause of neurologic deficit and mortality. The mechanisms that mediate the neuronal cell death include caspases and proapoptotic members of the Bcl-2 family (apoptosis), JNK and ATG orthologs (autophagy), PARP/AIF (PARP/AIF-dependent cell death), and neuroinflammation (Stoica and Faden 2010), among others. Future studies are needed to elucidate the detailed cellular mechanism and relationship between neuronal degeneration and circadian disruption.

Though rats in the TBI/LD group had increased numbers of Ki-67-, BrdU-, and DCX-positive cells in the subgranular zone of DG, constant light exposure significantly inhibited neuronal cell proliferation, newborn cell survival, and immature neuron production after TBI. In the hippocampus, adult neurogenesis plays an important role in hippocampal-dependent learning and memory functions and is thought to contribute to the spontaneous cognitive recovery observed after TBI. Our results showed that environmental circadian disruption inhibits hippocampal neurogenesis, delays motor functional recovery, and accelerates memory impairment after TBI.

Two limitations must be taken into account when interpreting the results of this study. First, we did not assess the effect of circadian disruption on neuronal loss and neurogenesis over time; the molecular mechanisms by which circadian rhythm disruption contributes to exacerbation of TBI need to be investigated. Second, we did not assess brain injury recovery in a constant dark state, which may help to reveal whether a normal circadian rhythm is beneficial to the treatment of brain injury.

In conclusion, our results demonstrate for the first time that circadian disruption induced by constant light exposure after TBI can exacerbate histologic and neurobehavioral deficits and inhibit hippocampal neurogenesis in adult rats. These negative effects of circadian disruption on TBI outcomes indicate that decreasing light exposure and reestablishing a normal

circadian rhythm in hospitalized TBI patients may be warranted, and designing drugs and strategies that help maintain circadian rhythm might offer a novel therapeutic option.

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Fig. 1. Environmental circadian disruption reduces body weight (a), water intake (b), food intake (c), and neurologic score (d) in a rat model of TBI. # $P < 0.05$ vs. Sham/LD group, * $P < 0.05$ vs. TBI/LD group, repeated measures ANOVA followed by Bonferroni post hoc test. Data are shown as mean $\pm$ SEM; $n = 15$ rats per group

Fig. 2.

Environmental circadian disruption delays motor function recovery and accelerates memory impairment after TBI. **a** Mean latency in the beam balance test. **b** Mean latency in the beam walking test. **c** Latency to find platform in the Morris water maze (MWM). **d** Mean time spent in the correct quadrant of the MWM. **e** Frequency of finding the platform in the MWM test on day 14. **f** Time spent in the platform quadrant during the probe trial of the MWM test on day 14. # $P < 0.05$ vs. Sham/LD group, * $P < 0.05$ vs. TBI/LD group, repeated measures ANOVA or one-way ANOVA followed by Bonferroni post hoc test. Data are presented as mean $\pm$ SEM; $n = 15$ rats/per group

Fig. 3.

Environmental circadian disruption exacerbates lesion volume on day 14 after TBI.

Representative Cresyl Violet-stained sections from TBI/LD (a) and TBI/LL (b) groups. c Quantification of lesion volume; # $P < 0.05$ vs. TBI/LD group, unpaired Student's t test. Data are presented as mean $\pm$ SEM; $n = 5$ rats per group. d Linear regression analysis shows a correlation between lesion volume and beam walking test performance on day 14 after TBI ($R^2 = 0.76$; $P < 0.05$)

Fig. 4.

Environmental circadian disruption reduces neuron survival in the cortex and hippocampus on day 14 after TBI. **a** Representative images of Cresyl Violet-stained brain sections. *Scale bar* 100 μ m. Quantification of surviving neurons in the cortex (**b**), dentate gyrus (DG; **c**), and hippocampal CA1 region (**d**). # $P < 0.05$, ### $P < 0.001$ vs. Sham/LD group; * $P < 0.05$, *** $P < 0.001$ vs. TBI/LD group; one-way ANOVA followed by Bonferroni post hoc test. Data are presented as mean $\pm$ SEM; $n = 8$ rats/group

Fig. 5.

Environmental circadian disruption reduces Ki-67-positive cells and BrdU-positive cells in the subgranular layer of DG on the contralateral side on day 14 after TBI. **a** Brain sections were immunohistochemically stained with Ki-67 to show cell proliferation. BrdU-positive cells represent surviving newborn cells. *Scale bar* 100 μm. Bar graphs represent number of Ki-67-positive cells (**b**) and number of BrdU-positive cells (**c**) in the subgranular zone of DG. Arrows indicate Ki-67 or BrdU-positive cells. # $P < 0.05$, ### $P < 0.001$ vs. Sham/LD group; * $P < 0.05$, *** $P < 0.001$ vs. TBI/LD group; one-way ANOVA followed by Bonferroni post hoc test. Data are presented as mean ± SEM; $n = 6$ rats/group

Fig. 6.

Environmental circadian disruption reduces immature neuron production in the subgranular layer of DG on the contralateral side on day 14 after TBI. **a** Representative images are shown of DCX-positive (+) cells. *Scale bar* 100 μ m. *Arrows* indicate DCX-positive cells **b** The bar graph shows the number of DCX-positive cells in the subgranular zone of DG. ### $P < 0.001$ vs. Sham/LD group; *** $P < 0.001$ vs. TBI/LD group; one-way ANOVA followed by Bonferroni post hoc test. Data are presented as mean $\pm$ SEM; $n = 6$ rats per group