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Automated monitoring of early neurobehavioral changes in mice following traumatic brain injury

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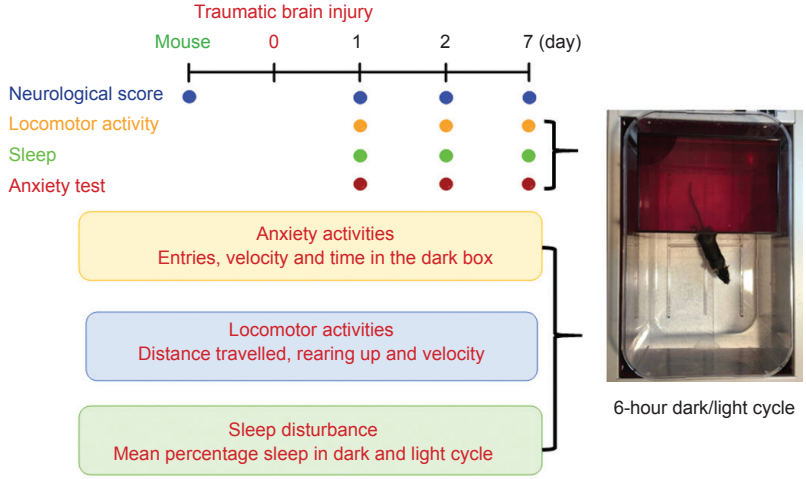
Graphical Abstract

SmartCage system can be used to detect early neurobehavioral changes in mice following traumatic brain injury



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Abstract

Traumatic brain injury often causes a variety of behavioral and emotional impairments that can develop into chronic disorders. Therefore, there is a need to shift towards identifying early symptoms that can aid in the prediction of traumatic brain injury outcomes and behavioral endpoints in patients with traumatic brain injury after early interventions. In this study, we used the SmartCage system, an automated quantitative approach to assess behavior alterations in mice during an early phase of traumatic brain injury in their home cages. Female C57BL/6 adult mice were subjected to moderate controlled cortical impact (CCI) injury. The mice then received a battery of behavioral assessments including neurological score, locomotor activity, sleep/wake states, and anxiety-like behaviors on days 1, 2, and 7 after CCI. Histological analysis was performed on day 7 after the last assessment. Spontaneous activities on days 1 and 2 after injury were significantly decreased in the CCI group. The average percentage of sleep time spent in both dark and light cycles were significantly higher in the CCI group than in the sham group. For anxiety-like behaviors, the time spent in a light compartment and the number of transitions between the dark/light compartments were all significantly reduced in the CCI group than in the sham group. In addition, the mice suffering from CCI exhibited a preference of staying in the dark compartment of a dark/light cage. The CCI mice showed reduced neurological score and histological abnormalities, which are well correlated to the automated behavioral assessments. Our findings demonstrate that the automated SmartCage system provides sensitive and objective measures for early behavior changes in mice following traumatic brain injury.

Key Words: *nerve regeneration; traumatic brain injury; controlled cortical impact; automated behavior; motor activity; anxiety; exploratory activity; sleep; neural regeneration*

Introduction

Traumatic brain injury (TBI) can result in a variety of sensory and motor deficits, emotional impairments, and sleep disturbances (Sherer et al., 2002; Verma et al., 2007). Consequences of TBI in adults have been well documented (Dikmen et al., 1995; Millis et al., 2001), but there is an insufficient literature addressing our ability to predict long-term outcomes. If we are able to identify early behavioural changes that predict the risk for lasting sequelae after TBI, preventive intervention strategies might be directed toward diminishing the rate of decline and enhancing functional recovery.

Well-designed behavioral evaluations in TBI animals are useful measures in identifying underlying mechanisms of functional recovery, which may be clinically relevant (Basso et al., 1995; Xiong et al., 2013b). Rodents exhibit behaviors similar to humans, and therefore they are commonly used to mimic behaviors of human disorders (Kochanek et al., 2002; Manley et al., 2006; Khroyan et al., 2012; Xiong et al., 2013b). To date, a large number of assessments have been developed to measure behavior function following TBI in mice (Chauhan et al., 2010; Liu et al., 2014; Bondi et al., 2015). However, it is disputed regarding which type of behavioral assessment is the most worthwhile or meaningful. Most of the current assessments use large and specific apparatuses with a limited time period and focus on a single behavioral domain, thus the subtle effects of the early phase of TBI are rarely detected (Liu et al., 2014; Bondi et al., 2015). Moreover, conventional behavioral tests measure an animal's responsiveness to a novel environment in which it is hard to determine whether the environment has an effect on the behavioral response. A home-like environment with an extended period of assessments is useful to detect behavior changes that may be more reliable than assessing animals in a novel environment during a short period (Khroyan et al., 2012). Therefore, there is a need to shift towards conducting rodent behavioral assessments in a home cage-like environment (Goulding et al., 2008; Kas et al., 2008; Jhuang et al., 2010; Khroyan et al., 2012). The SmartCage system (AfaSci, Inc., Burlingame, CA, USA) was successfully used for automated analysis of spontaneous activity, light/dark preference, and anxiety-related behavior in cerebral ischemia in mice in a home cage-like environment (Xiong et al., 2011, 2013a; Khroyan et al., 2012), but to the best of our knowledge, it was not used in mouse models of TBI. In the home cage, mice can walk freely providing an opportunity to objectively and simultaneously assess multiple behavior activities, which could be a powerful tool to detect early neurobehavioral changes following TBI. In this study, we sought to determine the sensitivity and reliability of a home-like cage system called SmartCage in detecting early neurobehavioral changes following a moderate controlled cortical impact (CCI) injury.

Materials and Methods

Ethics statement and experimental animals

All surgical interventions and postoperative animal care were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council) and the Guidelines of the Indiana University Institutional Animal Care and Use Committee (10951). Precautions were taken to minimize suffering and the number of animals used in each experiment. Fourteen female SPF C57/BL6 mice aged 8 weeks, weighing 20-25 g, (Jackson Laboratory, Bar Harbor, Maine, USA) were housed in a 12-hour dark/light cycle with food and water freely available.

CCI modelling

Mice were randomly divided into two groups: the sham (n = 7) and CCI groups (n = 7). The CCI in mice was performed according to our protocol as described previously (Liu et al., 2014). Briefly, mice were anesthetized with Avertin (2.5%, 0.2 mL/20 g) and placed in a stereotactic frame adapted for mice. A midline incision was made to expose the skull, a 4.5 mm (diameter) craniotomy was performed midway between the bregma and the lambda, and 2.5 mm lateral to midline over the left hemisphere. Mice were subjected to a CCI injury at a 1.0 mm impact depth using an electromagnetic impactor (39463920, Impactor OneTM, MyNeuroLab, Richmond, Illinois, USA; tip diameter: 3 mm; speed: 3 m/s; dwell: 50 ms). The skin incision was closed after the injury. For the sham-operated controls, mice received the same anesthesia and surgical procedure (craniotomy) without the impact. After surgery, the mice were immediately put into cages on the heating pad until they recovered from anesthesia. The mice were subjected to a series of behavior tests and sacrificed for histopathological examination 7 days later.

SmartCage system

SmartCage system is a non-invasive home cage rodent behaviour monitoring system. The SmartCage system provides an inner space of 36 cm × 23 cm × 9 cm for mice acting within the home cage (Khroyan et al., 2012). The infrared processor, motor control, instrument amplifier and microcontroller units are assembled in the platform (Khroyan et al., 2012). In this study, a single USB cable was linked to the host computer. Data were analyzed automatically using the CageScore (AfaSci, Inc. Burlingame, CA, USA). Home cage activity variables, including locomotion (distance travelled, locomotor velocity), activity time, rearing up counts, and sleep/wake states were recorded.

Activity and sleep assessments

The SmartCage enables simultaneous assessment of locomotion and wake/sleep assessments as previously published (Xie et al., 2012; Luo et al., 2014). Each mouse was placed into a freshly prepared cage for 12 hours (6-hour dark and 6-hour light). The animal activities, including distance travelled, locomotor velocity, and rearing up counts, were determined by photo-beam breaks. Automated data were analyzed using CageScore software (AfaSci, Inc.). For the wake/sleep assessment, a vibration floor sensor was used to monitor sleep and wake states, as previously published (Xie et al., 2012). The sleep pattern of rodents was steady because the floor sensor picked up the breathing pattern of a sleeping rodent. The activity assessments were performed at 1, 2, and 7 days after injury.

Anxiety-related behavior

The SmartCage system was also used to measure anxiety-related behavior. A dark red box (made in Plexiglass plastic; 16.5 cm × 11.0 cm × 13.5 cm) with an opening (3.0 cm × 3.0 cm) was inserted in a fresh mouse home cage (**Figure 1D**). The red acrylic walls did not interfere with the SmartCage sensors; and the position and movement of the tested mice could be monitored by the infrared sensors. Under enhanced light, the home cage could measure the place preference of a rodent in the light/dark cage (Rodgers and Shepherd, 1993; Tang et al., 2002; Schumacher et al., 2012; Xie et al., 2012). The mouse was placed in the cage for 10 minutes. The CageScore software automatically calculated the time spent in each compartment, as well as the number of entries between different compartments. The anxiety-related behavior was performed at 1, 2, and 7 days after injury.

Neurological test

A composite neurological test was used as a standard measure to assess injury severity on motor function in the TBI model as previously described (Longhi et al., 2004; Mbye et al., 2009; Liu et al., 2013). Testing was done at 1, 2, and 7 days after injury. Briefly, mice were given a score from 0 (severely impaired) to 4 (normal) for each of the following three indices: forelimb function, hindlimb function, and resistance to lateral pulsion. A composite neurological score (0–12 points) was generated by combining the scores for each of these three tests.

Measurement of volumetric tissue loss

Seven days after CCI, animals were anesthetised and perfused intracardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The brains were dissected out, embedded, and sectioned coronally and serially at 25 μm in six sets. One set of sections was stained with Cresyl-Violet-Eosin (Cayman Chemical Company, Ann Arbor, MI, USA). For each section, contours of ipsilateral cortical contusion (tissue lost) and corresponding contralateral sections were measured using a Neurolucida System (MBF Bioscience, Williston, VT, USA). Borders between healthy (right side) and necrotic cortex (left side) were generally sharp and easily identified. The percentage of volumetric tissue loss was calculated by the ratio of the cortical lesion volume in the ipsilateral cortex divided by the entire contralateral cortex volume.

Histology and immunohistochemistry

Fluoro-Jade B (FJB) was used for the histological staining of degenerating neurons as previously described (Hopkins et al., 2000; Schmued and Hopkins, 2000; Gao et al., 2008). For FJB staining, brain sections were first incubated in 1% alkaline (NaOH) in 80% ethanol for 5 minutes, followed by graded ethanol (75%, 50%, and 25%; 5 minites each) and distilled water. The slides were then transferred to 0.06% potassium permanganate for 10 minutes on a rotating stage. After rinsing in distilled water for 2 minutes, the slides were incubated in 0.0004% FJB solution (Histo-Chem Inc., Jefferson, AR, USA) and 0.0004% DAPI (Sigma, St. Louis, MO, USA) for 20 minutes. Sections were rinsed in distilled water for 2 minutes and then rapidly air dried on a slide warmer. The dry slides were dehydrated in xylene (2 minutes) and mounted with DPX (VWR, Radnor, PA, USA). For immunostaining, brain sections were rinsed in PBS three times and incubated in blocking solution (0.1% Triton X-100, 1% bovine serum albumin, and 5% normal goat serum in PBS) for 1 hour at room temperature. Sections were then incubated with primary antibodies overnight, including anti-ionized calcium binding adaptor molecule 1 (Iba-1) in PBST containing 5% normal goat serum for microglia (1:200, goat anti-mouse; Abcam, Cambridge, MA, USA) and anti-glial fibrillary acidic protein (GFAP) in PBST containing 5% normal donkey serum for astrocytes (1:1,000, rabbit anti-mouse; EMD Millipore, Billerica, MA, USA). The next day, Alexa Fluor® 488 (1:500, donkey anti-goat; Thermo Fisher, Grand Island, NY, USA) and Alexa Fluor® 594 (1:500, goat anti-rabbit; Thermo Fisher, Grand Island, NY, USA) were applied. The sections were analyzed using an inverted microscopy system (Zeiss Axiovert 200 M, Oberkochen, Germany) combined with apotome and interfaced with a digital camera (Zeiss Axio Cam MRc5) controlled by a computer, then images were captured using apotome microscopy (AxioVision, v4.8).

Statistical analysis

All data were presented as the mean ± SEM values and analyzed using GraphPad-Prism 6 (La Jolla, CA, USA). Two-way repeated measures analysis of variance and Tukey's *post-hoc* tests were used for analysis of rearing up counts, distance travelled and locomotor velocity, average percentage of sleep time spent in the dark and light cycles, and anxiety-related behavior. Student's *t*-test was used for histological analyses. A P value of < 0.05 was considered statistically significant.

Results

Spontaneous motor activity

We first used the SmartCage system to monitor motor activities of mice in their home cage for a 12-hour period with the data collected at each hour. At 1, 2, and 7 days post-injury, CCI mice exhibited a dramatic decrease in travelled distance during the dark phase (1–6 hours) but showed no difference during the light phase as compared with sham controls (**Figure 2A**, **D**, **G**, **J**, and **M**). Similarly, rearing up counts were significantly reduced in mice receiving CCI which occurred mainly during the dark phase at 1 and 2 days compared to the sham control (**Figure 2B**, **E**, **K**). The rearing up counts at 7 days were slightly, but not significantly, lower in the CCI group than in the sham group (**Figure 2H**, **K**). No significant changes in locomotor velocity were found between the CCI and sham groups (**Figure 2C**, **F**, **I**, and **L**). A strong correlation was found not only between motor activities and neurological scores (**Figure 3A**, **B**), but also between motor activities and tissue damage (**Figure 3D**, **E**). Individual representative travel patterns demonstrated that CCI decreased spontaneous activities (**Figure 2M**).

Sleep and wake states

Excessive sleepiness is a common sleep disturbance that was previously reported among TBI patients (Castriotta et al.,

2007; Verma et al., 2007; Baumann, 2012). We then investigated the daytime and nighttime sleepiness of the mice and determined the average percentage of sleep time spent in the dark and light boxes. At 1 day after injury, a significant increase in the average percentage of sleep time was found in CCI-injured mice during both the dark and light cycles, compared with the sham group (**Figure 4A**, **D–F**). At 2 days post-CCI, the average percentage of sleep time was greater in the CCI group than in the sham group mainly in the dark cycle (**Figure 4B**, **D–F**). At 7 days post-injury, no statistically significant difference in the average percentage of sleep time was found between the CCI and sham groups (**Figure 4C–F**). Repeated measures analysis of variance revealed a statistically significant difference in the scores of the CCI mice between 2 and 7 days in the dark cycle (**Figure 4D**), between 1 and 7 days in the light cycle (**Figure 4E**), and 1, 2, and 7 days post-injury in the dark and light cycles (**Figure 4F**). A significant correlation was obtained between the average percentage of sleep time and tissue damage (**Figure 3F**).

Anxiety-related behavior

We next assessed anxiety-related behavior by counting the number of entries between the two different compartments in the two groups (**Figure 1**). SmartCage analysis showed that the number of transitions between the two compartments in CCI mice was significantly less than in the sham mice at 1 day (**Figure 1A**). To further validate the assessment of injury-induced anxiety-like behavior, we compared the results with time spent in the dark compartment between the two groups. A significant increase in time spent in the dark compartment was observed in the CCI group at 1 and 7 days post-injury although it did not reach a statistically significant level at 2 days post-injury (**Figure 1B**). Three-dimensional plots for the anxiety-like behavior showed that the CCI mice spent less time in the dark compartment (**Figure 1E**, **F**). These results revealed that CCI mice were anxious. A strongly significant correlation was obtained between time in the dark box and tissue damage (**Figure 3C**).

Composite neurological score and histology

We examined neurological score and histology after CCI. The neurological score was significantly decreased at 1, 2, and 7 days after CCI ($P < 0.01$, *vs.* sham group; **Figure 5A**). Repeated measures analysis of variance also revealed a statistically significant difference of the scores between 1 and 7 days after CCI, suggesting that mice recovered to a certain extent on day 7 as compared with day 1. Consistent with the behavior results, remarkable cortical damage was found after CCI (**Figure 5B**, **D**, **E**). There was an inverse correlation between the neurological score and cortical lesion volume after the CCI ($r = –0.97$, $P < 0.001$; **Figure 5C**). We compared the appearance of neurons, astrocytes, and microglia between the two groups at 7 days after CCI. FJB-positive neurons were not detected in the cortex of the sham mice (**Figure 5D**') but were shown in the CCI mice (**Figure 5E**'). In the sham mice, microglia with typical spiny processes were found (**Figure 5D**"). Following the CCI, an increase in the number of activated microglia was observed (**Figure 5E**"). These reactive microglia showed enlarged cell bodies and intense immunoreactivity, and were predominately located near the cortical injury site. In the sham group, only a small number of scattered astrocytes exhibiting detectable levels of GFAP were found (**Figure 5D**"'). However, following CCI, robust reactive astrogliosis with markedly increased expression of GFAP was found particularly at the cortical lesion border (**Figure 5E**"').

Discussion

Early neurological deficits have clinically shown to be the major predictors of TBI outcomes (King et al., 2005). In the present study, the quantitative characterizations of behavior elements in a freely behaving mouse provide powerful means for addressing the impact of TBI on neurological deficits during the early phase of TBI. An important feature of the SmartCage system is its integration of activity level detection with non-invasive sleep monitoring. Furthermore, an anxiety-related behavior can also be examined using the SmartCage system throughout the entire testing period. To the best of our knowledge, this is the first time that behavior deficits following a moderate CCI were tested using the SmartCage system. We also showed for the first time the sleep/wake states after CCI using the SmartCage in mice. We found that the moderate CCI induced motor deficits including decreases in travelled distance and rearing up. We also found sleep and anxiety disorders in mice following the moderate CCI. Importantly, these behavior deficits were strongly correlated with tissue damage. These results collectively indicate that the SmartCageis a sensitive, objective, and reliable approach that can be used to detect early behavior changes following a moderate TBI.

Rodent CCI models are widely used in experimental TBI research. CCI is produced by driving a rigid impactor onto the exposed, intact dura and mimics cortical tissue loss, acute subdural hematoma, axonal injury, concussion, blood-brain barrier (BBB) dysfunction, and even coma (Dixon et al., 1991; Smith et al., 1995; Morales et al., 2005; Xiong et al., 2013b). The CCI model generates a reproducible injury with pathological features similar to human TBI (Kochanek et al., 2002; Manley et al., 2006). A localized tissue loss is a common phenomenon of such an injury. Using this injury model, we sought to determine whether a moderate CCI induced early neurological deficits can be reproduced using the SmartCage system.

There are several reasons for using a moderate CCI model in this study. First, moderate TBI is clinically one of the frequent TBI severities. Published studies have estimated that 80–85% of the TBI patients were diagnosed as mild, 10% as moderate, and 5–10% as severe TBIs (Miller, 1993; van der Naalt, 2001). Furthermore, diagnosis of moderate TBI is more reliable than diagnosis of mild TBI, and behavioral and emotional impairment measures were more reliable in moderate TBI than in mild TBI (Vakil, 2005). Importantly, the present study indicates that SmartCage can be reliably used to examine behavioral impairments associated with moderate TBI. Whether it can be used to differentially detect behavior impairments among different injury severities of

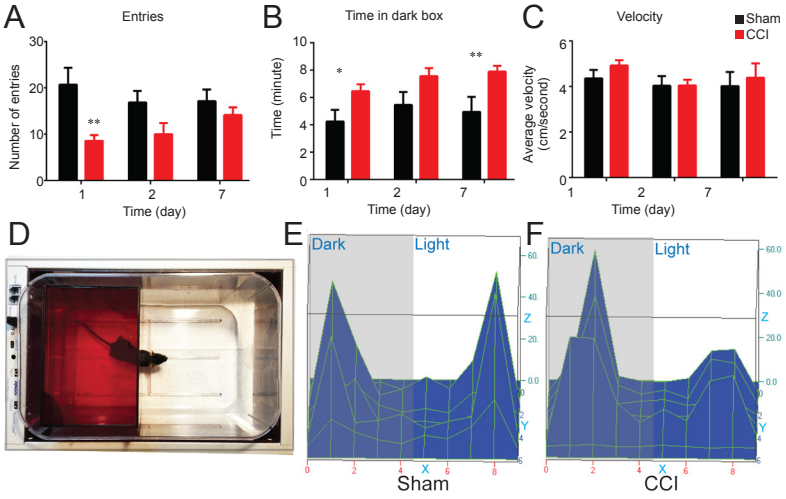

Figure 1 Controlled cortical impact (CCI) increased anxiety-like behavior in the SmartCage.
CCI mice actively avoided the light chamber as evidenced by a significant decrease in the number of entries between two compartments (A) and an increase in the time staying in the dark box (B). No significant difference in locomotor velocity was found between the sham and CCI mice (C). The dark box with an opening helped measure light/dark preference and monitor anxiety-related behavior (D). Anxiety-like behavior of mice in the sham (E) and CCI (F) groups in the dark compartment (gray area, left side) and light compartment (white area, right side). X-Y axis stands for the location in the SmartCage, and Z-axis is defined as time distribution. Data were expressed as the mean ± SEM and analyzed using two-way repeated measures analysis of varaince. $*P < 0.05$, $**P < 0.01$, *vs.* sham group (*post hoc* tests; n = 7 mice/group).

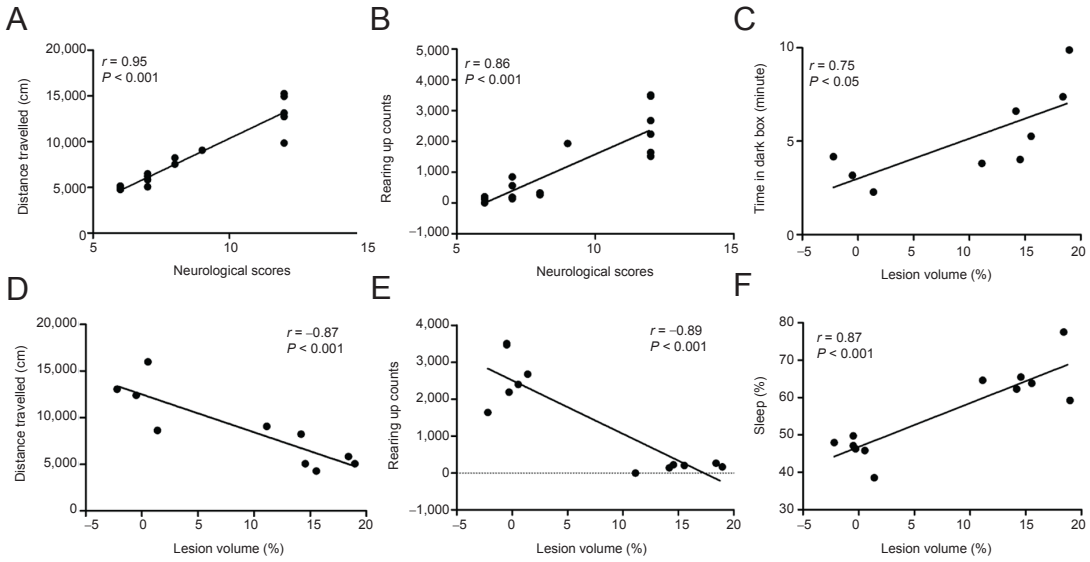

Figure 3 Correlation between neurological scores, tissue lesion volume , and automated activities.
High correlations were found between neurological scores and distance travelled (A), between neurological scores and rearing up counts (B), between lesion volume and time in dark box (C), between lesion volume and distance travelled (D), between lesion volume and rearing up counts (E), and between lesion volume and average perecentage of sleep time (F). The Pearson product moment correlation coefficient (r) and P-value are shown in A–F.

TBI remains to be determined.

To date, most of the studies in the TBI model have been short-term, in the range of hours to days, and have rarely extended beyond 1 month after injury (Marklund and Hillered, 2011; Xiong et al., 2013b). However, the behavioral data obtained at the early time points post-injury may not provide valid assessments of the long-term outcomes or clinical therapies for long-lasting efficacy. To verify whether early changes after TBI can predict the long-term outcomes, further studies evaluating injury responses and functional deficits over longer time periods should be conducted (Xiong et al., 2013b). In this study, we demonstrated that SmartCage was very sensitive in these behavior measures in short-term assessments. We will test long-term outcomes with SmartCage in future studies.

Locomotor activity is a key component in many behavior tests (Tang et al., 2002). In mice following TBI, neurological score is one of the most commonly used behavior assessments (Xiong et al., 2013a). In this study, neurological score was strongly correlated with tissue damage, consistent with a previous report (Xiong et al., 2013a). One advantage of using the SmartCage system is its ability to provide detailed quantitative assessments of spontaneous locomotor activity. We observed a greater reduction of spontaneous locomotor activity in mice receiving CCI at 1 and 2 days after CCI, which was strongly correlated with neurological score and tissue damage. The detection of locomotor activity may provide a powerful tool for early prediction of long-term outcomes. Taken together, these results support the interpretation that automated equipment of the SmartCage system is a useful tool to assess locomotor activity during the early phase of CCI. To evaluate whether the velocity influenced both

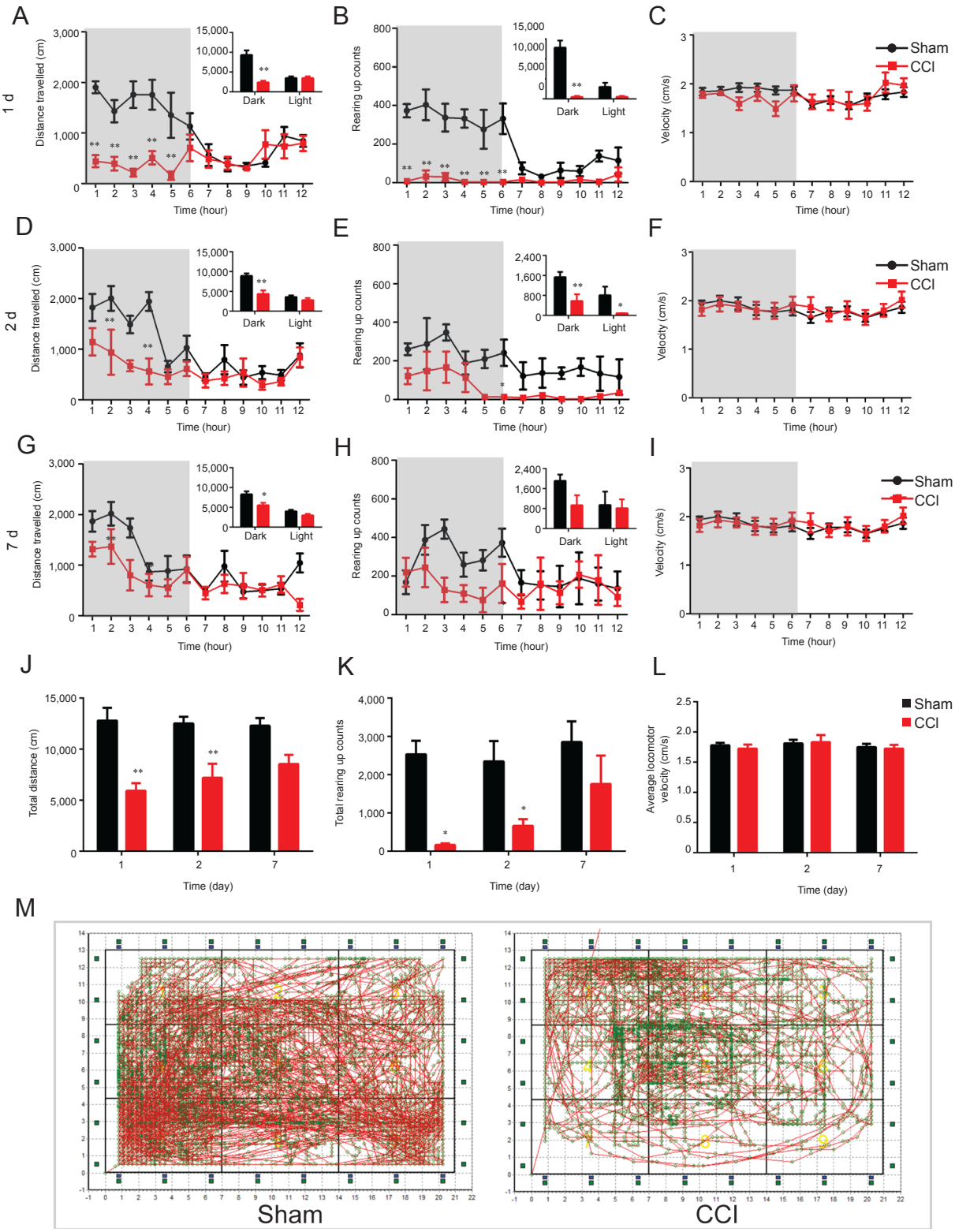

Figure 2 Controlled cortical impact (CCI) caused significantly reduced spontaneous activity in the SmartCage.
Spontaneous activity of mice in their home cages was measured over a 12-hour period during both the dark (gray area, left side) and light (white area, right side) phases in mice receiving either CCI or sham operation. Measurements include the distance travelled (left column), rearing up counts (middle column), and locomotor velocity (righ column) assessed at 1 (A–C), 2 (D–F), and 7 days (d) (G–I) post-injury. The total distance travelled (J), total rearing up counts (K), and average locomotor velocity (L) between the two groups at 1, 2, and 7 d were shown. Individual representative travel patterns demonstrated decreased spontaneous activity after CCI as compared with the sham group (M). Data were expressed as the mean ± SEM and analyzed by repeated measures two-way analysis of variance. *$P < 0.05$, **$P < 0.01$, *vs.* sham group (*post-hoc* tests; $n = 7$ mice/group).

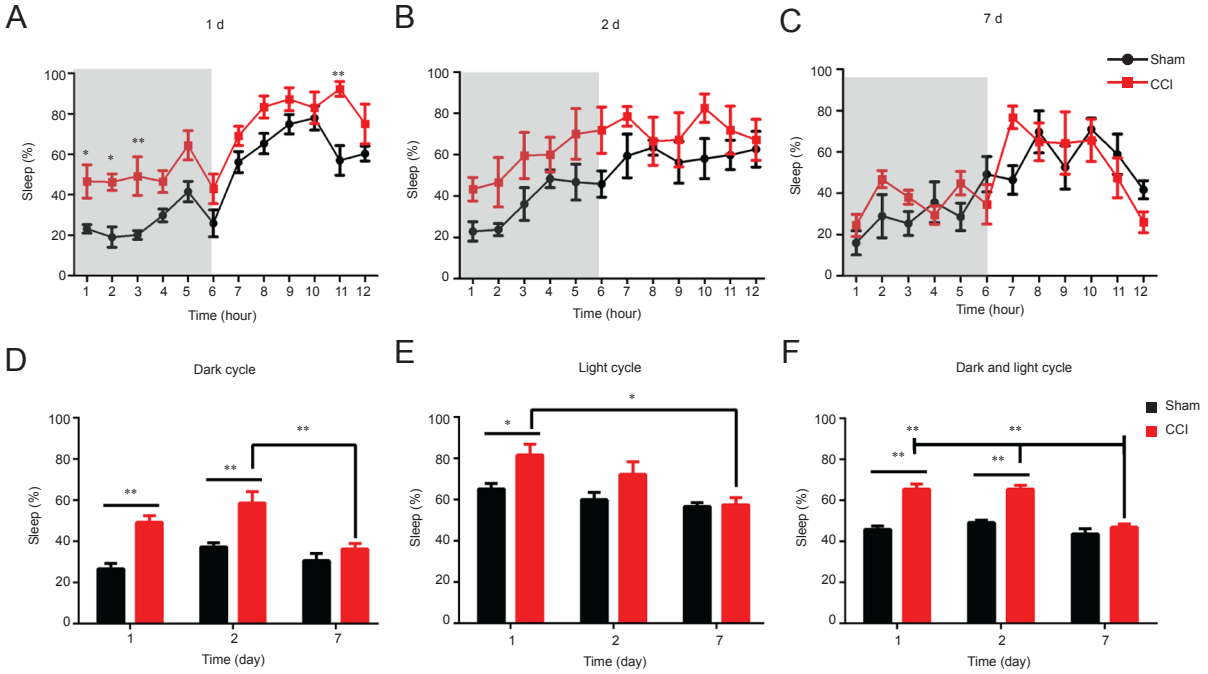

Figure 4 Controlled cortical impact (CCI) increased the average percentage of sleep time in the SmartCage.
Comparison of average percentage of sleep time at 1 (A), 2 (B), and 7 days (d) (C) in a 12 hour period using the SmartCage system. At 1 and 2 days post-CCI, changes in the average percentage of sleep time in the dark cycle (D), light cycle (E), and combined "dark and light" cycle (F) were found. CCI increased the average percentage of sleep time at 1 and 2 days post-injury, but showed no difference at 7 days post-injury. Data were expressed as the mean ± SEM and analyzed by repeated measures two-way analysis of variance. *$P < 0.05$, **$P < 0.01$, *vs.* sham group (*post hoc* tests; $n = 7$ mice/group) in A–C. *$P < 0.05$, **$P < 0.01$ in D–F. Grey area: dark cycle; white area: light cycle.

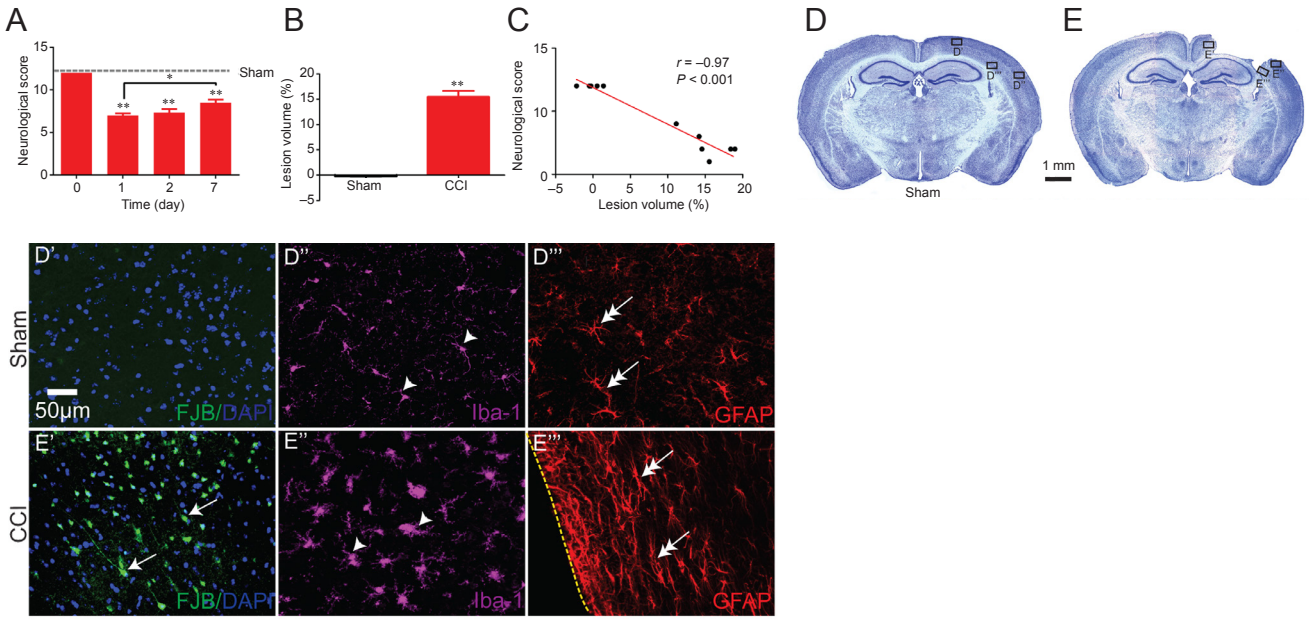

Figure 5 Controlled cortical impact (CCI) decreased neurological score and caused tissue damage.
After CCI, neurological score was significantly decreased at 1, 2, and 7 days (A) post-injury as compared with the sham group (black dash line) (data were expressed as the mean ± SEM and analyzed by repeated measures two-way analysis of variance. *$P < 0.05$. **$P < 0.01$, *vs.* sham group (0 day), *post hoc* tests; $n = 7$ mice/group). CCI induced significant percentage volumetric tissue loss in the ipsilateral cortex (B) (data were expressed as the mean ± SEM and analyzed by Student's *t*-test, **$P < 0.01$, *vs.* sham group, $n = 7$ mice/group). Significant correlation between neurological score and volumetric tissue lesion was found (C). Cresyl violet-stained coronal sections showed a sham (D) and a CCI mice (E) with brain tissue loss at the impact site at 7 days post-injury (scale bar: 1mm). Representative images of Fluoro Jade B (FJB) (green) and 4′,6-diamidino-2-phenylindole (DAPI) (blue) staining in the sham (D') and perilesional area of the CCI mice at 7 days post-injury (E'). FJB stained degenerating neurons were clearly seen (arrows). Ionized calcium-binding adapter molecule 1 (Iba-1) staining in the sham mice (D", arrowheads) and in the perilesional area of the CCI mice at 7 days post-injury (E", arrowheads). Glial fibrillary acidic protein (GFAP) staining in normal astrocytes (D"', double arrows) as well as in reactive astrocytes at the lesion border (E"', double arrows). After CCI, GFAP immunoreactivity was markedly increased particularly at the lesion border (E"', yellow dashed line). Scale bar: 50 μm.

spatial and temporal error detection in the current study, we studied the velocity using the SmartCage. The velocity of mice did not show any difference between both groups, demonstrating that CCI may lead to reduced alertness or neuropsychological impairment, which is in agreement with the previous study (Xiong et al., 2013a).

Sleep disturbances associated with TBI have been reported (Verma et al., 2007), affecting 30–70% of TBI survivors (Parcell et al., 2006). This disturbance significantly affects quality of life (Orff et al., 2009; Viola-Saltzman and Watson, 2012). Evidence suggests that the most common sleep-wake disturbances following TBI include fatigue, sleepiness, and posttraumatic hypersomnia (defined as increased sleep need per 24 hours) (Baumann, 2012). However, far less is known about the early association between TBI and sleep disturbance (Rowe et al., 2014b). In the current study, we used the non-invasive SmartCage to successfully monitor CCI-induced alterations in sleep during the early phase of the injury. It is noteworthy that sleep itself may be restorative and aid in the recovery of function following the injury (Rowe et al., 2014b). The average percentage of sleep time in the CCI group was significantly higher than in the sham group, indicating that more sleep is needed following CCI. Acute posttraumatic sleep significantly increased when compared with the sleep of the uninjured sham group in both dark and light cycles. It is possible that TBI-induced injury contributes to circadian rhythm sleep disorders (Boone et al., 2012; Rowe et al., 2014b). Furthermore, locomotor activity and sleep/wake states detected in the present study were assessed simultaneously to provide a framework for studying behavior integration and organization. So far, there has been no conclusion that posttraumatic sleep can enhance functional recovery (Rowe et al., 2014a). This is a good parameter for sleep monitoring during the early phase following TBI. Altogether, our data, for the first time, support the hypothesis that moderate CCI might improve acute posttraumatic sleep in the mice using the SmartCage system.

The increase in anxiety, a characteristic feature following TBI, is consistent with the previous results using various measures of anxiety (Silver et al., 2009; Mallya et al., 2014). In this study, we compared the number of entries and light-dark preference in mice, two assessments which are sensitive to detect the anxiety behavior induced by TBI (Washington et al., 2012). We found that CCI mice exhibited a significant decrease in terms of the number of entries at 1 day after injury, but showed similar velocity in the locomotion assessment. Other groups also reported similar anxiety-like behavior changes at early times (Malkesman et al., 2013). In the current study, CCI mice spent more time in the dark compartment than the sham mice during the entire observation period. These results further support the notion that CCI mice display lower exploration and higher anxiety-like behavior, which are consistent with previously reported anxiety-like behavior (Jones et al., 2008; Baratz et al., 2009; Schwarzbold et al., 2010; Khroyan et al., 2012). Lastly, the anxiety behavior observed in the present study was highly correlated with tissue damage.

In summary, we demonstrated early behavior changes after a moderate CCI using a novel SmartCagesystem. This system was shown to objectively and reliably detect motor, sleep, and anxiety changes in mice with SCI. The automated home cage can minimize an animal's stress and increase the consistency of results, which can be used to test global and subtle behavior changes. With the SmartCage system, more specialized behavior (sleep/wake states and anxiety-like behavior) can be assessed simultaneously with motor behavior (distance, rearing up, and velocity). This system could be used as a sensitive measure to examine different injury models and therapeutic efficacy following TBI.

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References

Baratz R, Rubovitch V, Frenk H, Pick CG (2009) The influence of alcohol on behavioral recovery after mTBI in mice. J Neurotrauma 27:555-563.

Basso DM, Beattie MS, Bresnahan JC (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 12:1-21.

Baumann CR (2012) Traumatic brain injury and disturbed sleep and wakefulness. Neuromolecular Med 14:205-212.

Bondi CO, Semple BD, Noble-Haeusslein LJ, Osier ND, Carlson SW, Dixon CE, Giza CC, Kline AE (2015) Found in translation: understanding the biology and behavior of experimental traumatic brain injury. Neurosci Biobehav Rev 58:123-146.

Boone DR, Sell SL, Micci MA, Crookshanks JM, Parsley M, Uchida T, Prough DS, DeWitt DS, Hellmich HL (2012) Traumatic brain injury-induced dysregulation of the circadian clock. PLoS One 7:e46204.

Castriotta RJ, Wilde MC, Lai JM, Atanasov S, Masel BE, Kuna ST (2007) Prevalence and consequences of sleep disorders in traumatic brain injury. J Clin Sleep Med 3:349-356.

Chauhan NB, Gatto R, Chauhan MB (2010) Neuroanatomical correlation of behavioral deficits in the CCI model of TBI. J Neurosci Methods 190:1-9.

Dikmen SS, Machamer JE, Winn HR, Temkin NR (1995) Neuropsychological outcome at 1-year post head injury. Neuropsychology 9:80-90.

Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL (1991) A controlled cortical impact model of traumatic brain injury in the rat. J Neurosci Methods 39:253-262.

Gao X, Deng-Bryant Y, Cho W, Carrico KM, Hall ED, Chen J (2008) Selective death of newborn neurons in hippocampal dentate gyrus following moderate experimental traumatic brain injury. J Neurosci Res 86:2258-2270.

Goulding EH, Schenk AK, Juneja P, MacKay AW, Wade JM, Tecott LH (2008) A robust automated system elucidates mouse home cage behavioral structure. Proc Natl Acad Sci U S A 105:20575-20582.

Hopkins KJ, Wang G, Schmued LC (2000) Temporal progression of kainic acid induced neuronal and myelin degeneration in the rat forebrain. Brain Res 864:69-80.

Jhuang H, Garrote E, Yu X, Khilnani V, Poggio T, Steele AD, Serre T (2010) Automated home-cage behavioural phenotyping of mice. Nat Commun 1:68.

Jones NC, Cardamone L, Williams JP, Salzberg MR, Myers D, O'Brien TJ (2008) Experimental traumatic brain injury induces a pervasive hyperanxious phenotype in rats. J Neurotrauma 25:1367-1374.

Kas MJ, de Mooij-van Malsen AJ, Olivier B, Spruijt BM, van Ree JM (2008) Differential genetic regulation of motor activity and anxiety-related behaviors in mice using an automated home cage task. Behav Neurosci 122:769-776.

Khroyan TV, Zhang J, Yang L, Zou B, Xie J, Pascual C, Malik A, Xie J, Zaveri NT, Vazquez J, Polgar W, Toll L, Fang J, Xie X (2012) Rodent motor and neuropsychological behaviour measured in home cages using the integrated modular platform SmartCage™. Clin Exp Pharmacol Physiol 39:614-622.

King JT, Carlier PM, Marion DW (2005) Early Glasgow Outcome Scale scores predict long-term functional outcome in patients with severe traumatic brain injury. J Neurotrauma 22:947-954.

Kochanek PM, Hendrich KS, Dixon CE, Schiding JK, Williams DS, Ho C (2002) Cerebral blood flow at one year after controlled cortical impact in rats: assessment by magnetic resonance imaging. J Neurotrauma 19:1029-1037.

Liu NK, Zhang YP, Zou J, Verhovshek T, Chen C, Lu QB, Walker CL, Shields CB, Xu XM (2014) A semicircular controlled cortical impact produces long-term motor and cognitive dysfunction that correlates well with damage to both the sensorimotor cortex and hippocampus. Brain Res 1576:18-26.

Liu NK, Zhang YP, O'Connor J, Gianaris A, Oakes E, Lu QB, Verhovshek T, Walker CL, Shields CB, Xu XM (2013) A bilateral head injury that shows graded brain damage and behavioral deficits in adultmice. Brain Res 1499:121-128.

Longhi L, Watson DJ, Saatman KE, Thompson HJ, Zhang C, Fujimoto S, Royo N, Castelbuono D, Raghupathi R, Trojanowski JQ, Lee VM, Wolfe JH, Stocchetti N, McIntosh TK (2004) Ex vivo gene therapy using targeted engraftment of NGF-expressing human NT2N neurons attenuates cognitive deficits following traumatic brain injury in mice. J Neurotrauma 21:1723-1736.

Luo J, Nguyen A, Villeda S, Zhang H, Ding Z, Lindsey D, Bieri G, Castellano JM, Beaupre GS, Wyss-Coray T (2014) Long-term cognitive impairments and pathological alterations in a mouse model of repetitive mild traumatic brain injury. Front Neurol 5:12.

Malkesman O, Tucker LB, Ozl J, McCabe JT (2013) Traumatic brain injury - modeling neuropsychiatric symptoms in rodents. Front Neurol 4:157.

Mallya S, Sutherland J, Pongracic S, Mainland B, Ornstein TJ (2014) The manifestation of anxiety disorders after traumatic brain injury: a review. J Neurotrauma 32:411-421.

Manley GT, Rosenthal G, Lam M, Morabito D, Yan D, Derugin N, Bollen A, Knudson MM, Panter SS (2006) Controlled cortical impact in swine: pathophysiology and biomechanics. J Neurotrauma 23:128-139.

Marklund N, Hillered L (2011) Animal modelling of traumatic brain injury in preclinical drug development: where do we go from here? Br J Pharmacol 164:1207-1229.

Mbye LH, Singh IN, Carrico KM, Saatman KE, Hall ED (2009) Comparative neuroprotective effects of cyclosporin A and NIM811, a nonimmunosuppressive cyclosporin A analog, following traumatic brain injury. J Cereb Blood Flow Metab 29:87-97.

Miller JD (1993) Head injury. J Neurol Neurosurg Psychiatry 56:440-447.

Millis SR, Rosenthal M, Novack TA, Sherer M, Nick TG, Kreutzer JS, High WM Jr, Ricker JH (2001) Long-term neuropsychological outcome after traumatic brain injury. J Head Trauma Rehabil 16:343-355.

Morales DM, Marklund N, Lebold D, Thompson HJ, Pitkanen A, Maxwell WL, Longhi L, Laurer H, Maegele M, Neugebauer E, Graham DI, Stocchetti N, McIntosh TK (2005) Experimental models of traumatic brain injury: do we really need to build a better mousetrap? Neuroscience 136:971-989.

Orff HJ, Ayalon L, Drummond SP (2009) Traumatic brain injury and sleep disturbance: a review of current research. J Head Trauma Rehabil 24:155-165.

Parcell DL, Ponsford JL, Rajaratnam SM, Redman JR (2006) Self-reported changes to nighttime sleep after traumatic brain injury. Arch Phys Med Rehabil 87:278-285.

Rodgers RJ, Shepherd JK (1993) Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. Psychopharmacology (Berl) 113:237-242.

Rowe RK, Harrison JL, O'Hara BF, Lifshitz J (2014a) Recovery of neurological function despite immediate sleep disruption following diffuse brain injury in the mouse: clinical relevance to medically untreated concussion. Sleep 37:743-752.

Rowe RK, Striz M, Bachstetter AD, Van Eldik LJ, Donohue KD, O'Hara BF, Lifshitz J (2014b) Diffuse brain injury induces acute post-traumatic sleep. PLoS One 9:e82507.

Schmued LC, Hopkins KJ (2000) Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. Brain Res 874:123-130.

Schumacher T, Krohn M, Hofrichter J, Lange C, Stenzel J, Steffen J, Dunkelmann T, Paarmann K, Fröhlich C, Uecker A, Plath AS, Sommer A, Brüning T, Heinze HJ, Pahnke J (2012) ABC transporters B1, C1 and G2 differentially regulate neuroregeneration in mice. PLoS One 7:e35613.

Schwarzbold ML, Rial D, De Bem T, Machado DG, Cunha MP, dos Santos AA, dos Santos DB, Figueiredo CP, Farina M, Goldfeder EM, Rodrigues ALS, Prediger RDS, Walz R (2010) Effects of traumatic brain injury of different severities on emotional, cognitive, and oxidative stress-related parameters in mice. J Neurot rauma 27:1883-1893.

Sherer M, Sander AM, Nick TG, High WM Jr, Malec JF, Rosenthal M (2002) Early cognitive status and productivity outcome after traumatic brain injury: findings from the TBI model systems. Arch Phys Med Rehabil 83:183-192.

Silver JM, McAllister TW, Arciniegas DB (2009) Depression and cognitive complaints following mild traumatic brain injury. Am J Psychiatry 166:653-661.

Smith DH, Soares HD, Pierce JS, Perlman KG, Saatman KE, Meaney DF, Dixon CE, McIntosh TK (1995) A model of parasagittal controlled cortical impact in the mouse: cognitive and histopathologic effects. J Neurotrauma 12:169-178.

Tang X, Orchard SM, Sanford LD (2002) Home cage activity and behavioral performance in inbred and hybrid mice. Behav Brain Res 136:555-569.

Vakil E (2005) The effect of moderate to severe traumatic brain injury (TBI) on different aspects of memory: a selective review. J Clin Exp Neuropsychol 27:977-1021.

van der Naalt J (2001) Prediction of outcome in mild to moderate head injury: a review. J Clin Exp Neuropsychol 23:837-851.

Verma A, Anand V, Verma NP (2007) Sleep disorders in chronic traumatic brain injury. J Clin Sleep Med 3:357-362.

Viola-Saltzman M, Watson NF (2012) Traumatic brain injury and sleep disorders. Neurol Clin 30:1299-1312.

Washington PM, Forcelli PA, Wilkins T, Zapple DN, Parsadanian M, Burns MP (2012) The effect of injury severity on behavior: a phenotypic study of cognitive and emotional deficits after mild, moderate, and severe controlled cortical impact injury in mice. J Neurotrauma 29:2283-2296.

Xie X, Zhang J, Zou B, Xie J, Fang J, Zaveri N, Khroyan T (2012) Rodent Behavioral Assessment in the Home Cage Using the SmartCage™ System. In: Animal Models of Acute Neurological Injuries II (Chen J, Xu XM, Xu ZC, Zhang JH, eds), pp 205-222. New York: Humana Press.

Xiong X, Barreto GE, Xu L, Ouyang YB, Xie X, Giffard RG (2011) Increased brain injury and worsened neurological outcome in interleukin-4 knockout mice after transient focal cerebral ischemia. Stroke 42:2026-2032.

Xiong X, White RE, Xu L, Yang L, Sun X, Zou B, Pascual C, Sakurai T, Giffard R, Xie X (2013a) Mitigation of murine focal cerebral ischemia by the hypocretin/orexin system is associated with reduced inflammation. Stroke 44:764-770.

Xiong Y, Mahmood A, Chopp M (2013b) Animal models of traumatic brain injury. Nat Rev Neurosci 14:128-142.

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