Prior housing conditions and sleep loss may affect recovery from brain injury in rats: A pilot study

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Abstract—The purpose of this study is to understand the effect of combat-associated conditions such as sleep deprivation (SD) on subsequent traumatic brain injury (TBI). Prior to TBI (or sham surgery) induced by controlled cortical impact (CCI), rats were housed singly in chambers that prevented rapid eye movement sleep or allowed unrestricted sleep (no SD). Sensorimotor function was tested pre-SD and retested on postoperative days (PDs) 4, 7, and 14. Two additional control groups were housed socially prior to either CCI or sham surgery. CCI resulted in immediate performance deficits on sensorimotor tasks. The PD on which performance returned to baseline depended on preinjury conditions. Overall, preinjury SD+CCI resulted in an earlier recovery than no SD+CCI, and the no SD+CCI group (housed singly under conditions comparable with the SD group) recovered slower than all other groups. These data are the first to raise the possibility that recovery of sensorimotor function following TBI is affected by preinjury conditions. The data suggest that preinjury SD 24 h in duration may result in faster recovery and that novel or social isolation conditions may impede recovery. Thus, the combat environment may contribute to complexities associated with TBIs common in U.S. servicemembers.

INTRODUCTION

Traumatic brain injury (TBI) has been labeled the signature injury of the conflicts in Iraq and Afghanistan [1–2]. While TBIs experienced by servicemembers in the combat theater are variable, several aspects of the environment are constant. Combat operations are associated with high stress and prolonged periods of sleep deprivation (SD) [3–4]. However, most clinical studies of TBI have been in populations where the environment is relatively constant and secure [5]. Individuals with sports-related TBI are often participating in activities for which they are typically well rested and they have physiologically and psychologically prepared their bodies [6–7].

Abbreviations: ANOVA = analysis of variance, CCI = controlled cortical impact, NH = normal housing, PBS = phosphate-buffered saline, PD = postoperative day, REM = rapid eye movement, SD = sleep deprivation, TBI = traumatic brain injury.

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While members of this population are relatively homogeneous in nature and similar in age to the typical service-member, they diverge from the servicemember in the injury environment, background physical state, and stress level [6].

Stress [8–9] and SD [10–11] have known effects on nervous system function of both humans [12] and animals [13]. Our pilot study sought to better define the effects of preinjury SD on recovery in an animal model of TBI. Animal models of SD have demonstrated altered glucose metabolism [14], increased pro-inflammatory cytokine production [15], abnormal neuronal excitability [16–17], and altered thermoregulation [18], all of which theoretically could be associated with increased pathology and behavioral deficits following TBI. Changes in glucose metabolism could increase tissue pH and place greater oxidative stress on the injured brain [19]. Altered thermoregulation with increases in temperature could also be deleterious to the injured brain [20]. Additionally, in animal models, SD yields significant changes in gene expression and neurogenesis [21–22], which could dramatically affect the injury response. Finally, both genetics [23–24] and biochemical changes induced by stressful conditions [25] are thought to have an effect on TBI severity. Combining validated models for rapid eye movement (REM) SD and TBI allows for a better understanding of the effect of the combat lifestyle on the quintessential combat injury. While other studies [26] have considered the effect of stress following injury, this is the first study to assess the effect of preinjury conditions. Although pre-injury conditions are virtually inaccessible in terms of treatment, the nature of their effect on disease progression may prove elemental to optimizing treatment strategies.

**METHODS**

We used 32 male Long Evans Blue Spruce rats (72–78 d of age and 250–315 g, Harlan Laboratories; Indianapolis, Indiana). Before experimental manipulation, we housed all animals socially with food and water available ad libitum.

We trained the rats daily for 5 d on both beam walk and adhesive removal tasks 7 d prior to performing controlled cortical impact (CCI) surgery to induce TBI. For the beam walk task, we trained the rats to traverse a 1.0 m-long narrow beam (width: 2.0 cm) elevated 90 cm for five trials per day. On day 5 of training, we recorded baseline performance measures. All rats were preoperatively able to transverse the beam without footslips and enter a goal box at the end of the beam. We allowed rats to stay in the goal box for 30 s before returning them to their home cage. We retested the rats on the beam on postoperative days (PDs) 4, 7, and 14. We recorded the time spent traversing the beam and distances reached and calculated speed as distance/time. We motivated the rats to cross the beam to escape white noise aversive stimuli and to receive a treat (8in1 Yogies for Rats, Ecotrition, United Pet Group; Cincinnati, Ohio). However, since after surgery all the rats fell off the beam before reaching the goal box, we made performance comparisons by assessing speed rather than time to reach the goal box. We placed the rats in their home cage for 2 min between each trial.

For the adhesive tab removal task, training also began 7 d prior to surgery and lasted 5 d. We trained rats to remove a round (0.75 in.) adhesive sticker (no. 5466, Avery; Brea, California) from the distal radial surface of each forelimb. On day 5 of training, we recorded baseline performance. We administered two trials per forelimb 5 min apart and terminated each trial when the adhesive tab was removed or 2 min had elapsed. We recorded latencies to remove each tab 3 d prior to injury and on PDs 4, 7, and 14.

On preoperative day 3, we completed training and baseline sensorimotor function assessment and randomly assigned the rats to one of four experimental groups: (1) 24 h of SD followed by CCI (SD+CCI group), (2) 24 h of housing in platform over water chambers with no SD followed by CCI (no SD+CCI group), (3) normal housing (NH; social housing in standard rodent cages) before CCI (NH+CCI group), or (4) NH before sham surgery (NH+sham group).

One day prior to surgery, we moved the rats in the SD+CCI and no SD+CCI groups from NH conditions to single housing in chambers with small or large platforms, respectively. We housed the SD+CCI group rats singly on a small platform (diameter: 10.0 cm) secured to a base placed in an opaque plastic 5 gal container at an elevation of 10.16 cm from the bottom of the container. Water filled the container to a level surrounding the platform. The small platform was large enough to support the rat when it was awake but too small to support the body and head while it was asleep. When the rat entered REM sleep and lost muscle tone, the rat’s head would fall into the water and wake it. Preinjury housing for the no SD+CCI group was the same as the SD+CCI group, except the platform was large enough (diameter: 14.0 cm) to allow the rat to
enter REM sleep without contacting the water [27]. Food and water were available ad libitum to the rats while they were in the platform over water conditions.

At the end of the 24 h SD and no SD period, the rats underwent a TBI via CCI while the NH rats received either a CCI or a sham surgery. We used the CCI model of TBI to produce a unilateral injury to the sensorimotor cortex. We collected sensorimotor performance measures on PDs 4, 7, and 14. On PD 15, we intracardially perfused the rats with phosphate-buffered saline (PBS) containing 4 percent paraformaldehyde. We removed the brains and postfixed them in 4 percent paraformaldehyde for 24 h, followed by incubation in cryoprotectant. We collected one 50 \( \mu \)m frozen section every 200 \( \mu \)m between +1.1 mm and −3.8 mm relative to bregma.

We conducted all surgical procedures using aseptic conditions. We placed the rats on a 37°C heating pad to maintain a physiologically normal body temperature and anesthetized them with 4 percent isoflurane in a mixture of 30 percent nitrous oxide and 70 percent oxygen delivered through a nose cone and mounted in a stereotaxic device, with their heads fixed in a horizontal position throughout the procedure. We used a high-speed dental drill to create a 5.0 mm craniotomy positioned at bregma and 2.0 mm from midline over the left sensorimotor cortex, keeping the dura intact. We used a probe (diameter: 3.0 mm) attached to an impact device (My Neurolab; St. Louis, Missouri) to compress the brain a depth of 2.0 mm at a speed of 3.0 m/s in order to cause mild to moderate trauma [28]. Sham-injured rats received the same anesthesia and craniotomy but were not subjected to TBI. After we sutured the scalp, we allowed all rats to recover from surgery on a heating pad maintained at 37°C. In all cases, the surgeon was blinded to the treatment status of the rat. We administered buprenorphine, an analgesic, at a dosage of 0.05 mg/kg subcutaneously for 24 h postoperatively.

On PD 15, we gave the rats a lethal overdose of sodium pentobarbital followed by cardiac perfusion. We collected the brains and postfixed them for 24 h in 4 percent paraformaldehyde followed by cryoprotection in 30 percent sucrose in PBS overnight. We prepared coronal brain sections 50 \( \mu \)m in thickness and stained them with cresyl violet. We took digital images of one section every 500 \( \mu \)m and analyzed percent lesion size by tracing the entire area of each hemisphere and calculating the percent area of the ipsilateral as compared with the contralateral hemisphere area using ImageJ (National Institutes of Health; Bethesda, Maryland).

We conducted a repeated-measures analysis of variance (ANOVA) (group × day) on the data to assess beam walk speeds and latency to remove adhesive tabs. Raw adhesive tab removal latencies were log-transformed because of lack of sphericity (Mauchly \( p < 0.01 \)). We conducted separate pairwise comparisons between baseline and PDs 4, 7, and 14 for each group, controlling for family-wise error rate across the tests at 0.05 level based on Bonferroni post hoc tests. For lesion volume, we conducted a one-way ANOVA. We calculated all tests using standard procedures (SPSS version 17.0 for Windows, IBM Corporation; Armonk, New York). We used a significance level of \( p < 0.05 \) for all statistical analyses.

RESULTS

We examined the effects of preinjury SD and no SD on recovery of hind-limb motor function using a modified beam walk task; results are plotted in Figure 1. For each test...
day (baseline and PDs 4, 7, and 14), we calculated beam speed as the distance traveled on the beam divided by time to reach the goal box or fall from the beam. There was a significant effect of day (Greenhouse-Geisser $F(1.64,37.81) = 29.99, p < 0.001$). All rats were able to traverse the length of the beam at comparable speeds (no significant differences) at baseline. Following CCI, however, few rats were able to reach the goal box before falling off the beam, making the distance each rat traveled variable. Thus, we presented these data as speed measurements rather than latency. We conducted separate pairwise comparisons using $t$-tests between baseline and PDs 4, 7, and 14 for each group. Based on $t$-tests, beam speeds were significantly slower between baseline and PD 4 for all groups (SD+CCI: $t(6) = 5.67, p = 0.01$; no SD+CCI: $t(5) = 4.89, p < 0.01$; NH+CCI: $t(5) = 5.51, p < 0.01$; NH+sham: $t(7) = 4.18, p < 0.01$). We expected no effect of surgery on performance in the NH+sham group. At PD 4, however, there appeared to be a significant effect of the craniotomy alone as has been reported by others [29].

On PD 7, the no SD+CCI group remained significantly ($t(5) = 3.18, p < 0.05$) slower than baseline speeds, while we found no significant differences in speed comparing baseline and PD 7 for any other group. This suggests that recovery had occurred by PD 7 in all groups except the no SD+CCI group. Also, we found no significant differences in speed for any groups between baseline and PD 14 ($p > 0.05$). There was no interaction between group $\times$ day (Greenhouse-Geisser $F(4.93,37.8) = 0.996, p > 0.05$). There were no significant differences between groups ($F(1.88) = 3, p > 0.05$). Speeds gradually increased from PD 4 to 14 for all groups, with all groups reaching speeds comparable with baseline by PD 14 ($p > 0.05$).

We assessed forelimb sensorimotor function using the adhesive tab removal task; data are plotted in Figure 2. These data revealed a significant effect of day (Greenhouse-Geisser $F(2.04,36.7) = 5.28, p < 0.01$) and between groups ($F(3,18) = 3.38, p < 0.05$), but no significant interaction for group $\times$ day ($F(6.1,24) = 1.73, p > 0.05$). Again, we conducted separate pairwise comparisons using $t$-tests of average tab removal latencies between baseline and PDs 4, 7, and 14 for each group, controlling for family-wise error rate across the tests at the 0.05 level based on Bonferroni post hoc tests. We expected that practice performance on the tab removal task might improve or that latency to remove would decrease. Rats with CCI showed no improvements in performance on any PD. However, rats with sham surgery only did show improved performance postoperatively.

On PD 4, the NH+sham group performed significantly ($t(6) = 12.94, p < 0.01$) better than baseline, suggesting a CCI injury-induced deficit on performance; there were no significant differences between baseline and PD 4 for the SD+CCI, no SD+CCI, or NH+CCI groups. However, the NH+CCI group removed tabs significantly faster on PD 14 than at baseline ($t(6) = 2.49, p < 0.05$). We found no significant differences between baseline and PD 7 for any group and no significant differences between baseline and PD 14 for the SD+CCI, no SD+CCI, or NH+sham groups ($p > 0.05$). Thus, no improvements from baseline levels occurred on any PD for the SD+CCI and the no SD+CCI groups, while performance improvement occurred on PD 4 for the NH+sham group and on PD 14 for the NH+CCI group. While results from sham surgery rats revealed temporary effects, at least one other study reported that effects remained at PD 14 [29].

These trends are similar to those noted with hindlimb function. Although latency averages are similar between all groups by PD 14, recovery patterns were different for individual groups. The SD+CCI group had a slight but not significant increase in latency or poorer performance on PDs 4 and 7 than at baseline, while the overall trend for all other groups was a decrease in latency or performance improvement on each PD. Sham surgery rats improved by PD 4, while the NH+CCI group improved at PD 14. This suggests that practice effects
had already resulted in performance improvement in the sham surgery rats on PD 4, while performance improvements in the NH+CCI group did not occur until PD 14; we observed no significant performance improvement in either the SD or no SD rats.

Calculated percent lesion volumes (ipsilateral hemispheric volume divided by the contralateral hemispheric volume × 100) revealed comparable injury sizes between all CCI groups and no measurable lesions present in sham-only rats (Figure 3). Based on Bonferroni post hoc tests \(F(3,20) = 8.251, p < 0.001\), the NH+sham group was significantly different from all other groups. We found no significant differences among the three other CCI groups.

DISCUSSION

These data support the hypothesis that preinjury conditions can affect recovery from TBI. We saw a somewhat unexpected protective effect of SD on the acute injury-induced effects for hind-limb motor function in this model. Despite the known and well-described neurophysiologic effects of SD, of all the groups receiving a CCI, the SD group had the least decline in beam walking speeds and the no SD+CCI group had the greatest decline in beam walking speeds. Both the no SD+CCI and SD+CCI groups experienced the stress of novel housing conditions, including social isolation. However, the SD group additionally experienced SD but still performed better than the no SD+CCI group on the beam walk task. This further supports the possibility of a neuroprotective effect. On the sensorimotor task of adhesive tab removal, the data did not show the same neuroprotective effects but also did not show statistical evidence of worsening. In fact, while the NH+sham and NH+CCI groups both showed performance improvements over baseline on PDs 14 and 4, respectively, the SD+CCI and no SD+CCI groups showed no improvements over baseline on any PD. To further investigate the unexpected neuroprotective effect, future experiments will include longer SD durations.

Analyzing sham surgery (craniotomy only) rats revealed an unexpected effect on behavioral measures, most likely because of the induction of a neurovascular injury as has been proposed by others [29]. Mechanical stress placed on the surface of the brain during drilling to create the bone flap could have triggered injury response mechanisms that led to secondary injury responses, resulting in a localized loss of function. Future studies will include additional control groups without craniotomy. A thorough analysis of brain integrity following craniotomy should be considered, as well as the inclusion of anesthesia-only rats as controls, for future studies.

The mechanisms for this potential neuroprotective effect are not clearly established, but future studies should seek to further validate trends demonstrated in our pilot study and better understand the neurochemical changes herein. It will be especially intriguing to investigate the underlying mechanisms of the potential neuroprotective properties of 24 h of SD. The trend for the no SD+CCI group to exhibit performance deficits may be related to the potentially stressful novel environment and social isolation conditions associated with the individual platform over water chambers. Repeated physical exercise has also been shown to diminish the harmful effects of social isolation, as demonstrated by changes to intracellular glucocorticoid receptor and its nuclear transporter protein 70 [30]. There is no question that under social isolation, novel environment, SD, or other potentially stressful conditions, the neurochemical milieu of the brain becomes altered. Adenosine, a ubiquitous neuromodulator, plays a key role in these changes by accumulating both during wakefulness and in

![Figure 3.](image-url)

**Figure 3.** Percent lesion volume for all experimental groups. Average lesion size for each group calculated from cresyl violet-stained sections through entire lesioned area. Sham-only group was significantly different from all groups \(p < 0.01\). No significant differences were found among three other controlled cortical impact (CCI) groups. Error bars represent standard error. NH = normal housing, SD = sleep deprivation.
response to ischemia, hypoxia, excitotoxicity, inflammation, and other types of TBI [31]. Interestingly, an agent that has significant effect on adenosine neurochemistry, caffeine, has also been demonstrated to exert neuroprotective effects after stroke [32] and other neurologic conditions [33–34]. The fact that one study found neuroprotective effects of caffeine when given chronically but not acutely [34] suggests that acute elevation of adenosine as seen with 24 h of SD may but protective, while longer duration SD may be detrimental to brain function. This constellation of information raises the possibility that adenosine may also play a role in the potential neuroprotective effect of REM SD.

In humans, mild TBI typically produces no permanent morphological or neurological deficits, while cognitive deficits may persist for years following the initial injury [35]. Severe injury in humans is conventionally considered to be associated with a period of unconsciousness or coma that lasts for ≥2 d and chronic neurological defects [36]. Although loss of consciousness cannot be compared between rat and human studies because injuries are performed under anesthesia, neurological and cognitive deficits provide a means for comparison. This protocol uses the CCI injury model originally developed by Dixon et al. [28]. Using this model, Dixon et al. defined injuries resulting from compression depths of 1, 2, or 3 mm as low, moderate, or severe and as comparable with mild, moderate, and severe TBI as defined in human patients [28,37].

Future studies should evaluate the effect of longer periods of REM SD and determine whether longer duration REM SD will lead to greater neuroprotective effects, or conversely, increased central nervous system damage. Use of multiplatform REM SD chambers will help to dissect at least the effects of social isolation. More sensitive measures of sensorimotor performance, particularly the use of behavioral tasks that are less sensitive to practice effects, may prove useful and may yield clearer results. Any effect of preinjury conditions on cognitive function would be particularly relevant to understanding the etiology of TBI in returning servicemembers. Additionally, the role of total SD has also yet to be evaluated and its effect on TBI should also be studied.

CONCLUSIONS

Although the effect reported in this study is mild, the implications may be quite significant. Understanding the unique effects involved in TBI incurred under common combat conditions is needed in order to generate a better animal model that considers the environment of the servicemember at the time of TBI. As new treatment strategies evolve, it will be imperative to consider their effectiveness in a model that includes these factors. Despite the plethora of data on alterations in cell death, inflammation, oxidative stress, and neurotransmitter systems following TBI, no treatments have proven to be clinically effective in improving mortality or limiting disability following injury [38]. It is possible that the condition of the combat servicemember is fundamentally different from the animal model and that the development of optimally effective treatments for combat-associated TBI depends on examining antecedent conditions.

Future studies should address the effect of chronic levels of SD, which more closely align with the combat servicemember’s experience. Additionally, emotional and cognitive function should be evaluated in addition to sensorimotor function. While this and numerous other studies have shown temporary effects of TBI on sensorimotor function, it is possible that deficits in emotional and cognitive function could be more long-lasting.

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