Lasting Neuroendocrine-Immune Effects of Traumatic Brain Injury in Rats

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ABSTRACT

Traumatic brain injury (TBI) is a principal cause of long-term physical, cognitive, behavioral, and social deficits in young adults, which frequently coexist with a high incidence of substance abuse disorders. However, few studies have examined the long-term effects of TBI on the neuroendocrine-immune system. TBI was induced in adult male rats under isoflurane anesthesia by cortical contusion injury with a pneumatic piston positioned stereotaxically over the left parietal cortex. Controls underwent sham surgery without injury. At 4 weeks post-injury, the plasma corticosterone response to 30-min restraint stress was significantly blunted in TBI rats compared to the sham controls. One week later, transmitters were implanted for continuous biotelemetric recording of body temperature and spontaneous locomotor activity. At 6 weeks post-injury, the febrile response to i.p. injection of the bacterial endotoxin, lipopolysaccharide (LPS; 50 μg/kg), was significantly lower in TBI than in sham rats. At 8 weeks, swimming in the forced swim test was significantly less in TBI than sham rats. At 9 weeks, rats were rendered ethanol (EtOH) dependent by feeding an EtOH-containing liquid diet for 14 days. Cosine rhythmometry analysis of circadian body temperature Midline Estimating Statistic of Rhythm (MESOR), amplitudes, and acrophases indicated differential effects of EtOH and withdrawal in the two groups. Light- and dark-phase activity analysis indicated that TBI rats were significantly more active than the sham group, and that EtOH and withdrawal differentially affected their activity. Given the extensive interactions of the neuroendocrine-immune systems, these results demonstrate that TBI produces lasting dysregulation amidst the central substrates for allostatics and circadian rhythmicity.

Key words: activity; body temperature; corticosterone; lipopolysaccharide; stress

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**INTRODUCTION**

Severe head injuries frequently lead to trauma of the hypothalamus (Crompton, 1971; Rudy, 1980). The hypothalamus can be injured as a result of direct or indirect damage, small hemorrhages, or ischemia (Lighthall et al., 1990). Secondary insults—such as hypotension, hypoxia, and diffuse brain swelling—often complicate traumatic brain injury (TBI) and have been shown to be associated with pituitary insufficiency (Kelly et al., 2000). Serious alterations in neuroendocrine (Agha et al., 1990) and in neurobehavioral processes (e.g., sleep phases, circadian rhythms) have been observed in clinical studies of patients with TBI (Frieboes et al., 1999); however, whether they are sustained long-term is not known.

Experimental studies have reported that corticotropin-releasing hormone (CRH) mRNA is upregulated at 2 and 4 h after fluid percussion injury (Roe et al., 1998; Grundy et al., 2001), and adrenocorticotropic (ACTH) and corticosterone (CORT) are increased up to 6 h after cortical contusion injury (CCI) (Gottesfeld et al., 2002; McCullers et al., 2002a) and within the first 8 h after closed head injury (Shohami et al., 1995). Along with this generalized activation of the hypothalamus-pituitary-adrenal (HPA) axis during the early post-injury period, TBI induces a constellation of early, acute-phase responses, which include fever and sickness behavior components (i.e., immobility, anorexia, weight loss) (Taylor et al., 2002a). These responses are mediated at least in part by proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor–α, which are released within minutes of the injury (Gottesfeld et al., 2002; Morganti-Kossmann et al., 2002). However, beyond the early effects of TBI on neuroendocrine-immune function, the responses of this system at later time points remain unknown.

To begin to determine the extent of long-term effects of TBI on neuroendocrine-immune regulation, we assessed rats at 1–3 months after CCI of the left parietal cortex. In addition to baseline levels of CORT and circadian rhythms of body temperature and spontaneous locomotor activity, we evaluated the capability of the neuroendocrine-immune system of TBI rats to respond to and recover from stressful stimuli, that is, the allostatic response (McEwen and Wingfield, 2003). Specifically, we assessed the adrenal glucocorticoid response to restraint stress, the febrile response to immune challenge with the bacterial endotoxin, lipopolysaccharide (LPS), and the behavioral response to the physical/emotional stress of forced swimming. Additionally, given the high incidence of alcohol and other drug abuse after TBI (Wilde et al., 2004), we examined the development of ethanol dependence and withdrawal following TBI. Preliminary reports have been presented (Taylor et al., 2005 a,b).

**METHODS**

**Subjects**

Adult male Sprague-Dawley rats (n = 27, 276 ± 2.01 g) from Charles River Breeding Labs (Hollister, CA) were used as experimental subjects. Upon arrival, rats were acclimated for 1–2 weeks to our standard temperature and lighting conditions (22 ± 1°C, 14 h/10 h light/dark cycle, lights on 0400–1800). All experimental procedures were approved by the UCLA and VAGLAHS Institutional Animal Care and Use Committees.

**Traumatic Brain Injury**

At 60–70 days of age, animals were anesthetized with isoflurane (2.0–2.5% in 100% O2, 2.0 mL/min flow rate) and placed into a stereotaxic frame (Kopf Instruments, Tujunga, CA) with the head positioned in a horizontal plane with respect to the interaural line. During all surgical procedures, body temperature was maintained at 37–38°C using a thermostatically controlled heating pad (Harvard Apparatus, Holliston, MA). All surgical procedures were performed under aseptic conditions. As we have done previously (Taylor et al., 2002a), following a midline incision, the skin, fascia, and temporal muscle were reflected. Animals receiving TBI were subjected to a 6-mm-diameter craniotomy of the left parietal cortex centered at 3 mm posterior and 4 mm lateral to bregma.

The cortical impact injury device, as described by Sutton et al. (1993), consisted of a small-bore, double-acting, pneumatic piston cylinder with a 40-mm stroke (Hydraulics Control, Inc., Emeryville, CA). The cylinder was mounted onto a stereotaxic micromanipulator (Trentwells Co., Coulterville, CA), allowing for precise determination of the impact center. The pneumatic-piston cylinder was angled 22.0° away from vertical enabling the flat, circular impactor tip (5-mm diameter) to be perpendicular to the surface of the brain at the site of injury, and adjusted so that the tip could be lowered to a depth of 2.0 mm below the cortical surface. At a pressure of 28 psi, the impactor tip penetrated the exposed brain at 2.75 m/sec for 250 msec (Lighthall, 1990; Sutton et al., 1993). Following the impact, the fascia and scalp were sutured closed, and the wound margins were infiltrated with local anesthetic (1% xylocaine). Sham-operated control rats (sham) underwent similar procedures, but did not receive trepanation or any impact. Animals were returned to their cages where they were pair housed and subsequently subjected to the experimental procedures outlined below in the time sequence of their occurrence.
Forelimb Use

The Forelimb Test was administered before and after TBI in order to assess injury-induced motor impairment (Schallert and Woodleee, 2005; Whishaw et al., 2004). Briefly, prior to TBI and at 2 weeks after surgery, rats were placed in a clear Plexiglas cylinder (height, 45 cm; internal diameter, 25 cm) for 5 min during which their behavior was recorded with a digital video camera. The tapes were scored by an observer, who was blind to the surgical treatment, for the number of times either one or both forepaws made contact with the cylinder wall during rearing. All testing occurred between 0900 and 1300 h.

Corticosterone Response to Restraint Stress

At week-4 post-TBI or sham surgery, rats were stressed with a modification of the restraint stress paradigm that we used previously (Weinberg et al., 1996). Rats were restrained for 30 min in Plexiglas holders (13 cm length $\times$ 8 cm width $\times$ 6 cm height). Blood samples were obtained by tail venipuncture immediately prior to stress and at 30, 60, and 90 min post-stress. After collection, blood samples were centrifuged at 2000 rpm for 15 min at 4°C, and plasma was separated and stored at $-70°C$ until assayed with a rat corticosterone $^{125}$I RIA kit (MP Biomedicals, Inc., Orangeburg, NY), as we have done previously (Taylor et al., 2002b).

Biotelemetry

Body temperature and spontaneous motor activity were monitored by a telemetric system (Mini-Mitter Co, Bend, OR) in individually caged animals, at an ambient temperature of $22 \pm 1°C$. At 5 weeks after TBI, calibrated transmitters were implanted intraperitoneally (i.p.) under isoflurane anesthesia. Body temperature, detected by a sensor imbedded in the battery-operated transmitter, was continuously recorded at 10-min intervals. Activity was measured by detecting the changes in signal strength that occur as the animal moved about its cage; the number of pulses generated by the transmitter were proportional to the amount of movement. After transmitter implantation, baseline temperature and activity data were sampled every 10 min and automatically stored on a personal computer using Vital View software (Mini-Mitter Co). The 10-min temperature data were subjected to rhythmometric circadian analysis. Both the 10-min temperature and activity data were pooled, and averaged every 30 min for presentation and for analysis of the activity data.

Febrile Response to Immune Challenge

At week-6 post-TBI or sham surgery, rats were injected i.p. at 0900 h with apyrogenic saline to serve as a temperature recording baseline for the injection 24 h later of LPS (50 $\mu$g/kg, E. coli serotype 026:B6, L3755; Sigma, St. Louis, MO). The febrile response to LPS was calculated as the difference between temperature measurements at comparable time points after injection of saline on day 1 and LPS (50 $\mu$g/kg) on day 2.

Behavior in the Forced Swimming Test

At 8 weeks post-TBI or sham surgery, the rats underwent a 2-day Forced Swimming Test (Porsolt et al., 1977), as modified by Lahmame et al. (1997). Rats were placed in a Plexiglas cylinder (height, 45 cm; internal diameter, 25 cm) containing water (25°C) up to a level of 35 cm for 15 min on the first day, and then for 5 min the following day. Behavior was recorded on videotape. The following types of behavior were distinguished and measured with a stopwatch by an experimenter who is blind to the treatment given to the animals: (1) struggling, which occurs when the rats are diving, jumping or strongly moving all four limbs, the front limbs breaking the surface of the water or scratching the walls, (2) mild swim, which occurs when the rats swim around the tank, moving all four limbs, and (3) immobility, which occurs when the rats remain motionless, which is widely used to predict the efficacy of antidepressant treatments (Lahmame et al., 1997). Total time spent in each category was analyzed using an analysis of variance (ANOVA).

Analysis of Body Temperature and Activity

Diurnal Rhythms during Ethanol Dependence and Withdrawal

Commencing at 9 weeks post-TBI or sham surgery, we induced ethanol (EtOH) dependence in TBI and sham rats by ad libitum feeding of a liquid diet containing EtOH (5% w/v, 35% EtOH-derived calories; BioServ Inc., Frenchtown, NJ) for 14 days. Control TBI and sham rats were pair fed the isocaloric diet without EtOH (PF), or normal rat chow and water ad libitum (N). Cosine rhythmometry analysis (COSINOR) (Mojon et al., 1992; Fernandez and Hermida, 1998) was performed on the 10-min temperature data. Three circadian rhythm parameters, i.e., MESOR (Midline Estimating Statistic of Rhythm, rhythm-adjusted mean value), amplitude (half the extent of rhythmic change, or the difference between the maximum value and the MESOR of the fitted curve), and acrophase (a measure of time, the lag from a defined reference time point to the crest time in the cosine-curve fitted to the data), were computed over the last 3 days of liquid diet/rat chow consumption and for each of the first 3 days of EtOH withdrawal. Rhythm detection was sought by testing the null hypothesis of zero amplitude with an $F$ test.
The 10-min activity data for each animal were averaged for the light and dark periods over the last 3 days of liquid diet/rat chow consumption and the first 3 days of EtOH withdrawal.

The pre-withdrawal data were subjected to two-way analysis of variance (ANOVA) for the factors of surgery and diet. As few significant differences were found between the N and PF rats, their data were combined to form the control baseline level to which the EtOH consumption and withdrawal data were compared.

**Histology**

At 12 weeks after TBI, animals were sacrificed by decapitation. Brains were quickly removed, frozen in dry-ice cooled isopentane (−35°C) and stored at −70°C until sectioning. Coronal sections (20 μm thick) were cut on a cryostat, and every 10th section (200-μm interval) was mounted onto SuperFrost slides, fixed in formalin for 1 h, washed, and stained with Cresyl violet. Differences in staining intensity provided a clear distinction between healthy and lesioned tissue at the injury site. In the present study, we did not quantify the extent of the lesions nor did we specifically examine the hypothalamus or related subcortical regions. Several sections through the center of the lesion were stained with the von Kossa stain to verify the presence of calcification in the dorsal thalamus that we had observed previously (cf. Fig. 3, Taylor et al., 2002a).

**Statistical Analysis**

One- and two-way analyses of variance (ANOVA) and repeated-measures ANOVA were carried out on the data, as indicated. Group means were compared using t tests under the Tukey-Fisher least significant difference (LSD) criterion with α set at 0.05 (two-sided).

**RESULTS**

**Forelimb Use**

The Forelimb Test was administered before and at 2 weeks after TBI in order to assess injury-induced motor impairment. Before TBI there were no differences in utilization of either front paw (Table 1). As expected, and confirmed by ANOVA, following CCI of the left sensorimotor cortex, utilization of the right (contralateral) front paw was significantly [F(1,28) = 17.81, p < 0.001] impaired, while that of the left (ipsilateral) paw was significantly [F(1,28) = 30.73, p < 0.0001] increased (Table 1).

**Corticosterone Response to Restraint Stress**

At 4 weeks post-TBI or sham surgery, plasma CORT was measured immediately prior to restraint stress and at 30, 60, and 90 min post-stress as an index of HPA func-
tion. Repeated-measures ANOVA indicated a significant effect of injury \([F(1,14) = 11.72, p = 0.004]\) and a significant interaction of injury and time \([F(3,42) = 9.69, p = 0.0001]\). As shown in Figure 1, baseline (0 min) levels of CORT did not differ between the TBI and sham rats. In both groups, CORT levels peaked at 30 min after stress onset. However, at 30 min and again at 90 min, TBI rats had significantly \((p = 0.001\) and \(p < 0.01\), respectively\) lower CORT levels than the sham rats. At this time, body weights of TBI rats \((396.44 \pm 5.20 \text{ g})\) and sham rats \((406.25 \pm 13.27 \text{ g})\) were not statistically different.

**Febrile Response to Immune Challenge**

Repeated-measures ANOVA of the febrile response of TBI and sham rats to 50 \(\mu\text{g/kg}\) LPS at 6 weeks post-TBI indicated a significant interaction of injury and time \([F(6,150) = 5.07, p = 0.0001]\) during the 3-h peak response period (Fig. 2). LPS-induced febrile responses of the TBI group were significantly \((p < 0.05)\) lower than those of the controls during the peak times.

**Behavior in the Forced Swimming Test**

Figure 3 shows the behavior of TBI and sham rats at 8 weeks post-TBI during 5 min in the forced swimming test on each of 2 days. ANOVA indicated a significant behavioral effect \([F(2,48) = 116.86\) and 378.96, \(p < 0.0001\), on the first and second days respectively\], and a significant interaction of injury and behavior \([F(2,48) =]

![FIG. 2. Lipopolysaccharide (LPS)–induced febrile response at 6 weeks after traumatic brain injury (TBI) or sham surgery. Shown are mean ± SEM body temperature responses presented as the difference between temperatures recorded biotelemetrically at comparable time-points after i.p. injection of saline on day 1 (the zero temperature line) and LPS (50 \(\mu\text{g/kg}\)) on day 2. The asterisks indicate that the febrile response of TBI rats was significantly \((^*p < 0.05, ^{*}*p < 0.01)\) lower than that of the controls during the peak of the response.]

![FIG. 3. Behavior of traumatic brain injury (TBI) and sham rats in the Forced Swim Test at 8 weeks after TBI or sham surgery. The mean amount of time (sec) that was spent during each minute of the two 5-min sessions in immobility, struggling, and mild swimming is shown. Standard errors are omitted from the immobility and struggling data, as there were no differences between the TBI and sham groups. The asterisks indicate significant \((p < 0.05)\) differences between TBI and sham rats for mild swimming behavior during the total 5-min period.]

\[ F(6,150) = 5.07, p = 0.0001 \] during the 3-h peak response period (Fig. 2, 1130–1430). LPS-induced febrile responses of the TBI group were significantly \((p < 0.05)\) lower than those of the sham rats during the peak times.
As is apparent (Fig. 3), there were no differences in total time spent by either group in immobility or struggling during each day’s 5-min test. However, TBI rats spent significantly \((p \leq 0.02)\) less total time in mild swimming than sham rats on both days.

Analysis of Body Temperature and Activity Diurnal Rhythms during Ethanol Dependence and Withdrawal

As can be seen from the continuous recordings of body temperature and locomotor activity taken at 10–11 weeks post-injury (Fig. 4), while there were no differences between TBI and sham groups in the control (no EtOH) condition, there were marked differences in the effects of EtOH during days 12–14 of exposure on body temperature in the TBI and sham rats (Fig. 4A): EtOH produced hypothermia (particularly during the dark-phase) in sham rats, whereas it produced light-phase hyperthermia in the TBI rats. These effects were confirmed by COSINOR analysis of the 10-min temperature data averaged over the last 3 days of EtOH exposure and during each of the first 3 days of withdrawal (Fig. 5). ANOVA of the MESORs averaged over the last 3 days of EtOH exposure indicated a significant effect of injury \([F(1,23) = 4.46, p = 0.046]\); the MESOR was significantly \((p = 0.02)\) higher in the TBI than in the sham rats. ANOVA of the average amplitudes showed a significant effect of diet \([F(1,23) = 6.15, p = 0.02]\) and a significant interaction of diet and injury \([F(1,23) = 6.66, p = 0.017]\); the average amplitude of the sham rats exposed to EtOH was significantly \((p \leq 0.02)\) lower than that of the control group or of the TBI-EtOH rats. ANOVA of the average acrophase indicated a significant effect of diet \([F(1,23) = 21.35, p = 0.0001]\); EtOH significantly \((p < 0.02)\) advanced the acrophases of both the TBI and sham EtOH groups compared to their non-EtOH controls.

The continuous recordings (Fig. 4A) show that EtOH withdrawal reversed the hypothermia of the sham rats, whereas the EtOH-induced hyperthermia of the TBI rats persisted during at least the first 2 days of withdrawal. Repeated-measures ANOVA of the rhythmometric parameters for each day of withdrawal and the control (no EtOH) condition indicated a marginally significant effect of injury \([F(1,23) = 3.74, p = 0.065]\) with a significant

![FIG. 4. Continuous biotelemetric recording of body temperature (A) and locomotor activity (B) in traumatic brain injury/ethanol (TBI-E) and sham-operated/ethanol (sham-E) rats during days 12–14 of exposure to an ethanol (EtOH)–containing liquid diet and the first 3 days of EtOH withdrawal (W1, W2, W3), and in non-EtOH-exposed TBI-control and sham-control rats at 10–11 weeks post-surgery. Recordings represent the means of 4–9 animals per group. For visual clarity, standard errors have been omitted. The solid bars on the x-axis indicate the dark portion of the daily light-dark cycle.](image-url)
interaction of day and diet \( F(3,69) = 8.33, p = 0.0001 \) on the MESORs. There were no significant effects of surgery or diet on the amplitudes. The acrophases showed a significant effect of diet \( F(1,23) = 4.51, p = 0.045 \), and significant interactions of day with surgery and day with diet \( F(3,69) = 3.13 \) and \( 5.91, p \leq 0.03 \), respectively. As shown in Figure 5, throughout the 3 days of withdrawal, MESORs of the TBI rats were significantly \( p < 0.05 \) elevated over the control (no-EtOH) condition, while amplitudes and acrophases remained at control levels. In the sham rats, MESORs and acrophases remained at control levels by the first withdrawal day (W1) and amplitude by the second withdrawal day (W2; Fig. 5).

To confirm our observation (Fig. 4B) that, in the control (no-EtOH) condition, activity of the TBI rats was consistently higher than that of the sham rats, we analyzed the average light- and dark-phase activity of each group during days 12–14 of EtOH/control diet feeding and during withdrawal days W1, W2, and W3 (Fig. 6). Repeated-measures ANOVA indicated a significant effect of injury in both the light- and dark-phases \( F(1,22) = 6.47 \) and \( 5.02, p = 0.019 \) and \( 0.036 \), respectively] and a significant interaction of surgery with diet in the dark-phase \( F(1,22) = 4.37, p = 0.048 \). Light-phase activity of TBI controls was significantly \( p < 0.001 \) higher than that of the sham controls; activity increased significantly \( p < 0.05 \) upon EtOH exposure of the sham rats, and remained significantly \( p \leq 0.05 \) elevated on W1 and W2 compared to the control condition. Dark-phase activity of TBI controls was significantly \( p < 0.01 \) higher than that of the sham controls. Upon EtOH exposure, dark-phase activity decreased significantly \( p < 0.02 \) in the TBI rats, and withdrawal produced no further effects. In contrast, dark-phase activity of the sham rats was not affected by EtOH exposure but did show a significant \( p < 0.05 \) increase over the control condition on W1.

**Histology**

At 12 weeks post-injury, there was a 5-mm diameter cavity of the left sensorimotor cortex, centered at 3 mm posterior to and 4 mm lateral to bregma. The cavity extended to a depth of 2 mm to the dorsal hippocampus (Paxinos and Watson, 2005). As in the previously published coronal section (Taylor et al., 2002a), a region of calcification (confirmed with the von Kossa stain) in the dorsal thalamus immediately below the lesion was repeatedly observed, supporting the findings of Osteen et al. (2001).

**DISCUSSION**

This screening of neuroendocrine-immune function at 1–3 months following TBI by left parietal CCI has revealed persistent deficits in stress-induced HPA function, in the behavioral response to a physical/emotional stressor, and in the host-defense response to immune challenge, as well as alterations of 24-h activity rhythms. We also report that TBI rats become hyperthermic during ethanol dependence and respond to EtOH withdrawal with a further increase in body temperature. These results provide some of the first experimental evidence suggest-
ing long-term disruptive effects of TBI on neuro-endocrine-immune mechanisms that mediate the frequently observed HPA dysfunction and sleep disturbances of TBI patients. Moreover, the adverse hyperthermic reaction to EtOH dependence and withdrawal may contribute to the high incidence of alcoholism in this population.

Responses of the neuro-endocrine-immune system involve numerous pathways and centers, including afferent neural pathways, the brainstem, the cortex, corticohypothalamic pathways, hypothalamic integrative centers, the pituitary gland, and efferent autonomic pathways (Hermer et al., 2003; Sternberg, 1997). TBI may disturb any part of these complex responses, depending on the severity and the location of primary and secondary injuries (Yuan and Wade, 1991). For example, trauma to the hypothalamus frequently occurs in severe head injuries (Crompton, 1971; Rudy, 1980). Indeed, endocrine dysfunction after TBI, affecting all hypothalamic-pituitary axes (i.e., corticotropin [ACTH], growth hormone, gonadotropin, thyrotropin, prolactin, and vasopressin) and manifested as hypopituitarism, has been described in clinical studies (Yuan and Wade, 1991; Childers et al., 1998; Kelly et al., 2000; Benvenga et al., 2000; Lieberman et al., 2001; Agha et al., 2005). Despite questions as to the applicability of brain trauma models in rodents to the human condition, in general, the CCI model appears to reproduce much of the pathophysiology of human TBI (Sutton et al., 1993; Goodman et al., 1994; Colicos et al., 1996; Hall et al., 2005a). Whether our CCI specifically affected any of the subcortical structures that mediate neuroendocrine-immune function or the hypothalamic-pituitary hormones, including CRH and ACTH, will require further investigation.

Specifically, with respect to the HPA axis, a high incidence of ACTH and adrenal insufficiencies has been reported (Benvenga et al., 2000; Cohan et al., 2005). These abnormalities, which occur soon after TBI, are transient in some patients, while the majority shows recovery at 6 months (Agha et al., 2005). Additionally, the extent of neuroendocrine impairment has been found to correlate with the severity of the neurological insult as assessed by the Glasgow Coma Score (Cernak et al., 1999). For example, plasma cortisol levels increase during the early postTBI period, but only in patients with minor to moderate injuries. In contrast, patients with severe trauma exhibit a significant decline in cortisol (Cernak et al., 1999). Generally, however, the clinical studies have defined alterations in baseline, but not stress-induced neuroendocrine function. Experimental data, cited above, confirm that TBI stimulates HPA function acutely, but the status of the HPA axis beyond the acute period remains unknown. Our data (Fig. 1) demonstrate that baseline plasma levels of the adrenal glucocorticoid, corticosterone (CORT), were no longer elevated at 4
weeks post-TBI but that the stress-induced CORT response was significantly blunted.

However, the source of the CCI-induced dysregulation of the stress-induced HPA response remains to be determined. In experimental studies, CCI has been shown to produce necrotic cell loss at the site of impact (Goodman et al., 1994; Sutton et al., 1993). Dystrophic cells have been identified in the dentate gyrus and the CA1 and CA3 subfields of the hippocampus for up to 1 week post-injury in mice (Hall et al., 2005a) and 2 weeks in rats (Colicos et al., 1996). There is also evidence that hippocampal adrenocorticotestosteroid receptor regulation of glucocorticoid receptor mRNA expression is suppressed acutely following CCI (McCullers et al., 2002b). Given the role of hippocampal adrenocorticosteroid receptors in mediating behavioral activation and providing negative feedback inhibition to the HPA axis (DeKloet et al., 1998), it is unlikely that their CCI-induced depletion would produce the observed suppression of the HPA-stress response. A more likely explanation is provided by the evidence that parietal CCI causes cellular dystrophy in the amygdala, entorhinal and piriform cortices, thalamic, and hypothalamic regions (Colicos et al., 1996), regions of known input to the CRH secreting cells of the paraventricular nucleus of the hypothalamus (Herman et al., 2003). Future specific histological analysis and receptor immunohistochemistry are required to determine the extent of the central impairments produced by parietal CCI.

It has been reported that immunosuppression occurs after severe TBI in rats (Sholkina et al., 2002). We observed that the LPS-induced febrile response is blunted after TBI (Fig. 2). Fever serves as a primary host defense response against the invasion of bacterial and viral pathogens (Blatteis and Sehic, 1998; Kluger, 1991), and in previous studies we have shown that blunting of the febrile response induced by the endotoxin, LPS, correlates with significant reductions in hypothalamic LPS-induced cytokine content (Taylor et al., 1999, 2002b). Consistent with the known immediate release of immune mediators of inflammation after TBI (Gottesfeld et al., 2002; Morganti-Kossman et al., 2002), in preliminary (unpublished) studies we also found that hypothalamic content of the proinflammatory cytokines IL-1β and TNFα, was increased acutely after TBI. Numerous immune mediators released within minutes of the primary injury determine and guide the neuro-immune sequence of events that follows and its temporal extent (Morganti-Kossman et al., 2002). Given that the LPS-induced febrile response is indicative of the host-defense response to infection, a blunted response suggests that TBI rats would show greater susceptibility to infection.

Our results indicate that one of the active behaviors demonstrated by rats in the FST, i.e., moderate swimming, was selectively altered by parietal CCI. It is unlikely that the CCI-induced motor impairment affected this behavior, given that there were no differences between TBI and sham rats in the amount of struggling in the FST. The CCI-induced reduction in moderate swimming suggests that parietal CCI may have impaired CNS activation mechanisms, such as sympathetic nervous system activity. Given that the mammalian response to stress involves both the HPA axis and the sympathetic nervous system (SNS) (Selye, 1946; Munck et al., 1984), our results (Figs. 1 and 3) suggest that CCI affected SNS inputs to the PVN from brain stem sites (i.e., locus coeruleus) to dopamine and noradrenergic receptors in limbic-forebrain regions. Indeed, evidence has been provided showing that pharmacologic interventions that enhance dopaminergic and noradrenergic transmission, such as amphetamine, can be beneficial in recovery from TBI (Goldstein, 2003; Sutton et al., 1987; Hornstein et al., 1996; Levin and Kraus, 1994; Phillips et al., 2003).

Sleep disturbances frequently occur in TBI patients several months after injury (Frieboes et al., 1999; Mahmood et al., 2004; Rao and Rollings, 2002). Moreover, the circadian rhythm of core temperature, which is normally synchronous with the sleep/wake cycle (Opp, 1998), appears to be free-running following TBI, i.e., no longer tied to the 24-h environmental cycle (Kropvyntsky et al., 2001). While the circadian rhythm of core body temperature did not appear to be affected by parietal CCI in this study, daily light- and dark-phase activity of the TBI rats was significantly greater than that of the sham rats (Fig. 6). Although circadian rhythms of body temperature and locomotor activity are generally correlated, evidence exists for their disassociation (Saper et al., 2005). For example, cell-specific lesions of the ventral subparaventricular zone, which receives input from the suprachiasmatic nucleus, the brain’s ‘master clock’, have been shown to disrupt the circadian rhythms of sleep and wakefulness, as well as locomotor activity, but have minimal effects on body temperature rhythms, while lesions of the dorsal subparaventricular zone impair circadian rhythms of body temperature, but not wake-sleep or locomotor activity (Saper et al., 2005). These data suggest that parietal CCI may have indirectly impacted the ventral rather than the dorsal subparaventricular zone, indicative of an altered sleep-wake cycle.

Confirming previous results (Taylor et al., 2002b), chronic EtOH exposure altered the amplitude of the circadian body temperature rhythm in sham rats, but it was without effect on this measure in the TBI rats (Fig. 5). Indeed, other than reduced activity during chronic EtOH exposure, TBI rats did not respond to EtOH withdrawal.
with the increased activity shown by the sham rats (Fig. 6). Moreover, TBI rats responded to EtOH exposure with atypical hyperthermia that persisted during withdrawal (Figs. 4 and 5). EtOH exposure and withdrawal are stressors that stimulate the HPA axis (Tabakoff et al., 1978; Redei et al., 1986, 1988; Rivier and Lee, 1996; Rasmussen et al., 2000). Given that glucocorticoids counteract stress-induced hyperthermia (Morrow et al., 1993), the lack of EtOH-induced hyperthermia in TBI rats and their marked hyperthermia upon withdrawal may reflect the blunted stress-induced HPA response of TBI rats. Such inappropriate temperature responses to EtOH exposure and withdrawal may contribute to the greater risk for alcohol abuse that has been documented in TBI subjects (Corrigan et al., 2004; Wilde et al., 2004). Taken together, the reported results are highly indicative of a prolonged disturbance in various aspects of allostasis following experimental TBI. Screening of neuroendocrine-immune function at 1–3 months following TBI revealed persistent deficits in stress-induced HPA function, in the behavioral response to a physical/emotional stressor, and in the host-defense response to immune challenge, as well as alterations of circadian activity rhythms. Given the extensive interactions of the neuroendocrine-immune systems, our results provide evidence that TBI produces lasting dysregulation amidst the central substrates for allostasis and circadian rhythmicity.

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REFERENCES


LASTING NEUROENDOCRINE-IMMUNE EFFECTS OF TBI


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