Experimental Traumatic Brain Injury Alters Ethanol Consumption and Sensitivity

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Abstract

Altered alcohol consumption patterns after traumatic brain injury (TBI) can lead to significant impairments in TBI recovery. Few preclinical models have been used to examine alcohol use across distinct phases of the post-injury period, leaving mechanistic questions unanswered. To address this, the aim of this study was to describe the histological and behavioral outcomes of a noncontusive closed-head TBI in the mouse, after which sensitivity to and consumption of alcohol were quantified, in addition to dopaminergic signaling markers. We hypothesized that TBI would alter alcohol consumption patterns and related signal transduction pathways that were congruent to clinical observations. After midline impact to the skull, latency to right after injury, motor deficits, traumatic axonal injury, and reactive astrogliosis were evaluated in C57BL/6J mice. Amyloid precursor protein (APP) accumulation was observed in white matter tracts at 6, 24, and 72 h post-TBI. Increased intensity of glial fibrillary acidic protein (GFAP) immunoreactivity was observed by 24 h, primarily under the impact site and in the nucleus accumbens, a striatal subregion, as early as 72 h, persisting to 7 days, after TBI. At 14 days post-TBI, when mice were tested for ethanol sensitivity after acute high-dose ethanol (4 g/kg, intraperitoneally), brain-injured mice exhibited increased sedation time compared with uninjured mice, which was accompanied by deficits in striatal dopamine- and cAMP-regulated neuronal phosphoprotein, 32 kDa (DARPP-32) phosphorylation. At 17 days post-TBI, ethanol intake was assessed using the Drinking-in-the-Dark paradigm. Intake across 7 days of consumption was significantly reduced in TBI mice compared with sham controls, paralleling the reduction in alcohol consumption observed clinically in the initial post-injury period. These data demonstrate that TBI increases sensitivity to ethanol-induced sedation and affects downstream signaling mediators of striatal dopaminergic neurotransmission while altering ethanol consumption. Examining TBI effects on ethanol responsiveness will improve our understanding of alcohol use post-TBI in humans.

Key words: alcohol; DARPP-32; loss of righting reflex; nucleus accumbens; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) can present with a number of perceptual symptoms that lack diagnosable, structural brain abnormalities.^{1–4} Despite injury symptoms that may be subtle or even undetectable, TBI can result in a wide range of disabilities with long-lasting behavioral consequences often developing in the brain-injured patient. Care associated with TBI ranges from \$600,000 to \$5 million over the lifetime of a person, as stated by the AEI-Brookings Joint Center for Regulatory Studies.⁵ While TBI affects both the older and younger populations, younger TBI victims are vulnerable to a lifetime (50–70 years) of disabilities, illustrating a critical need to examine the long-term effects of TBI.

In addition to the cognitive impairments and increased risk of both affective disorders and Alzheimer disease associated with

brain injury, alcohol abuse/dependence is the second most common Axis I disorder in persons with TBI.^{6–11} In one longitudinal clinical study, McKinlay and associates¹² identified significant increases in substance abuse after TBI. Limited data suggest increased rates of substance abuse disorders (SUDs) in a cohort of 100 civilian patients with TBI (in the absence of Axis I disorders before injury) compared with controls.⁸ Similar results were observed in self-reported severe TBI victims after controlling for alcohol use before injury.¹³ Likewise, the incidence of increased alcohol use disorders (AUDs) after TBI has been documented in the military population, including an increase in the frequency of binge drinking.^{14,15} Ommaya and colleagues¹⁶ reported that of 2000 discharged armed forces personnel with a diagnosis of TBI, those with moderate TBI were 5.4 times more likely to be discharged for alcoholism or drug use than those without TBI.

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While many reports have documented that drinking behaviors increase during the chronic post-injury period (2–3 years after TBI), rates of moderate and heavy alcohol use have been shown to be decreased within the first year post-TBI.^{17–23} Specifically, the frequency of drinking episodes per week and the number of drinks consumed per episode have been shown to decrease significantly from pre-injury values as early as 1 month after TBI, extending to a year.²² This dichotomy underscores the importance of understanding the consequences of TBI and the mechanisms by which the injured brain responds to alcohol exposure throughout all phases of the post-injury period.

Beyond examining the effect of TBI on consumption, understanding the mechanisms that underlie sensitivity to alcohol after TBI is also critical. For example, increases or decreases in sensitivity to alcohol in the post-TBI period can increase seizure frequency beyond that associated with TBI alone, facilitate occurrence of repeated TBI, and lead to increased rates of arrest for crimes or intoxication, while those patients with TBI with exaggerated responses to alcohol exhibit reduced societal functioning and delayed physical and cognitive recovery from the TBI.^{19,24,25}

Despite the abundance of rodent TBI models, the lack of data focused on mechanisms that underlie the response to alcohol after TBI has hampered our progress in this field. The aim of the present study was to characterize an experimental model of midline non-contusive TBI in the mouse and subsequent response to alcohol using histopathological and behavioral endpoints. To assess the behavioral effects of injury, mice were evaluated for latency to regain righting after removal of surgical anesthesia and neuromotor performance using the Rota-Rod. Histopathological endpoints included incidence of traumatic axonal injury using amyloid precursor protein (APP) immunohistochemistry, astrocyte reactivity using glial fibrillary acidic protein (GFAP) immunohistochemistry, and blood-brain barrier breakdown using immunoglobulin G immunohistochemistry.^{26,27} Particular emphasis was given to the striatal response to TBI, because this region plays a well-established role in alcohol-related behaviors.²⁸

To evaluate the behavioral effects of TBI on ethanol action, sham and injured mice were examined for alcohol sensitivity to acute high-dose ethanol exposure, binge-pattern drinking behaviors, and alterations in dopamine-related signal transduction, because dopamine is a critical mediator of ethanol action in the striatum. Using this model, we demonstrate that TBI significantly increases behavioral sensitivity to sedating doses of ethanol 14 days post-TBI and that this is associated with impairments in the phosphorylation of key signaling pathways that regulate dopaminergic neurotransmission and addiction behaviors in the striatum. When assessed at 17 days after TBI, injured mice demonstrate reduced consumption over a 7-day period compared with sham controls. Together, these data represent a novel model useful for examining the role of TBI as a risk factor for alterations in alcohol sensitivity and consumption that may lead to alcohol addiction behaviors.

Methods

Animals

All mice were progeny of C57BL/6J mice bred in our colony, originally purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained on a 12-h light/dark schedule with *ad libitum* access to food and water, except during ethanol consumption testing, when water was replaced by 15% ethanol as described. All experiments were performed using male mice, 6–7 weeks of age at the time of injury. Animal use protocols were in

accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at Wayne State University.

Traumatic brain injury

Mice were anesthetized with 5% isoflurane (Baxter Healthcare Corporation, Deerfield, IL) using an anesthesia induction chamber. After initial sedation, 2.5% isoflurane was used for maintenance during the surgical procedure and injury using a nose cone secured to the stereotaxic frame. Sterile lubricant ophthalmic ointment (Rugby Laboratories, Inc., Duluth, GA) was applied to prevent drying of the eyes immediately after the removal of fur from the surgical area with an electric razor.

On loss of the pain reflex, as assessed by paw pinch, mice were secured in a stereotaxic frame and a midline incision (1.0 cm) was made to expose the skull using sterile techniques. The periosteum was reflected after prepping the scalp with betadine and isopropyl alcohol solutions. The animal was supported by a soft pad to maintain proper orientation of the head with the body while receiving isoflurane via a nose cone. To induce an injury of moderate severity, an electromagnetically-driven impactor tip (Leica-Microsystems, Northbrook, IL) was positioned over the exposed skull at the midpoint between lambda and bregma along the midline suture and the stainless steel hemispheric indenter (5 mm diameter) was displaced 2 mm at 5 m/sec. Automated retraction of the impactor occurred 100 msec after impact.

Immediately after impact, ear bars were released, anesthesia was removed, and animals were monitored for length of apnea, respiration time, and seizure behaviors. Skulls of injured animals were evaluated for the presence of hairline fractures, herniation, and hematoma. Sham-injured animals (control) experienced all procedures except the impact. Scalp incisions were closed with tissue adhesive (3M, St. Paul, MN). Triple antibiotic ointment was applied to prevent infection at the incision site and the animals were placed on a heating pad maintained at 37°C during recovery from the injury/anesthesia.

The length of time until animals regained the righting reflex (to spontaneously orient onto four paws from a side-laying position) and the return of the pain reflex were monitored until mobility was recovered. After recovery, mice were returned to their home cages. The total time from initiation of anesthesia to removal of the nose cone after injury was approximately 15 min. The injury was the first procedure to occur in all experimental animals. See Table 1 for group sizes.

Tissue preparation and histopathology

Mice were anesthetized with 2.5% Avertin (0.7 mL) and perfused transcardially, first with 30 mL phosphate-buffered saline (PBS) followed by 50 mL 4% paraformaldehyde (PFA) in PBS at 6, 24, 48, 72 h, or 7 days post-injury (N = 3/time point). From an independent set of mice, tissues were similarly collected for histological analysis 24 h after ethanol treatment, which was administered 14 days after the TBI or sham procedure. After perfusion, brains were immediately post-fixed in 4% PFA for 24 h at 4°C then transferred to 30% sucrose solution. After 5 to 7 days in sucrose at 4°C, brains were sectioned in the coronal plane at 40 μ m using a cryostat and stored in PBS/0.1% sodium azide at 4°C until use. Sections were collected in a 10-slice series with one set stained with 0.3% cresyl violet and three additional sets used for immunohistochemistry as described individually in the following sections.

APP immunohistochemistry. After three washes in Tris-buffered saline (TBS), selected sections were placed in 60°C 10 mM sodium citrate, pH 6.5, in a water bath for 20 min, then allowed to cool at room temperature for 15 min. After another three washes in TBS, sections were incubated in hydrogen peroxide, methanol, and TBS (1:3:6, v/v) for 15 min at room temperature.

TABLE 1. LATENCY POST-SURGICAL ANESTHESIA

Experimental group	Injury status	N	Latency to righting (min)	Fractures (%)	Blood at impact site (%)	Hematoma (%)
Neurological impairment (Experiment 1)	Sham	9	1.9 ± 0.8	NA	NA	NA
	Injured	12	*24.1 ± 10.0	100	83	50
Immunohistochemistry (Experiment 2)	Sham	3	3.7 ± 3.8	NA	NA	NA
	Injured	15	*16.4 ± 5.3	100	80	40
LORR analysis (Experiment 3)	Sham	6	2.2 ± 1.0	NA	NA	NA
	Injured	10	*17.1 ± 8.0	100	60	30
DARPP-32 analysis (Experiment 4)	Sham	6	1.8 ± 0.4	NA	NA	NA
	Injured	7	*16.4 ± 4.1	100	43	0
Drinking-in-the-Dark (Experiment 5)	Sham	6	1.8 ± 0.4	NA	NA	NA
	Injured	7	*15.9 ± 3.1	100	80	0
ALL GROUPS	Sham	30	2.1 ± 1.3	NA	NA	NA
	Injured	51	*18.3 ± 7.4	100	69	24

NA, not applicable; LORR, loss of righting reflex.

*, $p < 0.05$ vs. respective sham control. Two-way ANOVA followed by a Tukey post-hoc test was used for statistical analysis.

Latency to right after removal of surgical anesthesia is increased after traumatic brain injury TBI in mice. Across all groups examined, mice subjected to TBI demonstrate an average latency to right of 18 min while sham controls regain righting an average of 2 min post-operatively. No significant differences were observed among groups used for individual experiments noted. Data presented are average time until righting reflex is regained after removal of anesthesia (min) ± standard deviation.

After an additional four washes in TBS, sections were blocked in 5% normal horse serum (NHS) in TBS with 0.001% Triton X-100 (TBS-T) for 60 min at room temperature with continuous agitation.

Tissues were incubated overnight at 4°C in anti-APP (C-terminal) (Invitrogen, Camarillo, CA) diluted 1:2000 in 5% NHS/TBS-T. Sections were incubated in biotin Donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:1000 in 5% NHS/TBS-T for 2 h at room temperature with continuous agitation after washing in TBS three times. After an additional 3 TBS washes, tissues were incubated for 1 h in 1:3 AB solution from the ABC Elite kit (Vector Laboratories, Burlingame, CA) and washed four times in TBS. Brain slices were then immersed in a solution containing 1 tablet of 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma-Aldrich, St. Louis, MO) and 15 mL 30% hydrogen peroxide solution for 7 min and rinsed twice with water and once with TBS-T. Tissues were stored in TBS-T at 4°C until mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA).

GFAP immunohistochemistry. After three washes in TBS, selected sections were blocked in a solution of 5% NHS, 0.1% Triton X-100, and TBS for 1 h and incubated in a 1:10,000 anti-GFAP (Sigma-Aldrich, St. Louis, MO) and 5% NHS/TBS-T solution overnight at 4°C with continuous agitation. Tissues were washed three times in TBS and immersed in 1:500 biotin-Donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) in 5% NHS/TBS-T for 2 h at room temperature. After an additional three washes in TBS, the selected sections were incubated in hydrogen peroxide, methanol, and TBS (1:3:6, v/v) for 15 min and rinsed four times in TBS. Tissue was immersed in 1:3 AB solution from the ABC Elite kit (Vector Laboratories, Burlingame, CA) with TBS/bovine serum albumin (BSA) for 1 h at room temperature and washed three times with TBS. Brain slices were incubated in a solution containing 1 DAB tablet (Sigma-Aldrich, St. Louis, MO) and 15 mL 30% hydrogen peroxide solution for 7 min, then rinsed twice with water. Finally, sections were washed once with TBS-T and stored at 4°C until mounted on Superfrost Plus slides.

IgG immunohistochemistry. After one rinse in TBS, selected brain slices were incubated in a blocking solution of 5% NHS, 0.1% Triton X-100, and TBS for 1 h at room temperature.

Tissue was exposed to 1:10,000 5% NHS/TBS-T diluted biotin-Donkey anti-Mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 h at room temperature. After washing three times in TBS, sections were immersed in hydrogen peroxide, methanol, and TBS (1:3:6, v/v) for 30 min at room temperature and washed six times in TBS. Tissue was incubated in 1:3 AB solution from the ABC Elite kit (Vector Laboratories, Burlingame, CA) with TBS/BSA for 1 h at room temperature, washed four times with TBS, and immersed in a solution comprising one tablet of DAB (Sigma-Aldrich, St. Louis, MO) dissolved in 15 mL 30% hydrogen peroxide solution for 5 min. Finally, sections were rinsed with water three times before being mounted on Superfrost Plus slides and stored at 4°C for at least 48 h.

Protein lysate preparation and immunoblotting

At 14 days post-injury, mice were administered a single intraperitoneal injection of saline or ethanol (20% [v/v] solution in 0.9% saline) to achieve a dose of 4.0 g/kg. Thirty minutes after saline or ethanol administration, mice were sacrificed by cervical dislocation. The striatum was rapidly dissected and frozen in liquid nitrogen. Using an electric pestle, tissues were homogenized in a buffer containing 4% (w/v) 3-[(3-cholamido-propyl)dimethylammonio]-1-propanesulfonate, 2 M thiourea, 7 M urea, and 30 mM Tris, pH 8.5. One tablet of Complete Protease Inhibitor mixture (Roche Products, Indianapolis, IN) was added to 50 mL of lysis buffer.

After homogenization, samples were centrifuged at 10,000 × g for 10 min at 4°C, and the supernatants were collected. A Pierce 660 Protein Assay kit (Thermo Scientific, Rockford, IL) was used to determine protein concentrations. Equal amounts of protein were submitted to 4–12% SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were probed with primary antibodies at the following concentrations: anti-phosphorylated DARPP-32 (Thr34) and anti-DARPP-32 at 1:2000 (Cell Signaling Technology, Beverly, MA) and anti-actin at 1:5000 (Sigma-Aldrich, St. Louis, MO). Antibodies were detected using horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibodies and signals were visualized using chemiluminescence (SuperSignal West Dura kit; Pierce, Rockford, IL).

Densitometric analysis was performed using Image J Software. For each sample, phosphoprotein signals were normalized to total protein signals and averaged within groups. In addition,

phosphoprotein and total protein signals were independently normalized to actin signals and averaged within groups (data not shown).

Accelerating Rota-Rod

Motor performance of an independent group of injured and sham animals was evaluated using an accelerating Mouse Rota-Rod (Ugo Basile, Collegeville, PA). Mice were tested repeatedly at consecutive time points of 24 and 48 h; 7 and 14 days post-injury. The rod (3 cm in diameter and 14 cm above a platform) accelerated from 3.5 rotations per minute (RPM) to 36 RPM at a constant rate over a 5 min testing interval. Latency to fall from the rotating rod or to traverse around the rod for one full rotation was recorded for each mouse. This motor performance test was administered once per testing time point, with no pre-injury training session.

Determination of behavioral ethanol sensitivity and blood ethanol concentration

At 14 d after TBI, a separate group of mice was evaluated for the loss of righting reflex (LORR) after acute ethanol challenge. Mice were given an intraperitoneal injection of ethanol to achieve a dose of 4.0 g/kg (20% [v/v] solution in 0.9% saline) and placed on their back in a U-shaped trough after losing the ability to right themselves. Sedation time was defined as the time until the mice regained the ability to right themselves onto three of four paws three times within 30 sec. On regaining the righting response, blood samples, taken either from tail or trunk, were collected in lithium heparin-coated tubes (Sarstedt, Newton, NC) to evaluate blood ethanol concentration (BEC). At 14 days post-TBI, blood was similarly collected from an additional group of mice 30 min after acute ethanol administration (4.0 g/kg), regardless of sedation status. Ethanol concentration was determined using the Pointe Scientific Alcohol Reagent (Pointe Scientific, Canton, MI) according to manufacturer instructions.

Ethanol consumption: Drinking-in-the-Dark (DID) paradigm

One week before injury, mice were introduced to a 12-h reversed light:dark schedule (lights on at 1800 h) and maintained on this schedule for the duration of the DID paradigm. Immediately after injury or sham surgery, which occurred at the start of the light cycle, mice were allowed to recover in group housing for 1 week, then switched to single-housing conditions for an additional week in preparation for DID evaluation.

For DID assessment, beginning at 14 d post-TBI, each mouse was provided with a single bottle containing tap water for 3 days followed by 7 days of 15% ethanol ([v/v] in tap water) for a 2-h limited access period starting 3 h into the dark cycle (0900–1100 h). Mice were weighed on the first and last day of DID testing, and bottles were weighed before and after each DID session to measure intake volume. Volume consumed was calculated from weight of solution consumed daily and normalized to body weight. After rapid decapitation, brains and trunk blood samples were collected immediately after the last DID drinking session. BEC measurements were performed as previously described for the LORR study.

Statistical analysis

All statistical analyses were performed using SigmaPlot 12 software, unless noted. Two-way repeated-measures analysis of variance (ANOVA) followed by the Tukey *post-hoc* test was used for statistical analysis of Rota-Rod data to assess neurological impairment and volume of water or ethanol consumed across days (Statistica 6.0.668.0). Two-way ANOVA followed by a Tukey *post-hoc* test was used for statistical analysis of surgical righting time across TBI studies and immunoblot data. Student *t* test was

used for all other measurements. Effects were considered to be statistically significant at $p \leq 0.05$. In some instances, statistical outliers (greater than 2 standard deviations $\pm$ the group mean) were excluded from analysis. Likewise, in the case of BEC measurements, animals that did not yield sufficient blood volumes for BEC measurement were excluded from analysis.

Results

Injury assessment—Experiment 1

All injuries resulted in skull fractures at the impact site, which was accompanied by minimal, localized bleeding in 69% of injured mice. Visual observation of dark discoloration under the skull (hematoma) was noted in 24% of injured mice. Statistical analysis of latency to regain righting after removal of surgical anesthesia indicated a significant main effect of injury ($p < 0.001$; $F[1,80] = 131.07$, $N = 30-51$). No significant differences were observed among treatment groups used for individual experiments performed in this study ($[p = 0.19$; $F[4,80] = 1.57$, $N = 3-15$]; see Table 1 and figure legends for individual group sizes).

Latency to right after removal of surgical anesthesia was significantly increased in injured mice compared with sham controls ($t[79] = -11.81$, $p < 0.001$) (Table 1). Sham controls demonstrated the righting reflex within 2 min after removal from isoflurane, while injured mice needed greater than 18 min to regain the righting reflex, a nine-fold increase compared with sham controls. Similarly, significant motor deficits were detected using the accelerating Rota-Rod assay at 24 and 48 h after TBI compared with sham controls (Fig. 1).

Statistical analysis indicated main effects of injury ($p = 0.026$; $F[1, 78] = 5.79$, $N = 6-10$) and time point ($p < 0.001$; $F[3, 78] = 9.04$, $N = 6-10$) with an injury $\times$ time point interaction ($p = 0.006$; $F[3, 78] = 4.60$, $N = 6-10$). No significant differences were detected among sham controls across testing sessions. The observed motor deficits in injured mice were transient, resolving to sham levels by 7 days after injury.

Injury-induced histopathology—Experiment 2

No gross tissue cavitation was observed at the impact site in injured brains, either in the cortex or hippocampus, as assessed by cresyl violet staining (Fig. 2, top row). At 24 h post-TBI, areas of

FIG. 1. Neurological function is impaired after traumatic brain injury (TBI) (Experiment 1). Injured mice demonstrate significantly impaired motor performance in the accelerating Rota-Rod paradigm at 24 and 48 h post-TBI compared with respective sham controls that resolves by 7 days after injury to sham levels. Data presented are average time spent on the Rota-Rod in seconds (sec) $\pm$ standard error of the mean (SEM), with maximal time being 300 sec. (*, $p < 0.05$ vs. respective sham control, $N = 10$ injured, 6 sham). Two-way repeated-measures analysis of variance followed by the Tukey *post-hoc* test was used for statistical analysis.

FIG. 2. Histopathological alterations are observed at the site of impact after traumatic brain injury (TBI) (Experiment 2). Top row: Cresyl violet staining at 24 h and 7 days post-TBI reveals no gross histopathological alterations in cortical or hippocampal regions surrounding the impact site compared with sham controls. Cellular thinning (arrows) was observed in midline cortical areas under the impact site at 24 h that resolved by 7 days post-TBI. Images acquired at 4 $\times$ magnification, scale bar=200 μ m. Middle row: Breakdown of the blood–brain barrier as indicated by IgG immunoreactivity is increased following brain injury with maximal effects at 24 h post-injury compared to sham controls. Images acquired at 4 $\times$ magnification, scale bar=200 μ m. Bottom row: Robust glial fibrillary acidic protein (GFAP) immunoreactivity (reactive astroglia) was observed at 24 h in white matter regions under the impact site with intense staining persisting at 7 days post-injury in adjacent cortical and white matter regions of brain-injured animals compared with sham controls. Images acquired at 20 $\times$ magnification, scale bar=100 μ m. (N=3 injured/time point, 3 sham.) Color image is available online at www.liebertpub.com/neu

sparse cellularity were detected in the cortex, under the site of impact, which resolved to sham levels by 7 d after impact.

After injury, tissue immunoglobulin G (IgG) immunoreactivity, as a marker of blood–brain barrier breakdown, was observed in all cortical layers and in subcortical white matter (WM) tracts surrounding the region of impact (Fig. 2, middle row). Immunoreactivity for IgG was evident as early as 6 h post-injury in the cortex, WM, and hippocampus (data not shown) and robustly present in these regions at 24 h post-TBI. Diffuse IgG labeling was still observed at 72 h post-injury in most animals, but had largely resolved by 7 days (Fig. 2).

Robust reactive astroglia, as measured by GFAP immunoreactivity, was detected after TBI in cortical and subcortical regions surrounding the impact site as early as 24 h after injury and persisted beyond 7 days post-injury (Fig. 2, bottom row and Fig. 6, top row). GFAP $^{+}$ astrocytes with morphology consistent with activation were present throughout all layers of the cortex and in the underlying WM tract at these time points. Sham controls exhibited little evidence of GFAP $^{+}$ cells with similar activated morphology in this region. At 72 h post-TBI, intense GFAP $^{+}$ immunoreactivity was observed in deeper subcortical structures, such as both the nucleus accumbens core and shell subregions, in the absence of gross cell loss (Fig. 3).

While GFAP $^{+}$ cells were observed in the anterior commissure of sham animals, staining in this region intensified and spread to adjacent areas by 72 h after injury, with additional morphological evidence for persistent reactive astroglia at 7 days post-TBI (Fig. 3, bottom row).

Sham-injured animals exhibited no evidence of APP accumulation at any time point or in any region examined. Intra-axonal accumulation of APP, an indicator of traumatic axonal injury, was

observed in the cingulum, lateral WM tracts, external capsule, and corpus callosum of both hemispheres of the injured brain (Fig. 4). Both swellings in contiguous axons and terminal bulbs were observed in these various WM tracts of brain-injured mice (Fig. 4, bottom row inset). APP accumulation was detected as early as 6 h, with peak accumulation at 24 h after TBI. By 72 h post-TBI, little to

FIG. 3. Histopathological alterations are observed in striatal structures after traumatic brain injury (TBI) (Experiment 2). Top row: Cresyl violet staining reveals no evidence of cell loss in the nucleus accumbens (Acb) after injury compared with sham controls (anterior commissure [ac]). Bottom row: Robust glial fibrillary acidic protein (GFAP) immunoreactivity was observed at 72 h and 7 days post-injury in the Acb of brain-injured animals while sham controls exhibited little GFAP $^{+}$ staining. Reactive astrocytes were present in the ac of sham animals, with injury intensifying this response. Images acquired at 10 $\times$ magnification, scale bar=200 μ m. (N=3 injured/time point, 3 sham.) Color image is available online at www.liebertpub.com/neu

FIG. 4. Traumatic axonal injury is increased after traumatic brain injury (TBI) (Experiment 2). Intra-axonal accumulation of amyloid precursor protein (APP) was observed at 6 and 24 h, but largely resolved by 72 h in the cingulum (top), lateral subcortical white matter (WM) tracts (external capsule, middle), and corpus callosum (bottom) in both hemispheres of brain-injured animals. Sham-injured animals exhibited no evidence of traumatic axonal injury, while APP+ staining peaked at 24 h in injured mice. Images acquired at 20 $\times$ magnification, scale bar=100 μ m. High magnification image (60 $\times$) illustrates the presence of APP+ swellings and terminal bulbs at 24 h post-TBI in the corpus callosum (inset). Scale bar=25 μ m. (N=3 injured/time point, 3 sham.) Color image is available online at www.liebertpub.com/neu

no intra-axonal APP accumulation was observed in any WM tracts of brain-injured mice.

Sensitivity to ethanol-induced sedation—Experiment 3

At 14 days after injury, mice demonstrated increased behavioral sensitivity after acute ethanol administration (4.0 g/kg) as evidenced by significantly longer sedation times compared with sham controls using the LORR assay ($t[14]=2.14$, $p=0.05$ N=6–10) (Fig. 5A). Sham controls regained the righting reflex an average of 28 min after ethanol treatment, while injured mice regained righting after 44 min on average, representing an approximately 50% increase in sedation time. A similar effect was observed at 7 days post-TBI with no differences observed in latency to sedation between sham and injured mice at either time point (data not shown). Measurement of BEC at 14 days after injury revealed no differences between sham and injured mice from samples collected either at 30 min after ethanol administration ($t[6]=-0.03$, $p=0.98$ N=3–5) (Fig. 5B), or at the time of righting ($t[10]=0.56$, $p=0.59$, N=2–10) (data not shown).

To examine the effects of ethanol exposure on TBI-induced pathology, sham and injured mice from Experiment 1 were evaluated for APP and GFAP immunoreactivity at 24 h after a single ethanol injection (4 g/kg, intraperitoneally) administered at 14 days after injury. Ethanol did not exacerbate TBI-induced GFAP+ immunoreactivity compared with that observed in saline-treated injured mice in any region examined (Fig. 6). Of note, reactive astrocytes were still present in cortical and subcortical regions surrounding the impact site at 15 days in all injured mice, irrespective of ethanol treatment, compared with sham controls (Fig. 6, top row). Ethanol treatment alone did not induce astrocyte reactivity in sham animals in any region examined. Reactive astrogliosis observed in all injured mice at 72 h post-TBI in the nucleus accumbens (Fig. 3, bottom row) had resolved by 15 days after injury, irrespective of ethanol treatment, although GFAP+ cells were still present in the anterior commissure (Fig. 6, bottom row). At 24 h after ethanol treatment, no evidence of traumatic axonal injury was observed in sham or injured WM tracts or the nucleus

accumbens (data not shown). Likewise, no effects of injury on APP accumulation were detected at this time point (15 days post-TBI, data not shown).

Phosphorylation of DARPP-32—Experiment 4

Statistical analysis of phosphorylated DARPP-32 protein expression normalized to total DARPP-32 levels as determined by immunoblot detection indicated a main effect of injury ($p=0.006$; $F[1,12]=13.09$, N=6–7) with an injury $\times$ treatment interaction ($p=0.018$; $F[1, 12]=8.25$, N=3–4). *Post-hoc* tests revealed that sham mice demonstrated significantly increased DARPP-32 phosphorylation relative to total DARPP-32 protein levels in the striatum 30 min after acute 4.0 g/kg ethanol administration ($p=0.035$; N=3–4) (Fig. 7).

TBI alone significantly increased basal levels of phospho-DARPP-32 (pDARPP-32) protein levels compared with respective sham controls ($p=0.001$). Unlike sham controls, TBI mice demonstrated impaired DARPP-32 phosphorylation after ethanol administration, exhibiting no further increase after treatment compared with ethanol-treated sham controls or saline-treated TBI controls. No differences in total DARPP-32 protein levels were detected between any groups when normalized to actin (data not shown), indicating that the increase in DARPP-32 phosphorylation did not result from a concomitant reduction in total DARPP-32 levels. These findings were confirmed using an independent group of samples (data not shown).

Ethanol consumption—Experiment 5

At 17 days post-TBI, injured mice demonstrated decreased ethanol intake compared with sham controls when assessed across all 7 days of ethanol exposure (main effect of injury) using the DID paradigm ($p=0.009$; $F[1, 11]=10.09$; N=6–7) (Fig. 8A). No significant changes in water intake (days 1–3 of DID) between sham and injured mice were identified before ethanol exposure. Measurement of BEC revealed no differences between sham and injured mice from samples collected immediately after the

FIG. 5. TBI results in increased sensitivity to the sedative effects of ethanol (Experiment 3). **(A)** Loss of righting duration measured after acute injection of ethanol (4 g/kg, intraperitoneally) demonstrates a significant increase in sedation duration in injured mice at 14 days post-traumatic brain injury (TBI) compared with sham controls. Data presented are average sedation time (min) $\pm$ SEM. *, $p < 0.05$. (N = 10 injured, 6 sham). Student *t* test was used for statistical analysis. **(B)** TBI does not affect ethanol metabolism after acute exposure. Blood ethanol concentration (BEC) levels are equivalent between sham and injured mice 30 min after acute ethanol administration (4 g/kg, intraperitoneally) at 14 days post-TBI. No significant differences were detected between injured mice and sham controls. Data presented are average BEC (mg/dL) $\pm$ SEM, N = 5 injured, 3 sham). Student *t* test was used for statistical analysis.

limited-access exposure on day 7 of DID testing ($p = 0.164$; $t[10] = 1.503$; N = 6/group) (Fig. 8B).

Discussion

We present a mouse model of TBI that demonstrates features of traumatic axonal injury (TAI) and transient motor deficits that is accompanied by decreased ethanol consumption and increased acute sensitivity to high-dose ethanol administration with a deficit in acute ethanol-induced striatal DARPP-32 phosphorylation. The observed reduction in intake and the prolonged sedation response to high-dose ethanol after injury was not accompanied by changes in blood ethanol concentrations compared with sham controls. Likewise, acute ethanol exposure did not exacerbate injury-induced pathology assessed 24 h later. To our knowledge, this is the first

rodent model to examine the effects of TBI on ethanol sensitivity and consumption.

In this model, mice were exposed to midline impact over the intact skull. This resulted in transient motor deficits that resolved by 7 days post-injury. While sham controls demonstrated no dependence on learning to perform the Rota-Rod task, it is possible that injured mice exhibited neuromotor deficits because of a learning, and not a motor, impairment. Additional studies to investigate the cognitive effects of injury in this model would be needed to address this possibility.

Beyond neuromotor performance, this injury resulted in TAI in WM regions directly under and adjacent to the impact site in both hemispheres. APP+ profiles were detected as early as 6 h, peaked at 24 h, and were largely resolved by 72 h post-TBI. These data are in accordance with other models of diffuse injury in the mouse.^{29,30} Robust astrogliosis was detected by 24 h post-TBI and remained evident at 15 days after injury under the impact site. Interestingly, GFAP immunoreactivity was also detected in regions distant from the impact site, including the nucleus accumbens region of the striatum.

Select studies have explored the effects of TBI on the striatum, the major entry into the basal ganglia, demonstrating cellular pathology in this region after TBI.^{31–36} Our data are in accordance with these reports, because we demonstrate robust astrogliosis in response to TBI in the nucleus accumbens. Striatal damage is associated with deficits in executive function, attention, emotional control, learning and memory.^{37,38} Striatal injury is of particular importance to the current study, because striatal circuits largely contribute to acute intoxication, alcohol seeking, dependence, and withdrawal.²⁸ After TBI, the structure and/or function of these circuits may be altered and contribute to alterations in ethanol sensitivity in the delayed post-TBI period.

The primary neuronal subtype in the striatum, medium spiny neurons (MSNs), receives glutamatergic cortical and thalamic inputs as well as dopaminergic innervation from the ventral tegmental area.³⁹ Therefore, it must be considered that damage to cortical afferents may account for secondary injury within the striatum. This would suggest that the striatal astrogliosis observed in the present study is not a direct response to the injury, but rather a downstream consequence of cortical injury, which could even be independent of cortical cell loss. As the primary mechanism for glutamate elimination in the synapse, astrocyte action is integral to maintaining glutamatergic tone.⁴⁰

Dopaminergic signaling is intimately linked to glutamatergic signaling in the striatum, notably in the context of ethanol exposure.⁴¹ It follows, then, that dopaminergic signaling could be indirectly affected by alterations in glutamatergic signaling resulting from reactive astrogliosis. Glutamatergic and dopaminergic signaling within the striatum regulate corticostriatal synaptic plasticity and ethanol sensitivity.^{41,42}

In the current study, the pathological changes observed in the striatum are in accordance with the disrupted dopaminergic tone reported in previous studies. For example, reduced synthesis and release of dopamine occurs 1 week after TBI, with disruption of dopaminergic signaling occurring chronically after injury.^{43–45} We report a robust and persistent increase in astrocyte reactivity within the nucleus accumbens at 7 days post-TBI associated with heightened ethanol sensitivity.

Ethanol sensitivity, commonly measured using duration of LORR to quantify ethanol-induced sedation in rodents, is altered by initial brain sensitivity, rate of ethanol metabolism, rapid development of neuroadaptation (functional tolerance), and glutamatergic and

FIG. 6. Acute ethanol administration does not increase reactive astrogliosis. Top row: Injured mice from Experiment 1 demonstrate increased glial fibrillary acidic protein (GFAP+) staining at the site of impact 15 d post-traumatic brain injury (TBI) compared with sham controls, with no added effect of ethanol. Acute ethanol administration had no effect on GFAP immunoreactivity in sham mice compared with saline controls. Images acquired at 10 $\times$ magnification, scale bar=200 μ m. Bottom row: Little to no GFAP+ staining was observed in the nucleus accumbens (Acb) at this time point, irrespective of injury status or ethanol treatment. Images acquired at 20 $\times$ magnification, scale bar=100 μ m. (N=3 injured/time point, 3 sham.) ac, anterior commissure. Color image is available online at www.liebertpub.com/neu

FIG. 7. TBI impairs DARPP-32 phosphorylation after acute ethanol administration (Experiment 4). (A) Quantification of immunoblot analysis reveals a significant increase in striatal DARPP-32 phosphorylation (pDARPP-32) in sham but not injured mice 30 min after an acute injection of ethanol (EtOH, 4.0 g/kg, intraperitoneally). At 14 days post-injury, TBI alone significantly increases DARPP-32 phosphorylation compared with respective sham controls, with no further effect of ethanol. Data presented are average band intensity in arbitrary units (AU) of pDARPP-32/DARPP-32 $\pm$ SEM. *, $p < 0.05$ vs. saline (Sal)-treated sham control. (N=3–4/group) (B) Representative immunoblot of whole cell striatal lysates demonstrating increased expression of pDARPP-32 after ethanol treatment in sham controls or after TBI. Two-way analysis of variance followed by a Tukey *post-hoc* test was used for statistical analysis.

dopaminergic tone.^{41,46–49} The present data suggest that ethanol metabolism is not affected by TBI, because no differences in BEC were detected between sham and injured mice either at the time of righting or at a fixed interval after ethanol exposure, such as 30 min (a time at which sham animals are waking but injured mice are still sedated).

The concept of altered neuroadaptation after acute ethanol exposure is particularly relevant to the response of the injured brain to ethanol. Numerous studies have demonstrated features of neuronal plasticity after TBI in regions including the cortex and hippocampus.^{50–55} Evidence of acute neuroplasticity in both the hippocampus and cortex has also been reported after acute ethanol exposure in rodents.⁵⁶ Disruption of this observed ethanol-induced neuroplasticity by impairment in the protein kinase A (PKA) signaling pathway, for example, results in heightened sensitivity to ethanol, which is manifested as extended sedation durations.^{56,57} These data suggest that the increase in LORR time in injured mice may be subserved by a similar impairment in neuroplasticity that, in TBI, results from the trauma itself, or possibly secondary effects, such as reduced cAMP and/or PKA signaling, which has been observed after experimental TBI.⁵⁸

In the present study, the injured brain is unable to phosphorylate DARPP-32, a downstream mediator of the dopamine D1 receptor, after acute ethanol challenge. This may represent a ceiling effect, because injured mice given saline have significantly more phospho-DARPP-32 than do sham-saline controls. Alternatively, injured mice may be exhibiting an impaired neuroplastic response to ethanol administration. DARPP-32, which is predominantly expressed in MSNs, is particularly important for controlling striatal neuroplasticity in the form of long-term depression and potentiation, regulating striatal dopaminergic neurotransmission and mediating ethanol sensitivity.^{41,59,60} Importantly, reductions in DARPP-32 activity are associated with reduced motivation for drinking, with concurrent increases in sensitivity to ethanol, which are in accordance with the sensitivity and consumption effects observed after TBI in the present study.^{41,61}

DARPP-32 activity is regulated by the phosphorylation state of the protein. For example, PKA phosphorylation of DARPP-32 at threonine 34 leads to inhibition of protein phosphatase-1 activity and ultimately, loss of direct inhibition of the N-methyl-D-aspartate glutamate receptor (NMDAR) channel by ethanol.⁶² In this way, a deficit in DARPP-32 phosphorylation would promote ethanol-induced inhibition of NMDAR channel activity, thereby enhancing

FIG. 8. Traumatic brain injury (TBI) results in decreased ethanol (EtOH) intake (Experiment 5). (A) Volume of EtOH consumed/body weight in 2-h limited-access Drinking-in-the-Dark (DID) paradigm beginning at 17 days post-TBI demonstrates a significant decrease in consumption after TBI compared with sham controls. Data presented are average volume consumed (mL/g) $\pm$ SEM. *, $p < 0.05$ of EtOH volume consumed across days 4–10 compared with sham controls using a two-way repeated-measures analysis of variance (ANOVA) followed by the Tukey *post-hoc* test. No significant differences in water intake across days 1–3 were observed as determined using a two-way repeated-measures ANOVA. (N=7 injured, 6 sham.) (B) TBI does not affect ethanol metabolism after limited-access DID exposure. Blood samples were obtained immediately after the last session of DID for assessment of blood ethanol concentration (BEC) from sham and injured mice. No significant differences were detected between injured mice and sham controls as determined using the Student *t* test. Data presented are average BEC (mg/dL) $\pm$ SEM. (N=6 injured, 6 sham.)

the sedating properties of ethanol exposure, an interpretation that is in accordance with previous studies.⁴¹ Therefore, this mechanism may explain the increased sensitivity (sedation) to high-dose ethanol administration observed after TBI in the present study.

Reductions in DARPP-32 phosphorylation, however, have been reported after controlled cortical impact (CCI) in the rat.⁶³ While

these data are in contrast to the current results that demonstrate a basal upregulation of pDARPP-32 in the injured brain, it is important to consider the differences in injury model (contusive vs. noncontusive; lateral vs. midline) and species (rat vs. mouse). These differences become critical when considering that DARPP-32 phosphorylation is dependent on glutamatergic and dopaminergic tone and that dopamine release is selectively impaired in the ipsilateral striatum after CCI.⁴⁵ The bilateral alterations observed in DARPP-32 phosphorylation in the current study reflect the diffuse nature of the injury and could be a likely source of discrepancy between these findings. The complexities associated with dopaminergic-glutamatergic interactions, the wide range of signaling pathways affected and the spectrum of deficits in neuroplasticity after TBI establish that any alterations in dopaminergic signaling (whether increased or decreased) could significantly impact ethanol sensitivity after TBI.

To assess binge-pattern drinking, mice were exposed to the DID paradigm, a well-established model of binge consumption that induces voluntary oral ingestion of ethanol for intoxicating effects over a fixed time interval and produces pharmacologically relevant BECs.⁶⁴ Robust consumption of high concentrations of ethanol is observed in the DID model without training, a requirement for operant-based paradigms, that could be problematic in the post-TBI setting. Similarly, DID does not rely on sucrose fading and does not appear to be a result of dietary demand, but does result in physical signs of intoxication.^{65,66} Using the DID paradigm, TBI reduced overall intake at 17 days post-injury compared with that of sham controls.

While this result may seem contrary to the clinical observations of increased consumption after TBI, specifically in the delayed post-TBI period, there are several interpretations that must be considered. For example, in this paradigm, the increased sensitivity to ethanol after TBI may act to limit the overall amount of ethanol consumed. That is, increased sensitivity to ethanol may cause injured mice to reach intoxication before sham controls, limiting intake behaviors. Similarly, frequency of binge drinking in humans is reported to increase proportionally with TBI severity.¹⁵

Injured mice exposed to the DID protocol may be demonstrating “front-loading” behaviors in which drinking rates have been shown to be maximal within the first 15 min of drinking.^{64,67} In this way, TBI could be increasing binge drinking frequency, which could be assessed using shorter limited-access durations.

Finally, it must be recognized that in humans who sustain TBI, rates of moderate and heavy alcohol use have been shown to decline in the early post-TBI period (up to a year), while drinking increases during the delayed post-injury period (2–3 years post-TBI).^{17–23} This suggests that our observations at the 17 day time point after experimental TBI may represent this initial clinical condition, and future experiments will evaluate the consumption behavior up to 1 year post-TBI in mice.

The temporal nature of alcohol consumption patterns may differ from the traditionally defined post-injury (acute vs. delayed) periods in animal models. That is, what is typically referred to as the delayed post-TBI period with respect to histopathological outcomes or motor performance may still only reflect the acute post-TBI period with respect to alcohol consumption. In this way, the novel data presented here may represent a re-interpretation of the post-TBI period, with respect to alcohol use behaviors as measured using animal models.

Future studies will focus on consumption behaviors and signaling pathways that regulate the neuronal responses to ethanol after experimental TBI to develop a more comprehensive

understanding of the relationship between TBI, ethanol sensitivity, and ethanol intake. Further, few studies have examined the development of alcohol sensitivity and use disorders after related conditions, such as stroke, Alzheimer disease, or other models of trauma. These data suggest that ethanol responsiveness may be generally affected by chronic neurodegenerative diseases and should be examined more closely in these models. Together, forthcoming studies that further explore the ability of brain insult to alter ethanol-related behaviors would contribute to our understanding of alcohol use in the brain-injured population.

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References

- Lyeth, B.G., Jenkins, L.W., Hamm, R.J., Dixon, C.E., Phillips, L.L., Clifton, G.L., Young, H.F., and Hayes, R.L. (1990). Prolonged memory impairment in the absence of hippocampal cell death following traumatic brain injury in the rat. *Brain Res.* 526, 249–258.
- Hamm, R.J., Lyeth, B.G., Jenkins, L.W., O'Dell, D.M., and Pike, B.R. (1993). Selective cognitive impairment following traumatic brain injury in rats. *Behav. Brain Res.* 59, 169–173.
- Kibby, M.Y., and Long, C.J. (1996). Minor head injury: attempts at clarifying the confusion. *Brain Inj.* 10, 159–186.
- Margulies, S. (2000). The postconcussion syndrome after mild head trauma: is brain damage overdiagnosed? Part 1. *J. Clin. Neurosci.* 7, 400–408.
- Wallsten, S., and Kosek, K. (2005). The Economic Costs of the War in Iraq. In: *AEI-Brookings Joint Center for Public Policy Research Working Paper Series*, pps. 5–19.
- McDonald, B.C., Flashman, L.A., and Saykin, A.J. (2002). Executive dysfunction following traumatic brain injury: neural substrates and treatment strategies. *NeuroRehabilitation* 17, 333–344.
- Salmond, C.H., and Sahakian, B.J. (2005). Cognitive outcome in traumatic brain injury survivors. *Curr. Opin. Crit. Care* 11, 111–116.
- Hibbard, M.R., Uysal, S., Kepler, K., Bogdany, J., and Silver, J. (1998). Axis I psychopathology in individuals with traumatic brain injury. *J. Head Trauma Rehabil.* 13, 24–39.
- Rogers, J.M., and Read, C.A. (2007). Psychiatric comorbidity following traumatic brain injury. *Brain Inj.* 21, 1321–1333.
- Van Den Heuvel, C., Thornton, E., and Vink, R. (2007). Traumatic brain injury and Alzheimer's disease: a review. *Prog. Brain Res.* 161, 303–316.
- Koponen, S., Taiminen, T., Portin, R., Himanen, L., Isoniemi, H., Hinkkunen, H., Hinkka, S., and Tenovuori, O. (2002). Axis I and II psychiatric disorders after traumatic brain injury: a 30-year follow-up study. *Am. J. Psychiatry* 159, 1315–1321.
- McKinlay, A., Grace, R., Horwood, J., Fergusson, D., and MacFarlane, M. (2009). Adolescent psychiatric symptoms following pre-school childhood mild traumatic brain injury: evidence from a birth cohort. *J. Head Trauma Rehabil.* 24, 221–227.
- Silver, J.M., Kramer, R., Greenwald, S., and Weissman, M. (2001). The association between head injuries and psychiatric disorders: findings from the New Haven NIMH Epidemiologic Catchment Area Study. *Brain Inj.* 15, 935–945.
- Adams, R.S., Corrigan, J.D., and Larson, M.J. (2012). Alcohol use after combat-acquired traumatic brain injury: what we know and don't know. *J. Soc. Work Pract. Addict.* 12, 28–51.
- Adams, R.S., Larson, M.J., Corrigan, J.D., Horgan, C.M., and Williams, T.V. (2012). Frequent binge drinking after combat-acquired traumatic brain injury among active duty military personnel with a past year combat deployment. *J. Head Trauma Rehabil.* 27, 349–360.
- Ommaya, A.K., Salazar, A.M., Dannenberg, A.L., Chervinsky, A.B., and Schwab, K. (1996). Outcome after traumatic brain injury in the U.S. military medical system. *J. Trauma* 41, 972–975.
- Kreutzer, J.S., Wehman, P.H., Harris, J.A., Burns, C.T., and Young, H.F. (1991). Substance abuse and crime patterns among persons with traumatic brain injury referred for supported employment. *Brain Inj.* 5, 177–187.
- Kreutzer, J.S., Witol, A.D., and Marwitz, J.H. (1996). Alcohol and drug use among young persons with traumatic brain injury. *J. Learn Disabil.* 29, 643–651.
- Corrigan, J.D. (1995). Substance abuse as a mediating factor in outcome from traumatic brain injury. *Arch. Phys. Med. Rehabil.* 76, 302–309.
- Corrigan, J.D., Lamb-Hart, G.L., and Rust, E. (1995). A programme of intervention for substance abuse following traumatic brain injury. *Brain Inj.* 9, 221–236.
- Corrigan, J.D., Smith-Knapp, K., and Granger, C.V. (1998). Outcomes in the first 5 years after traumatic brain injury. *Arch. Phys. Med. Rehabil.* 79, 298–305.
- Dikmen, S.S., Machamer, J.E., Donovan, D.M., Winn, H.R., and Temkin, N.R. (1995). Alcohol use before and after traumatic head injury. *Ann. Emerg. Med.* 26, 167–176.
- Kolakowsky-Hayner, S.A., Gourley, E.V., 3rd, Kreutzer, J.S., Marwitz, J.H., Cifu, D.X., and McKinley, W.O. (1999). Pre-injury substance abuse among persons with brain injury and persons with spinal cord injury. *Brain Inj.* 13, 571–581.
- Kolakowsky-Hayner, S.A., Gourley, E.V., 3rd, Kreutzer, J.S., Marwitz, J.H., Meade, M.A., and Cifu, D.X. (2002). Post-injury substance abuse among persons with brain injury and persons with spinal cord injury. *Brain Inj.* 16, 583–592.
- Ellerd, D.A., and Moore, S.C. (1992). Follow-up at twelve and thirty months of persons with traumatic brain injury engaged in supported employment placements. *J. Appl. Rehab. Counsel* 23, 48–50.
- Buki, A., and Povlishock, J.T. (2006). All roads lead to disconnection?—Traumatic axonal injury revisited. *Acta Neurochir (Wein)* 148, 181–194.
- Saatman, K.E., Feeko, K.J., Pape, R.L., and Raghupathi, R. (2006). Differential behavioral and histopathological responses to graded cortical impact injury in mice. *J. Neurotrauma* 23, 1241–1253.
- Chen, G., Cuzon Carlson, V.C., Wang, J., Beck, A., Heinz, A., Ron, D., Lovinger, D.M., and Buck, K.J. (2011). Striatal involvement in human alcoholism and alcohol consumption, and withdrawal in animal models. *Alcohol. Clin. Exp. Res.* 35, 1739–1748.
- Greer, J.E., McGinn, M.J., and Povlishock, J.T. (2011). Diffuse traumatic axonal injury in the mouse induces atrophy, c-Jun activation, and axonal outgrowth in the axotomized neuronal population. *J. Neurosci.* 31, 5089–5105.
- Creed, J.A., DiLeonardi, A.M., Fox, D.P., Tessler, A.R., and Raghupathi, R. (2011). Concussive brain trauma in the mouse results in acute cognitive deficits and sustained impairment of axonal function. *J. Neurotrauma* 28, 547–563.
- Dietrich, W.D., Alonso, O., Busto, R., Prado, R., Zhao, W., Dewanjee, M.K., and Ginsberg, M.D. (1998). Posttraumatic cerebral ischemia after fluid percussion brain injury: an autoradiographic and histopathological study in rats. *Neurosurgery* 43, 585–594.
- Ding, Y., Yao, B., Lai, Q., and McAllister, J.P. (2001). Impaired motor learning and diffuse axonal damage in motor and visual systems of the rat following traumatic brain injury. *Neurol. Res.* 23, 193–202.
- Dunn-Meynell, A.A., and Levin, B.E. (1997). Histological markers of neuronal, axonal and astrocytic changes after lateral rigid impact traumatic brain injury. *Brain Res.* 761, 25–41.
- Hinzman, J.M., Thomas, T.C., Quintero, J.E., Gerhardt, G.A., and Lifshitz, J. (2012). Disruptions in the regulation of extracellular glutamate by neurons and glia in the rat striatum two days after diffuse brain injury. *J. Neurotrauma* 29, 1197–1208.
- Shah, S., Yallampalli, R., Merkley, T.L., McCauley, S.R., Bigler, E.D., Macleod, M., Chu, Z., Li, X., Troyanskaya, M., Hunter, J.V., Levin, H.S., and Wilde, E.A. (2012). Diffusion tensor imaging and volumetric analysis of the ventral striatum in adults with traumatic brain injury. *Brain Inj.* 26, 201–210.

36. Yan, H.Q., Ma, X., Chen, X., Li, Y., Shao, L., and Dixon, C.E. (2007). Delayed increase of tyrosine hydroxylase expression in rat nigrostriatal system after traumatic brain injury. *Brain Res.* 1134, 171–179.
37. Baldo, B.A., and Kelley, A.E. (2007). Discrete neurochemical coding of distinguishable motivational processes: insights from nucleus accumbens control of feeding. *Psychopharmacology* 191, 439–459.
38. Ridley, R.M., Cummings, R.M., Leow-Dyke, A., and Baker, H.F. (2006). Neglect of memory after dopaminergic lesions in monkeys. *Behav. Brain Res.* 166, 253–262.
39. Pennartz, C.M., and Kitai, S.T. (1991). Hippocampal inputs to identified neurons in an in vitro slice preparation of the rat nucleus accumbens: evidence for feed-forward inhibition. *J. Neurosci.* 11, 2838–2847.
40. Papa, M., De Luca, C., Petta, F., Alberghina, L., and Cirillo, G. (2014). Astrocyte-neuron interplay in maladaptive plasticity. *Neurosci. Biobehav. Rev.* 42C, 35–54.
41. Maldiv, R.E., Zhang, T.A., Ferrani-Kile, K., Schreiber, S.S., Lippmann, M.J., Snyder, G.L., Fienberg, A.A., Leslie, S.W., Gonzales, R.A., and Morrisett, R.A. (2002). DARPP-32 and regulation of the ethanol sensitivity of NMDA receptors in the nucleus accumbens. *Nat. Neurosci.* 5, 641–648.
42. Calabresi, P., Picconi, B., Tozzi, A., and Di Filippo, M. (2007). Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends Neurosci.* 30, 211–219.
43. Shin, S.S., Bray, E.R., Zhang, C.Q., and Dixon, C.E. (2011). Traumatic brain injury reduces striatal tyrosine hydroxylase activity and potassium-evoked dopamine release in rats. *Brain Res.* 1369, 208–215.
44. Wagner, A.K., Drewencki, L.L., Chen, X., Santos, F.R., Khan, A.S., Harun, R., Torres, G.E., Michael, A.C., and Dixon, C.E. (2009). Chronic methylphenidate treatment enhances striatal dopamine neurotransmission after experimental traumatic brain injury. *J. Neurochem.* 108, 986–997.
45. Wagner, A.K., Sokoloski, J.E., Ren, D., Chen, X., Khan, A.S., Zafonte, R.D., Michael, A.C., and Dixon, C.E. (2005). Controlled cortical impact injury affects dopaminergic transmission in the rat striatum. *J. Neurochem.* 95, 457–465.
46. Kakhana, R., Brown, D.R., McClearn, G.E., and Tabershaw, I.R. (1966). Brain sensitivity to alcohol in inbred mouse strains. *Science* 154, 1574–1575.
47. Keir, W.J., and Deitrich, R.A. (1990). Development of central nervous system sensitivity to ethanol and pentobarbital in short- and long-sleep mice. *J. Pharmacol. Exp. Ther.* 254, 831–835.
48. Ponomarev, I., and Crabbe, J.C. (2002). Ethanol-induced activation and rapid development of tolerance may have some underlying genes in common. *Genes Brain Behav.* 1, 82–87.
49. Tabakoff, B., and Ritzmann, R.F. (1979). Acute tolerance in inbred and selected lines of mice. *Drug Alcohol Depend.* 4, 87–90.
50. Phillips, L.L., and Reeves, T.M. (2001). Interactive pathology following traumatic brain injury modifies hippocampal plasticity. *Restor. Neurol. Neurosci.* 19, 213–235.
51. Scheff, S.W., Price, D.A., Hicks, R.R., Baldwin, S.A., Robinson, S., and Brackney, C. (2005). Synaptogenesis in the hippocampal CA1 field following traumatic brain injury. *J. Neurotrauma* 22, 719–732.
52. Ansari, M.A., Roberts, K.N., and Scheff, S.W. (2008). Oxidative stress and modification of synaptic proteins in hippocampus after traumatic brain injury. *Free Radic. Biol. Med.* 45, 443–452.
53. Ansari, M.A., Roberts, K.N., and Scheff, S.W. (2008). A time course of contusion-induced oxidative stress and synaptic proteins in cortex in a rat model of TBI. *J. Neurotrauma* 25, 513–526.
54. Thompson, S.N., Gibson, T.R., Thompson, B.M., Deng, Y., and Hall, E.D. (2006). Relationship of calpain-mediated proteolysis to the expression of axonal and synaptic plasticity markers following traumatic brain injury in mice. *Exp. Neurol.* 201, 253–265.
55. Jones, T.A., Liput, D.J., Maresh, E.L., Donlan, N., Parikh, T.J., Marlowe, D., and Kozlowski, D.A. (2012). Use-dependent dendritic regrowth is limited after unilateral controlled cortical impact to the forelimb sensorimotor cortex. *J. Neurotrauma* 29, 1455–1468.
56. Conti, A.C., Maas, J.W., Jr., Moulder, K.L., Jiang, X., Dave, B.A., Mennerick, S., and Muglia, L.J. (2009). Adenylyl cyclases 1 and 8 initiate a presynaptic homeostatic response to ethanol treatment. *PLoS One* 4, e5697.
57. Maas, J.W., Jr., Vogt, S.K., Chan, G.C., Pineda, V.V., Storm, D.R., and Muglia, L.J. (2005). Calcium-stimulated adenylyl cyclases are critical modulators of neuronal ethanol sensitivity. *J. Neurosci.* 25, 4118–4126.
58. Atkins, C.M., Oliva, A.A., Jr., Alonso, O.F., Pearce, D.D., Bramlett, H.M., and Dietrich, W.D. (2007). Modulation of the cAMP signaling pathway after traumatic brain injury. *Exp. Neurol.* 208, 145–158.
59. Calabresi, P., Gubellini, P., Centonze, D., Picconi, B., Bernardi, G., Chergui, K., Svenningsson, P., Fienberg, A.A., and Greengard, P. (2000). Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. *J. Neurosci.* 20, 8443–8451.
60. Greengard, P., Allen, P.B., and Nairn, A.C. (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron* 23, 435–447.
61. Risinger, F.O., Freeman, P.A., Greengard, P., and Fienberg, A.A. (2001). Motivational effects of ethanol in DARPP-32 knock-out mice. *J. Neurosci.* 21, 340–348.
62. Ron, D. (2004). Signaling cascades regulating NMDA receptor sensitivity to ethanol. *Neuroscientist* 10, 325–336.
63. Bales, J.W., Yan, H.Q., Ma, X., Li, Y., Samarasinghe, R., and Dixon, C.E. (2011). The dopamine and cAMP regulated phosphoprotein, 32 kDa (DARPP-32) signaling pathway: a novel therapeutic target in traumatic brain injury. *Exp. Neurol.* 229, 300–307.
64. Rhodes, J.S., Best, K., Belknap, J.K., Finn, D.A., and Crabbe, J.C. (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiol. Behav.* 84, 53–63.
65. Lyons, A.M., Lowery, E.G., Sparta, D.R., and Thiele, T.E. (2008). Effects of food availability and administration of orexigenic and anorectic agents on elevated ethanol drinking associated with drinking in the dark procedures. *Alcohol. Clin. Exp. Res.* 32, 1962–1968.
66. Rhodes, J.S., Ford, M.M., Yu, C.H., Brown, L.L., Finn, D.A., Garland, T., Jr., and Crabbe, J.C. (2007). Mouse inbred strain differences in ethanol drinking to intoxication. *Genes Brain Behav.* 6, 1–18.
67. Wilcox, M.V., Cuzon Carlson, V.C., Sherazee, N., Sprow, G.M., Bock, R., Thiele, T.E., Lovinger, D.M., and Alvarez, V.A. (2014). Repeated binge-like ethanol drinking alters ethanol drinking patterns and depresses striatal GABAergic transmission. *Neuropsychopharmacology* 39, 579–594.

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