Hypersensitive Glutamate Signaling Correlates with the Development of Late-Onset Behavioral Morbidity in Diffuse Brain-Injured Circuitry

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Abstract

In diffuse brain-injured rats, robust sensory sensitivity to manual whisker stimulation develops over 1 month post-injury, comparable to agitation expressed by brain-injured individuals with overstimulation. In the rat, whisker somatosensation relies on thalamocortical glutamatergic relays between the ventral posterior medial (VPM) thalamus and barrel fields of somatosensory cortex (S1BF). Using novel glutamate-selective microelectrode arrays coupled to amperometry, we test the hypothesis that disrupted glutamatergic neurotransmission underlies the whisker sensory sensitivity associated with diffuse brain injury. We report hypersensitive glutamate neurotransmission that parallels and correlates with the development of post-traumatic sensory sensitivity. Hypersensitivity is demonstrated by significant 110% increases in VPM extracellular glutamate levels, and 100% increase in potassium-evoked glutamate release in the VPM and S1BF, with no change in glutamate clearance. Further, evoked glutamate release showed 50% greater sensitivity to a calcium channel antagonist in brain-injured over uninjured VPM. In conjunction with no changes in glutamate transporter gene expression and exogenous glutamate clearance efficiency, these data support a presynaptic origin for enduring post-traumatic circuit alterations. In the anatomically-distinct whisker circuit, the injury-induced functional alterations correlate with the development of late-onset behavioral morbidity. Effective therapies to modulate presynaptic glutamate function in diffuse-injured circuits may translate into improvements in essential brain function and behavioral performance in other brain-injured circuits in rodents and in humans.

Key words: amperometry; brain trauma; fluid percussion glutamate; thalamocortical system

Introduction

DIFFUSE TRAUMATIC BRAIN INJURY (TBI) occurs in a significant proportion of the population, without sufficient understanding of the anatomical and circuit mechanisms responsible for the chronic morbidities that emerge (Scheid and von Cramon, 2010). More than one million Americans survive a TBI each year, of which 20–50% develop persistent functional morbidity within 1 month post-injury (Langlois et al., 2004; McAllister, 1992). Post-traumatic morbidity is the long-term neurological consequence of injury-related pathological processes that impair brain circuit function and activation, including problems with cognition, sensory processing, communication, and behavior/mental health (Chen and D'Esposito, 2010; McAllister, 1992). The first step in preventing or attenuating

post-traumatic morbidity is to investigate causal mechanisms in functionally-relevant neuronal circuits.

Diffuse TBI arises from abrupt deceleration forces, which mechanically shear axons, vasculature, and plasma membranes, without overt cavitation (Farkas and Povlishock, 2007; Graham et al., 2005; Povlishock and Katz, 2005). These primary injury components and acute secondary processes, including excitatory neurotransmitter release, oxidative stress, mitochondrial dysfunction, metabolic dysregulation, and inflammation (Faden et al., 1989; Farkas and Povlishock, 2007; Katayama et al., 1990; Povlishock and Katz, 2005), explain acute deficits, but not late-onset gain-of-function morbidities. Morbidity with delayed phenotypic expression likely arises from ongoing, unregulated homeostatic or neuroplastic processes in the wake of primary and secondary injuries to maintain cellular viability (Emery et al., 2000; Erb and Povlishock, 1991; Lifshitz et al., 2007; Singleton et al., 2002).

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Midline fluid percussion injury (mFPI) in rodents provides a clinically relevant model of diffuse brain injury that reproduces key acute features of human brain injury (Cernak, 2005; Morales et al., 2005), such as metabolic dysregulation, hypotension, and subdural hematoma (Dixon et al., 1987; McIntosh et al., 1987). Neuropathology includes axonal injury, membrane permeability, and an acute elevation of extracellular glutamate concentrations (Faden et al., 1989; Hinzman et al., 2010; Katayama et al., 1990; Kelley et al., 2006; Singleton and Povlishock, 2004; Singleton et al., 2002). Brain-injured rats show early neurological impairments in learning acquisition and sensorimotor processes (Hamm, 2001; Wiley et al., 1996; Yamaki et al., 1998). Over the first month after moderate mFPI, rodents develop sensory sensitivity to constant manual whisker stimulation, as observed by robust avoidance and apprehensive behaviors, in contrast to ambivalent or curious behavior by uninjured animals (McNamara et al., 2010). Whisker deflection drives mechanoreceptors on sensory axons that synapse in the “barrelettes” of the principal sensory trigeminal nucleus (Pr5) that innervate neuronal clusters in the ventral posterior medial (VPM) thalamus, called “barreloids” (Land et al., 1995). The VPM neurons project topographically to barrel fields in layer IV of primary somatosensory cortex (S1BF; Woolsey and Van der Loos, 1970). This whisker-barrel circuit encompasses large anatomical volumes that facilitate recordings, suffer diffuse axonal injury after mFPI (Hall and Lifshitz, 2010; Kelley et al., 2006; Lifshitz et al., 2007), and underlie the behavioral responses to whisker stimulation (McNamara et al., 2010). Since synapses throughout the afferent circuit are primarily glutamatergic (Kidd and Isaac, 1999), we propose that alterations in glutamate regulation contribute to late-onset post-traumatic behavioral morbidity. Novel glutamate-selective microelectrode arrays capable of sub-second *in vivo* electrochemical recordings by amperometry can be used to determine transient changes in extracellular glutamate neurotransmission as a gauge for circuit function (Burmeister et al., 2004; Pomerleau et al., 2003; Thomas et al., 2009).

These experiments explore mechanisms contributing to brain injury-induced, late-onset sensory sensitivity by investigating the functional consequences of diffuse brain injury on glutamatergic neurotransmission in the whisker-barrel circuit. We uncover hypersensitivity of glutamate neurotransmission across this circuit, which parallels and correlates with post-traumatic behavioral morbidity.

Methods

Subjects

A total of 57 adult male Sprague-Dawley rats (weight 300–350 g; Harlan, Indianapolis, IN) were used in these experiments, with 31 animals used to test behavior followed by *in vivo* amperometric recordings of glutamate (group I), 20 animals for behavior and real-time polymerase chain reaction (rtPCR, group II), and 6 animals for conotoxin studies (group III), as indicated in Figure 1. The animals were allowed access to food and water *ad libitum*, and all procedures and animal care were conducted according to an approved Institutional Animal Care and Use Committee protocol which is consistent with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

Diffuse brain injury: Midline fluid percussion injury (mFPI)

On day 0, experimental brain injury procedures were conducted as previously described (Hinzman et al., 2010; Kelley et al., 2006, 2007; Lifshitz et al., 2007). Under isoflurane anesthesia (2%), a craniotomy was performed on the sagittal suture midway between the bregma and the lambda. The female portion of a Luer-Loc needle hub was secured in the craniotomy using dental cement (cranial hub). Animals were either brain-injured (fluid percussion), or the cranial hub was removed without injury (sham). For injury, the cranial hub was filled with saline and attached to the male Luer-Loc on the end of the injury device. Animals recovering from isoflurane anesthesia (return of paw withdrawal reflex) were injured by a rapid (~20 msec) fluid pressure pulse (~1.9 atm) delivered onto the intact dura under the sagittal suture, as previously described (Kelley et al., 2006, 2007; Lifshitz et al., 2007). Moderate brain-injured animals exhibited a positive fencing response and a righting reflex time greater than 5 min (Hosseini and Lifshitz, 2009). One animal was euthanized due to post-injury complications because the righting reflex time was greater than 10 min and regular respiration did not return.

Whisker nuisance task (WNT)

At 1 week or 1 month post-injury (1 day prior to *in vivo* amperometric glutamate recordings in group I; immediately prior to tissue dissection for rtPCR in group II; Fig. 1), uninjured and moderate brain-injured rats were evaluated for behavioral responses to whisker stimulation using the WNT

FIG. 1. Experiments were conducted in three groups of animals. All animals were sham or brain-injured on day 0 using midline fluid percussion injury (mFPI). Group I rats were tested in the whisker nuisance task (WNT) at 6 and 27 days after FPI followed by amperometric recordings (Amp) at 7 and 28 days post-injury, respectively. Group II rats were tested in the WNT and had tissue taken for real-time polymerase chain reaction (PCR) within an hour of WNT completion at 7 days and 28 days post-FPI. Group III rats were tested for conotoxin sensitivity at 1 month post-mFPI, without behavioral evaluation.

(McNamara et al., 2010). After a 5-min acclimation period to the test cage, the whiskers on both mystacial pads were stimulated with a wooden applicator stick for three consecutive 5-min periods (15 min total). The predominant behavioral responses were recorded on 0–2-point non-parametric scales (0, absent, 1, present, 2, profound). The maximum whisker nuisance score was 16, with higher scores indicating multiple abnormal behavioral responses. Behavioral testing was conducted at the same time of day by the same observer, who was blinded to injury status. WNT data were analyzed using Kruskal-Wallis analysis of variance (ANOVA) with Dunn's *post-hoc* comparison.

Microelectrode array preparation

Ceramic-based microelectrode arrays (MEAs) that contained four platinum (Pt) recording surfaces ($15\ \mu\text{m} \times 333\ \mu\text{m}$) in a paired configuration were prepared to measure glutamate. These electrodes were fabricated and selected for *in vivo* recordings using previously published methods (Burmeister et al., 2000, 2002; Thomas et al., 2009). Pt sites 1 and 2 were coated with a solution containing glutamate oxidase (GluOx), bovine serum albumin (BSA) and glutaraldehyde, enabling these sites to selectively detect glutamate levels with low limits of detection (Nickell et al., 2005). Pt sites 3 and 4 were coated only with BSA and glutaraldehyde and served as sentinels, recording everything channels 1 and 2 recorded except for glutamate (Burmeister and Gerhardt, 2001; Day et al., 2006). On the day prior to amperometric recordings, all four recording sites were electroplated with a size-exclusion layer, 1,3-phenylenediamine (mPD; Thomas et al., 2009). In the presence of GluOx, glutamate is broken down into α -ketoglutarate and peroxide (H_2O_2). The H_2O_2 traverses the mPD exclusion layer and is readily oxidized and recorded as current using the FAST-16 instrument (see below).

Microelectrode array (MEA) calibration

The glutamate-selective MEA was submerged in 40 mL of 0.05 M PBS (pH 7.1–7.4), warmed to 37°C using a circulating water bath (Gaymar Industries, Inc., Orchard Park, NY), and was stirred using a magnetic stir bar and battery operated stir plate. Following 20 min of equilibration, aliquots of stock solutions in 500 μL ascorbic acid (AA; 20 mM), three 40- μL aliquots of L-glutamate (20 mM), 40 μL dopamine (2 mM), and 40 μL H_2O_2 (8.8 mM) were added to the PBS to calibrate the MEA to produce final concentrations of 250 μM AA, 20, 40, and 60 μM glutamate, 2 μM dopamine (DA), and 8.8 μM H_2O_2 (Thomas et al., 2009). From the calibration, the slope (electrode sensitivity to L-glutamate), selectivity (capability of recording glutamate over AA), and limit of detection (LOD; lowest amount of detectable glutamate) were determined; average values for slope were $4.7 \pm 0.3\ \text{pA}/\mu\text{M}$, for selectivity were 220 ± 37 to 1, and for LOD were $1.6 \pm 0.1\ \mu\text{M}$ ($n = 47$ electrodes; 80 glutamate recording sites). Calibrations were performed prior to experiments, as MEA performance is not compromised as a result of implantation (Hinzman et al., 2010).

Micropipette attachment

After the MEA was calibrated, a single-barrel glass capillary with filament ($1.0 \times 0.58\ \text{mm}^2$, 6", A-M Systems, Inc., Sequim, WA) was pulled using a Kopf Pipette Puller (David

Kopf Instruments, Tujunga, CA). The pulled micropipette was bumped against a glass rod so that the inner diameter of the micropipette was 10–14 μm (average = 12 μm). Clay was used to place the tip of the micropipette between the four Pt recording sites, approximately 50–80 μm (average = 73 μm) away from the MEA surface. This alignment was secured using Sticky Wax (Kerr Manufacturing Co., Romulus, MI). The measurements were taken under a microscope with a calibrated reticule. Immediately prior to *in vivo* placement of the MEA-micropipette assembly, the micropipette was filled with the appropriate solution (see below).

Surgeries for amperometric recordings of glutamate

At 1 week or 1 month post-injury, sham and brain-injured animals were anesthetized using 2.5% isoflurane in 100% O_2 at $\sim 3\ \text{L}/\text{min}$ flow rate. They were placed in a stereotaxic frame (David Kopf Instruments) fitted with an isoflurane nose cone adapter to maintain the isoflurane anesthetized state (2–2.5% isoflurane in 100% O_2 at $\sim 2\ \text{L}/\text{min}$ flow rate). An isothermal heating pad (Braintree Scientific, Braintree, MA) and rectal temperature probe was used to maintain body temperature at 37°C. After a midline scalp incision, the skin, fascia, and temporal muscles were reflected and a bilateral craniotomy over the S1BF and VPM was completed. The Ag/AgCl reference electrode was placed in a posterior subcutaneous pocket formed using blunt dissection and held in position by sutures (Moussy and Harrison, 1994; Quintero et al., 2007).

Amperometry: *in vivo* electrochemical recordings

The amperometry recording procedures were similar to previously published methods (Hinzman et al., 2010; Thomas et al., 2009). Constant-voltage amperometry was performed using a FAST-16 Mk-I electrochemistry instrument (Quanteon, LLC, Nicholasville KY) using software (Fast Analytical Sensor Technology FAST; Quanteon, LLC) developed for concurrent four-channel recordings. For *in vivo* recordings, a potential of +0.7 V was applied versus a Ag/AgCl reference electrode and the data were recorded at a frequency of 2 Hz. Current signals were converted to voltage by the headstage (2 pA/mV) and a secondary gain of 10 times, for a final gain of 0.2 pA/mV.

In vivo experimental protocol for measurements of glutamate

Prior to *in vivo* placement of the MEA-micropipette assembly, the micropipette was filled with isotonic 100- μM glutamate (100 μM L-glutamate in 0.9% physiological saline; pH 7.2–7.4), or isotonic 120 mM potassium (KCl) solution (120 mM KCl, 29 mM NaCl, and 2.5 mM CaCl_2 ; pH 7.2–7.4), using a combination of a 1-mL syringe filled with glutamate or KCl solutions, a 0.22- μm sterile syringe filter, and a 4" stainless steel needle (30 gauge, beveled tip; Popper and Son, Inc., NY). Sham and brain-injured animals were prepared for amperometric recordings 7 or 28 days post-sham or injury procedure and 1 day following WNT (see above). The MEA-micropipette assembly was positioned in the brain according to stereotaxic coordinates for the appropriate region where all anteroposterior (AP) measures were from the bregma, mediolateral (ML) measures were from midline, and

dorsal-ventral (DV) measures were from the dural surface (VPM: -3.5 ± 3.0 , -5.6 – 6.4 mm; S1BF: -2.0 ± 5.0 , -1 – 3.0 mm; Paxinos and Watson, 2007). Glutamate clearance and KCl-evoked release were recorded in opposite hemispheres in a balanced experimental design to control for any hemispheric variations, time under anesthesia, influence (if any) of exogenous glutamate/KCl, or regional disturbances in surrounding regions.

Nanoliter volumes of glutamate or KCl were locally applied to tissue by pressure ejection using a Picospritzer II connected to the open end of the micropipette by Tygon tubing (Parker Hannifin Corp., General Valve Corporation, Mayfield Heights, OH). Pressure was applied at 5–25 psi for 1 sec in all experiments. The volume of glutamate or KCl delivered was measured by determining the amount of fluid ejected from the micropipette using a dissection microscope fitted with a calibrated reticule (Cass et al., 1992; Friedemann and Gerhardt, 1992).

In vivo measurements of extracellular glutamate levels

Upon stereotaxic placement of the MEA-micropipette assembly, 10–20 min of baseline data were recorded. Tonic extracellular levels of glutamate were measured by averaging 30 sec of baseline recordings into a single data point prior to application of glutamate or KCl solutions. The microelectrode was moved in 400- μ m increments using a micromanipulator (MO-10; Narishige Scientific Instruments, Tokyo, Japan), and allowed us to establish a stable baseline for approximately 10 min between locations.

Glutamate clearance parameters

Once a steady-state signal was achieved, glutamate solution was locally applied every 30–60 sec for a total of 5 ejections. The MEA was then lowered in 400- μ m increments by an attached micromanipulator, baseline was acquired, and the recordings were repeated. Parameters from two of the five signals ranging from 5–20 μ M in amplitude were averaged for each Pt electrode site at each depth. The recordings were analyzed for the uptake rate, rise time, and time it took for 80% of the signal to decay from maximum amplitude (T_{80}). Amplitude-matched signals from brain-injured rats were compared to sham animals across time points (Hascup et al., 2006; Thomas et al., 2009).

KCl-evoked release of glutamate

Once a steady-state signal was achieved, the effect of a single local application of KCl solution on glutamate release was determined (Gerhardt et al., 1985; Thomas et al., 2009). Data regarding amperometric recordings were volume-matched prior to data analysis.

Pharmacological blockade of injury-induced alterations with ω -conotoxin

Double-barrel micropipettes with a 360° twist were fabricated from fused glass capillaries (1.0×0.58 mm², 6", A-M Systems, Inc.) pulled on a Narishige Electrode Puller (Narishige International USA, Inc., East Meadow, NY; McCann and Rogers, 1990). The inner diameter of each pipette was adjusted to 7–12 μ m. ω -Conotoxin MVIIA (C1182; Sigma-Aldrich, St. Louis, MO) was dissolved in sodium acetate

buffer and diluted to 1 μ M in 0.9% physiological saline. Solution pH was adjusted between 7.0 and 7.6 using sodium hydroxide. KCl and ω -conotoxin were filled into a double-barrel micropipette and secured 50–100 μ m away from the MEA recording sites as described above. At 1 month post-injury, sham and brain-injured animals were anesthetized and prepared for amperometric recording as described above. Local applications of 120 mM KCl separated by 1–2 min were used to establish the amount of a KCl-evoked glutamate release. Then 200–300 nL of 1 μ M ω -conotoxin was locally applied, followed by several additional local applications of 120 mM KCl spaced 1–2 min apart. Percent change in maximum amplitude of evoked glutamate release before and after ω -conotoxin application was compared between sham and mFPI animals. These experiments were conducted in the VPM at the following stereotaxic coordinates: AP -3.5 mm, ML ± 2.8 mm, DV -5.6 – -6.0 mm.

MEA placement verification

Animals used for electrochemistry were overdosed with sodium pentobarbital (150 mg/kg intraperitoneally) and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde immediately following the conclusion of experimental recordings. Electrode tracks were identified and registered with a standard rat brain atlas to confirm MEA placement in Giemsa-stained cryosections. No animals were excluded due to electrode misplacement.

Statistical analysis for amperometric recordings

Data from the paired recording sites on the MEA were averaged and used as a single data point. If only one microelectrode site provided usable data, then single site recordings were used. Multiple depths within anatomical regions were included as distinct regions in a two-way ANOVA with Bonferroni post-tests, as well as averaged for determination of differences in the whole anatomical structure (one-way ANOVA with Tukey's post-hoc comparison). Sham animals from one week and one month time points were combined for the sham animal group since no significant differences were measured between animal groups. Numbers of animals differed between groups for a variety of reasons: electrode recording failure due to blood contacting electrode, loss of exclusion layer (mPD) as indicated by the sentinel channels and outlier status (using Grubb's test). For behavioral correlations each animal was required to have a corresponding whisker nuisance score and electrochemical recording to be included in any analysis. For anatomical correlations, recordings from both the S1BF and VPM of the same animal were necessary. In all cases, significance was defined as $p < 0.05$.

Quantitative gene expression by rtPCR

At one week and one month post-FPI, sham and brain-injured animals were transcardially perfused with cold saline, the brains rapidly removed from the skull, dissected into 2 mm slices using a chilled rat brain matrix and a 1 mm diameter punch taken from VPM and S1BF. The mRNA content was stabilized (RNAlater, Qiagen Corp) and stored frozen. Isolated mRNA (RNeasy, Qiagen Corp) was quantified (NanoDrop ND-1000 spectrophotometer), converted to

complementary DNA (cDNA; High Capacity cDNA Archive Kit, Applied Biosystems Inc.) and then used as a template for selected commercially-available gene expression assays for quantitative real-time PCR (StepONE, Applied Biosystems). Samples were run in triplicate according to manufacturer's instructions. The Applied Biosystems TaqMan® Gene Expression Assays (GLT-1: Rn00568080_m1; GLAST: Rn00570130_m1; EAAC: Rn00564704_m1; 18s rRNA: 4352930E) have been optimized to run under universal thermal cycling conditions, with amplification efficiencies of 100%. Within each animal, relative gene expression is normalized to the 18s rRNA endogenous control and the expression level in the sham/vehicle group using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001), which relates gene expression to the PCR cycle number at which the fluorescence signals exceed a threshold above baseline. Relative gene expression was compared between sham, one week post-injury and one month post-injury rats using a one-way ANOVA with Tukey's post-hoc analysis. Sham animals from the one week and one month post-injury groups were combined for final analysis.

Results

Progressive development of post-traumatic sensory sensitivity to whisker stimulation

To demonstrate the progressive development of sensory sensitivity after diffuse brain injury, we tested uninjured and brain-injured rats in the WNT at 1 week and 1 month post-injury (McNamara et al., 2010). In their home cages, brain-injured animals showed no changes in grooming, motor, or exploratory behavior compared to uninjured animals. In an open field, the whiskers were manually stimulated for 3 consecutive 5-min periods (a total of 15 min), and aberrant behavioral responses (i.e., freezing, guarded body position, forced breathing, whisker retraction, or evasion of stimulation), were recorded in eight categories on a pre-determined scale, for which low scores demonstrated behavior that was soothing and high scores represent sensory sensitivity. Performance on the WNT showed the development of significant post-traumatic morbidity by 1 month post-injury (6.8 ± 0.6 , $n=18$) compared to uninjured rats (3.8 ± 0.6 , $n=16$; $KW_{(2,48)}=10.03$, $p=0.007$; Fig. 2), and animals 1 week post-injury (4.4 ± 0.8 , $n=17$). These data confirm behavioral morbidity that develops over 1 month post-injury, as previously reported (McNamara et al., 2010), and provide a behavioral correlate for studies of glutamate release in the diffusely-injured brain. These animals were divided into two groups to investigate glutamate neurotransmission ($n=31$), and glutamate transporter gene expression ($n=20$). Overall, the development of sensory sensitivity provides time points for the evaluation of circuit function prior to (1 week) and after (1 month) the expression of behavioral morbidity that can be correlated with functional changes in glutamate neurotransmission.

Progressive increase in extracellular glutamate levels in the VPM over 1 month post-injury

To evaluate if glutamate homeostasis is affected by diffuse brain injury in the time frame required to develop sensory sensitivity to whisker stimulation, measurements of tonic extracellular glutamate levels were recorded from the thalamocortical relays of the whisker-barrel circuit at 1 week and 1 month after mFPI using MEAs in anesthetized rats. For each relay, recordings were averaged from multiple depths; 3 depths within the VPM and 4 depths within the S1BF.

FIG. 2. Sensory sensitivity develops by 1 month after fluid percussion injury (FPI). Behavioral performance in response to whisker stimulation in sham ($n=16$), 1-week ($n=17$), and 1-month post-FPI rats ($n=18$) shows the development of significant behavioral morbidity over the 1-month time course, where higher numbers represent expression of more aberrant behaviors (* $p<0.05$ compared to sham animals; † $p<0.05$ compared to 1-week post-FPI by Kruskal-Wallis analysis of variance with Dunn's *post-hoc* comparison; bar graphs represent the mean $\pm$ standard error of the mean).

For individual animals, tonic extracellular glutamate levels in the VPM directly correlated with levels in S1BF (Pearson's coefficient $r^2=0.784$; $p<0.001$, Fig. 3E), which confirmed consistent tonic glutamate activity along the thalamocortical circuit. Despite time-dependent increases in the tonic extracellular levels of glutamate and the expression of behavioral morbidity, no significant correlations between these data were observed (correlations not shown).

Glutamate clearance and transporter expression remain unaffected by diffuse brain injury

To evaluate the contribution of glutamate transporter function to injury-induced increases in tonic glutamate levels,

In the VPM, a progressive increase in extracellular levels of glutamate occurred over 1 week ($6.7 \pm 0.7 \mu M$, $n=10$) to 1 month post-injury ($9.6 \pm 1.5 \mu M$, $n=10$), becoming significant at 1 month post-injury compared to sham animals ($4.6 \pm 0.9 \mu M$, $n=7$; $F_{(2,24)}=4.69$, $n=0.02$; Fig. 3A). The depth profile through the dorsal-ventral extent of the VPM revealed that the most superior and inferior regions of the VPM were susceptible to brain injury ($F_{(2,72)}=9.09$; $p=0.0003$; Fig. 3B).

In the S1BF, a trend toward elevated extracellular levels of glutamate was evident at 1 week ($6.6 \pm 0.7 \mu M$, $n=11$) and 1 month ($6.8 \pm 1.2 \mu M$, $n=10$) post-injury, but was not statistically significant compared to uninjured sham animals ($4.5 \pm 0.7 \mu M$, $n=8$; $F_{(2,26)}=1.70$, $p=0.20$; Fig. 3B). However, the depth profile analysis is significant ($F_{(2,100)}=3.29$; $p=0.0413$; Fig. 4E), with *post-hoc* comparisons revealing significant differences between uninjured sham and 1-week post-injured animals at a depth corresponding to cortical layer VIb (-2.8 to -3.5 mm; * $p<0.05$).

Glutamate clearance and transporter expression remain unaffected by diffuse brain injury

To evaluate the contribution of glutamate transporter function to injury-induced increases in tonic glutamate levels,

FIG. 3. Tonic levels of extracellular glutamate along the somatosensory whisker thalamocortical relays in diffuse brain-injured rats demonstrate changes in glutamate homeostasis post-injury. **(A)** Tonic levels of extracellular glutamate are significantly elevated in the VPM of the thalamus by 1 month post-injury (sham: $n = 7$; 1 week FPI: $n = 10$; 1 month FPI: $n = 10$). **(B)** The depth profile indicates the most robust effects occurred at depths of -5.6 mm and -6.4 mm from the dorsal surface of the rat brain. **(C)** Tonic levels of extracellular glutamate in the S1BF show a trend toward elevation after mFPI (sham: $n = 8$; 1 week FPI: $n = 11$; 1 month FPI: $n = 10$). **(D)** Analysis of the depth profile indicates a significant injury effect, however, post-hoc comparisons did not reveal significant differences between uninjured sham and 1 month post-injured animals at any specific depth. A significant difference does occur between sham and 1 week post-injured animals at a depth corresponding to layer VIb (-2.8 to -3.5 mm) **(E)** A positive correlation exists between tonic extracellular glutamate levels within the VPM and the S1BF ($r^2 = 0.784$; $p < 0.0001$). *, $p < 0.05$ compared to sham, one-way ANOVA with Tukey's post-hoc comparison, two-way ANOVA with Bonferroni post-tests for depth profile with depth and injury as variables. Bar graphs represent the mean $\pm$ SEM.

glutamate clearance was measured by amperometry during the local application of exogenous glutamate by pressure ejection from a micropipette in the relays of the thalamocortical circuit (Hascup et al., 2006; Nickell et al., 2005; Thomas et al., 2009). Glutamate signals from sham, 1-week, and 1-month brain-injured rats were amplitude-matched to allow for valid comparisons of glutamate clearance (Hascup et al., 2006; Thomas et al., 2009). Rise time, uptake rate, and the time to clear 80% (T_{80}) of the maximum glutamate signal did not significantly change over time or in response to diffuse brain injury in the VPM or S1BF (Table 1). For the VPM and S1BF, the range for rise time was 1.7–1.9 sec, the uptake rate was 1.3–2.3 μ M/sec, and T_{80} was 2.5–3.2 sec. With glutamate transporter function unaffected by diffuse brain injury at 1 week

and 1 month post-injury, we next considered glutamate transporter gene expression levels.

Glutamate transporter gene expression was measured using quantitative rtPCR in 1-week and 1-month post-injured animals in comparison to sham as a surrogate marker of glutamate transporter composition. In the VPM, the glutamate aspartate transporter (GLAST) was significantly elevated by 46% at 1 week post-injury, and returned to baseline by 1 month post-injury in comparison to sham animals ($F_{(2,15)} = 4.46$; $p = 0.03$; Fig. 4). The glial glutamate transporter (GLT-1; $F_{(2,14)} = 3.21$; $p = 0.07$) and the neuronal excitatory amino acid carrier (EAAC; $F_{(2,5)} = 1.65$; $p = 0.23$) gene expression were unaffected in the VPM at these time points. In the S1BF, neither GLT-1 ($F_{(2,11)} = 1.27$; $p = 0.32$), GLAST

TABLE 1. GLUTAMATE CLEARANCE PARAMETERS AFTER LOCAL APPLICATION OF 100 μ M GLUTAMATE IN UNINJURED SHAM AND THE BRAIN-INJURED VPM AND S1BF

VPM	Sham	1 week FPI	1 month FPI
Peak amplitude (μ M)	11.1 $\pm$ 1.1	10.6 $\pm$ 1.2	11.6 $\pm$ 1.2
Rise time (sec)	1.9 $\pm$ 0.3	1.7 $\pm$ 0.1	2.0 $\pm$ 0.2
Uptake rate (μ M/sec)	1.3 $\pm$ 0.3	2.3 $\pm$ 0.9	1.7 $\pm$ 0.4
T ₈₀ (sec)	2.5 $\pm$ 0.2	2.7 $\pm$ 0.3	3.0 $\pm$ 0.3
S1BF			
Peak amplitude (μ M)	10.4 $\pm$ 1.1	10.8 $\pm$ 1.1	11.6 $\pm$ 0.9
Rise time (sec)	1.9 $\pm$ 0.2	1.9 $\pm$ 0.2	2.0 $\pm$ 0.4
Uptake rate (μ M/sec)	1.5 $\pm$ 0.4	2.0 $\pm$ 0.7	2.0 $\pm$ 0.5
T ₈₀ (sec)	3.1 $\pm$ 0.4	3.1 $\pm$ 0.3	3.2 $\pm$ 0.4

Glutamate concentrations, from locally-applied exogenous glutamate as measured by the microelectrode arrays (MEAs), were empirically adjusted to achieve equivalent peak amplitudes.

In the VPM and S1BF, none of the parameters are significantly different between sham ($n=8$; 10), 1-week FPI ($n=10$; 9), and 1-month post-FPI ($n=9$; 10). Values are mean $\pm$ standard error of the mean.

FPI, fluid percussion injury; Rise time, time to reach maximum amplitude of extracellular glutamate concentration; Uptake rate, first-order decay kinetics of glutamate uptake; T₈₀, time for 80% of the maximal glutamate amplitude to decay; VPM, ventral posterior medial thalamus.

($F_{(2,11)}=0.85$; $p=0.45$) nor EAAC ($F_{(2,7)}=0.59$; $p=0.58$) gene expression levels were altered due to brain injury (Fig. 4). In addition, gene expression for vesicular glutamate transporters 1 and 2 were not altered in the VPM (vGluT1: $F_{(2,13)}=0.90$, $p=0.43$; vGluT2: $F_{(2,15)}=0.04$, $p=0.96$), or S1BF (vGluT1: $F_{(2,11)}=0.53$, $p=0.61$; vGluT2: $F_{(2,11)}=3.27$, $p=0.08$) in brain-injured animals compared to uninjured sham animals. Thus, injury-induced change in glutamate uptake and transporter gene expression cannot explain the behavioral sensory sensitivity or alterations in tonic glutamate levels.

Delayed increase in evoked glutamate release in the VPM and S1BF at 1 month post-injury

Since glutamate clearance did not appear responsible for the injury-induced elevation of tonic extracellular glutamate

levels after brain injury, we next examined whether injury-induced changes in presynaptic glutamate release underlie the elevated tonic glutamate levels. Whisker stimulation under isoflurane anesthesia did not elicit glutamate transients (data not shown), as reported in the awake animal (Pomerleau et al., 2003). Therefore, small amounts of isotonic potassium (KCl) solution (~ 40 nL) were locally applied using an attached micropipette to depolarize pre-synaptic terminals. The maximum amplitude of evoked glutamate release was measured by MEAs at 1 week and 1 month post-injury in comparison to sham animals. For each relay, several recordings were made at multiple depths in the dorsal-ventral axis (3 VPM depths and 4 S1BF depths).

Representative traces of real-time extracellular glutamate concentrations show a marked increase in maximum glutamate concentration with the application of 40 nL of 120-mM isotonic KCl solution in the 1-month brain-injured VPM compared to sham and 1-week brain-injured VPM (Fig. 5A). In uninjured sham animals, KCl solution elicited a transient, three- to six-fold rise in extracellular glutamate levels above tonic levels.

In the VPM of 1-month brain-injured animals, evoked glutamate release was significantly elevated by over 100% ($49.7 \pm 8.0 \mu$ M; $n=8$) compared to 1-week brain-injured ($24.4 \pm 3.1 \mu$ M; $n=10$) and sham animals ($24.5 \pm 3.5 \mu$ M, $n=9$; $F_{(2,24)}=8.16$, $p=0.002$; Fig. 5B). Pre-synaptic glutamate release from 1-week brain-injured animals was not significantly different from uninjured sham animals. These averaged results represent significant differences in glutamate signaling at all three recording depths within the VPM ($F_{(2,62)}=18.86$; $p<0.0001$; Fig. 5C).

In the S1BF of 1-month brain-injured animals, evoked glutamate release was significantly elevated by $\sim 100\%$ ($26.1 \pm 5.9 \mu$ M; $n=7$) compared to sham ($13.1 \pm 2.1 \mu$ M, $n=7$; $F_{(2,21)}=3.61$, $p=0.045$; Fig. 5D). At 7 days post-injury, evoked glutamate release in the S1BF ($17.3 \pm 1.4 \mu$ M; $n=10$) was not significantly different from uninjured sham animals. These averaged results arise from non-significant increases in evoked glutamate release at four depths through the S1BF ($F_{(2,81)}=4.52$; $p=0.0137$; Fig. 5E). As with tonic glutamate levels, a significant positive correlation existed between evoked glutamate release in the VPM and S1BF for individual animals (Pearson's coefficient $r^2=0.62$; $p=0.002$; Fig. 5F).

FIG. 4. Glutamate transporter gene expression was assessed in the ventral posterior medial (VPM) nucleus of the thalamus and primary somatosensory barrel cortex (S1BF) using quantitative real-time PCR. In the VPM, a significant 50% increase in GLAST mRNA expression was evident at 1 week post-injury, which returned to baseline by 1 month post-injury (sham: $n=4-6$; 1 week FPI: $n=4-6$; 1 month FPI: $n=4-6$). No significant gene expression changes in comparison to sham were found at the 1 month time point for GLT-1, GLAST, or EAAC in the VPM or S1BF. *, $p<0.05$ compared to sham, one-way ANOVA with Tukey's post-hoc comparison. Bar graphs represent the mean $\pm$ SEM.

FIG. 5. Potassium (KCl)-evoked glutamate release uncovers circuit dysfunction after midline fluid percussion injury (mFPI). (A) Representative raw traces of KCl-evoked glutamate release in the ventral posterior medial (VPM) thalamus. Baseline levels have been adjusted to emphasize transient changes in evoked glutamate concentrations. The arrow indicates the application of KCl, which depolarizes neurons and releases glutamate as recorded by a single electrode site on the microelectrode array. The maximum amplitude of glutamate measured in the VPM at 1 month post-injury (triangles) was greater than that of sham (circles) and 1-week post-injury (squares) animals. (B and D) In the VPM (sham: $n=9$; 1 week FPI: $n=10$; 1 month FPI: $n=8$), and S1BF (sham: $n=7$; 1 week FPI: $n=10$; 1 month FPI: $n=7$), the amplitudes of KCl-evoked glutamate release were significantly elevated above sham levels at 1 month post-injury. In the VPM, evoked release of glutamate at 1 month post-injury was also significantly greater than at 1 week post-injury. (C) The depth profile in the VPM indicates that brain injury contributes to 36% of the total variance. Significant increases in evoked glutamate release at 1 month after moderate diffuse brain injury were evident at all depths tested. (E) The depth profile in the S1BF revealed that diffuse brain injury contributed to 10% of the total variance. However, while the overall analysis of variance (ANOVA) was significant, *post-hoc* comparisons did not reveal significant differences between uninjured sham and injured animals at any specific depth. (F) There is a positive correlation between evoked glutamate release within the VPM and the S1BF ($r^2=0.62$; $p=0.002$; * $p<0.05$ compared to sham; † $p<0.05$ compared to 1 week FPI by one-way ANOVA with Tukey's *post-hoc* comparison, two-way ANOVA with Bonferroni *post-hoc* tests for depth profile with depth and injury as variables; bar graphs represent the mean $\pm$ standard error of the mean; note the different scales for VPM and S1BF).

Thus, the injured brain exhibits enhanced evoked glutamate release, which represents injury-induced augmentation of glutamate neurotransmission in the diffusely-injured thalamocortical circuit.

Evoked glutamate release correlates with post-traumatic sensory sensitivity

Diffuse brain injury altered evoked glutamate release along the somatosensory thalamocortical circuit in a time course that parallels the development of behavioral sensory sensitivity to whisker stimulation. The intra-animal study design allowed direct correlation between behavioral expression of post-traumatic morbidity and glutamatergic neurotransmission in the associated circuit. In the VPM, evoked glutamate release directly correlated with whisker nuisance scores in individual animals (-6.4 mm depth, ventral; Pearson's coefficient $r^2=0.41$; $p=0.019$; Fig. 6). In the S1BF, evoked glutamate release directly correlated with whisker nuisance scores in individual animals (average of all depths; $r^2=0.36$; $p=0.024$; Fig. 6). Thus, significant correlations between injury-induced sensory sensitivity (whisker nuisance score) and hypersensitive glutamate neurotransmission (maximum amplitude of potassium-evoked glutamate release) indicate functional alterations in the circuitry associated with the expression of behavioral morbidity.

Inhibition of pre-synaptic glutamate release blocked the increased evoked release in the brain-injured VPM

By 1 month post-injury, KCl-evoked glutamate release in the VPM and S1BF was increased concomitantly with the development of sensory sensitivity to whisker stimulation. To verify that injury-induced alterations in presynaptic glutamate release contribute to the elevated evoked release of glutamate at 1 month post-injury in the VPM, a calcium channel inhibitor, ω -conotoxin, was locally applied *in vivo*. Reproducible traces of KCl-evoked glutamate release were established and were immediately followed by local application of 200–300 nL of ω -conotoxin (Fig. 7A). After inhibitor application, repeated local applications of KCl solution were

delivered every 60–90 sec to evaluate the efficacy of inhibition on evoked glutamate release. Maximum amplitudes of KCl-evoked glutamate release after ω -conotoxin were markedly reduced compared to pre-treatment measurements in both sham and brain-injured animals. The percentage reduction in peak evoked glutamate release at 1 month post-injury ($61.7\% \pm 3.3\%$) was significantly greater compared to sham animals ($39.3\% \pm 6.2\%$ by two-tailed unpaired Student's *t*-test, $T_4=3.18$, $p=0.034$; Fig. 7B). Thus, the injury-induced increases in evoked glutamate release may have a neuronal origin and appear to have an altered sensitivity to inhibition of voltage-gated calcium channels.

Discussion

The data support a pre-synaptic locus for the glutamate neurotransmission hypersensitivity that paralleled and correlated with the expression of behavioral morbidity after diffuse TBI in a functionally-relevant circuit of the rat. Using state-of-the-art glutamate-sensitive microelectrode arrays, we showed functional alterations in glutamate neurotransmission (tonic and evoked levels) along the whisker-barrel circuit. A robust 110% increase in tonic glutamate levels was seen in the thalamic relay, concomitant with a 100% increase in KCl-evoked pre-synaptic glutamate release in both the thalamus and cortex (see Figure 8 for summary). Inhibition of voltage-gated calcium channels had a significantly greater effect in the brain-injured VPM, indicating hypersensitive pre-synaptic glutamate release as a likely site for injury-induced damage. Together these results demonstrate that injury-induced hypersensitivity of glutamate neurotransmission may mediate post-traumatic behavioral morbidity, in part through augmented pre-synaptic glutamate release.

The whisker-barrel circuit serves as a prototype to study circuit disruption following diffuse brain injury

The somatosensory whisker-barrel circuit serves as a simplified *in vivo* model system to isolate and test mechanisms underlying glutamatergic circuit disruption and long-term morbidity as a result of diffuse brain injury. Neurons in the

FIG. 6. Injury-induced alterations in evoked glutamate release correlate with post-traumatic sensory sensitivity. **(A)** For the VPM, a significant positive correlation exists between KCl-evoked glutamate release and whisker nuisance scores in uninjured sham and 1 month mFPI rats ($r^2 = 0.41$; $p = 0.0192$), supporting that altered glutamate neurotransmission may be associated with behavioral morbidity. **(B)** For the S1BF, KCl-evoked glutamate release and whisker nuisance scores also showed a significant positive correlation ($r^2 = 0.36$; $p = 0.024$). These data indicate that increased evoked glutamate release at 1 month post-FPI occurs in accordance with the developmental progression of behavioral morbidity. Note different scales for VPM and S1BF.

FIG. 7. Pharmacological blockade of calcium channels attenuates the injury-induced increase in KCl-evoked glutamate release. (A) Representative traces show KCl-evoked glutamate release before (Pre) and after (Post) the local application of 1 μ M ω -conotoxin to block voltage-dependent calcium channels in the uninjured sham and 1-month post-injured ventral posterior medial (VPM) thalamus. Isotonic KCl solution application (indicated by the arrows) evoked glutamate release that is reproducible both before and after drug application. (B) The average percent reduction in the amount of KCl-evoked glutamate release by ω -conotoxin (sham, $n=3$; injured, $n=3$) was significantly greater in injured animals in comparison to sham animals (* $p < 0.05$ by two-tailed unpaired Student's t -test; Bar graphs represent the mean $\pm$ standard error of the mean).

FIG. 8. Summary of experiments in the VPM: Our studies are indicative of a late-onset injury-induced behavioral morbidity that correlates with altered presynaptic glutamate neurotransmission. Schematically, we illustrate possible cellular sites that may explain the observed behavioral and neurochemical changes after brain injury. Tonic levels of glutamate (1) gradually increased over time post-injury becoming significant by 1 month. Glutamate clearance was evaluated by clearance kinetics of locally applied exogenous glutamate and gene expression of associated transporters and uptake mechanisms. Glutamate clearance kinetics were not significantly altered at 1 week and 1 month post-injury; however, GLAST gene expression (2) at 1 week was significantly increased and returned to sham levels by 1 month post-injury. GLT-1 (3) and EAAC (4) gene expression were not altered. Increased levels of KCl-evoked pre-synaptic glutamate release were evident by 1 month post-injury (5) and confirmed by voltage-gated calcium channel inhibition (not illustrated). Gene expression for vesicular glutamate transporters (6; vGluT1, vGluT2) were unchanged after injury, indicating that vesicle number or neurotransmitter transport into vesicles is likely unaffected by brain injury (data not illustrated).

VPM and S1BF share morphological and physiological similarities with glutamatergic neurons in more complex circuits (Danbolt, 2001; Waite and Tracey, 1995). Without overt cavitation, this circuit undergoes bilateral indiscriminate diffuse axonal injury, including complete axotomy after mFPI (Hall and Lifshitz, 2010; Kelley et al., 2006; Lifshitz et al., 2007). Injury-related neuropathology would impair tactile facial whisker somatosensation, as expressed by aberrant behavioral responses to whisker stimulation in brain-injured animals (McNamara et al., 2010). The present communication exploits the unique anatomy and diffuse brain injury pathology to uncover pre-synaptic pathological processes contributing to the development of late-onset morbidity.

Glutamate neurotransmission in the whisker thalamocortical relays convey injury-induced circuit disarray

In individual animals, tonic glutamate levels in the VPM correlated with levels in the S1BF, indicating circuit, rather than regional, dysfunction. S1BF tonic glutamate levels in sham animals were comparable to values previously reported for the prefrontal cortex, despite different anesthetics (urethane versus isoflurane; Hinzman et al., 2010). Interestingly, published values for baseline extracellular glutamate levels measured by microdialysis in the S1BF of Wistar rats under urethane anesthesia ($6.9 \pm 1.0 \mu\text{M}$) were 50% higher than our recorded concentrations in sham S1BF of Sprague-Dawley rats (Homola et al., 2006), which may be due to strain differences, methodology, and the sampling of different pools of extracellular glutamate (Hascup et al., 2010). Additionally, depth profiles of tonic glutamate levels were more affected by injury in the dorsal-most and ventral-most regions of the VPM, rather than the core of the region, extending prevailing hypotheses of injury vulnerability at gray-white matter interfaces (Graham et al., 2002). Microelectrode array recordings of tonic glutamate were sensitive and reproducible, thereby aiding a detailed investigation of injury-induced alterations in glutamate neurotransmission in the whisker-barrel circuit.

Increased tonic and phasic glutamate levels at 1 month post-FPI could result from changes in glutamate transporter expression and/or their function. If reduced glutamate transporter expression was responsible for increased glutamate concentrations, we would have measured slower glutamate clearance parameters with exogenous glutamate application in the extracellular space. Synaptic glutamate transporter function or concentration could differ from that outside the synapse, though current *in vivo* techniques cannot differentiate these loci. In diffuse brain injury, however, glutamate uptake rates and glutamate clearance times (T_{80}) remained unaffected by brain injury. In the rat, glutamate clearance is predominantly mediated by glutamate transporters (GLT-1, GLAST, and EAAC in the rodent; Danbolt, 2001). Gene expression of GLT-1, GLAST, and EAAC were unchanged at 1 month after diffuse TBI. In contrast, glutamate transporter protein expression was downregulated by 20–50% in contused or peri-contusional tissue from humans and rodents up to 72 h post-injury (Rao et al., 1998; van Landeghem et al., 2001, 2006; Yi and Hazell, 2006), indicating a role for glutamate dysregulation in necrotic contusion formation. Similarly, KCl-evoked glutamate overflow (microdialysis)

was elevated in an established kainic acid-induced epilepsy model as a result of downregulated glutamate transporters (GLT-1 and GLAST; Ueda et al., 2001). Thus, in diffuse brain injury without contusion, glutamate transporter gene expression and function may not substantially contribute to the late-onset increase seen in tonic and evoked glutamate concentrations.

Injury-induced changes in the VPM were greater than in the S1BF and correlated with sensory sensitivity. Additionally, post-traumatic thalamic damage could be related to thalamic pain syndrome (Formisano et al., 2009; Nicholson and Martelli, 2004). To demonstrate a presynaptic locus for injury-induced alterations in VPM glutamate neurotransmission, we inhibited presynaptic glutamate release in the VPM with the voltage-gated calcium channel inhibitor ω -conotoxin. As predicted, calcium channel inhibition reduced KCl-evoked release in the uninjured VPM, likely attenuating action potentials and calcium-mediated vesicular release. In the injured brain, calcium channel inhibition significantly reduced evoked glutamate release compared to uninjured values, indicating that ion channel dysfunction may contribute to post-traumatic pre-synaptic alterations in glutamatergic neurotransmission. The remaining KCl-evoked glutamate release after conotoxin application may represent a pool of conotoxin-insensitive glutamate release, with the conotoxin-sensitive pool vulnerable to diffuse TBI. However, astrocyte modulation of neuronal release cannot be completely ruled out, as N-type calcium channels (blocked by ω -conotoxin) are also present on a small subset of astrocytes in the CNS (Latour et al., 2003). Regardless, the injury-induced increase in the sub-second evoked release of glutamate was likely of neuronal origin. Glutamatergic inputs to the VPM from the brainstem (the principal nucleus of the trigeminal nerve) and S1BF likely contributed to the observed local effects of KCl.

Several reports of thalamic disruption after lateral fluid percussion have been demonstrated from 1 week to 12 months post-injury, identifying ongoing wallerian degeneration, apoptosis, and hemodynamic fluctuations (Bramlett and Dietrich, 2002; Conti et al., 1998; Hallam et al., 2004; Hayward et al., 2011; Osteen et al., 2001; Sato et al., 2001; Smith et al., 1997). One month post-moderate midline FPI, stereological assessments confirmed the presence of neuronal atrophy in the VPM (Lifshitz et al., 2007) and S1BF (Lifshitz and Lissabee, 2011), without significant neuronal loss. These initial subtle differences between fluid percussion impact sites may have a profound influence on the time course and severity of thalamic neurodegeneration. Future work will explore degeneration and remodeling of specific pathway inputs to the VPM in order to identify the locus of the injury-induced effects seen after moderate midline fluid percussion injury.

An increase in KCl-evoked glutamate release could represent changes in several parts of the network: an increase in pre-synaptic release, an increase in cellular excitability, an increase in post-synaptic receptor number/function, or an increase in synapse number. The first two options could cause a direct increase in KCl-evoked glutamate release, and all but the first option could cause an increase via greater network excitability. Deafferented neurons have been documented to exhibit delayed-onset and long-lasting hyperexcitability attributable to axonal and synaptic reorganization, failure of synaptic inhibition, intrinsic regulation of excitability, and

homeostatic changes in receptors, channels, and transporters (Cai et al., 2007). Injury-induced alterations in presynaptic release could also arise from impaired extracellular buffering capacity (Santhakumar et al., 2003), or vesicular loading (high quantal transmitter concentration or more released vesicles; Watt et al., 2000). GABA-ergic inputs are received by the VPM from the reticular nucleus. In the cortex, several classes of GABA-ergic interneurons refine sensory signals associated with whisker activation (Schubert et al., 2007), such that functional consequences of injury-induced damage to sensory afferents may be augmented or attenuated by these local GABA-ergic relays, and explain the post-injury hypersensitivity. Further studies addressing the previously mentioned topics could tease out the molecular mechanisms associated with the functional hypersensitivity associated with post-traumatic morbidity.

Conclusions

In humans, the acute treatment of brain injury has advanced tremendously, reducing mortality rates, which has increased the prevalence of post-traumatic morbidity. Our data indicate an association between injury-induced late-onset behavioral morbidity and altered presynaptic glutamate signaling in rats, as evidenced by increased extracellular and evoked glutamate concentrations. Several mechanisms underlying the increased evoked release were explored, including glutamate clearance, transporter gene expression, and pre-synaptic release. Glutamate uptake and transport remain intact after diffuse brain injury, but pre-synaptic release mechanisms appeared to be more vulnerable to brain injury (Fig. 8). These data imply that hypersensitive glutamate neurotransmission plays a pivotal role in the development or maintenance of post-traumatic morbidity. Additional structural, molecular, electrophysiological, and awake-behaving experiments are required to identify rational pharmacological targets. However, similarly to post-traumatic epilepsy and neuropathic pain, these data implicate hypersensitive glutamate neurotransmission in the maintenance of functional morbidity.

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Author Disclosure Statement

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