Lithium Improves Dopamine Neurotransmission and Increases Dopaminergic Protein Abundance in the Striatum after Traumatic Brain Injury

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Abstract

Experimental models of traumatic brain injury (TBI) recapitulate secondary injury sequela and cognitive dysfunction reported in patients afflicted with a TBI. Impairments in neurotransmission are reported in multiple brain regions in the weeks following experimental TBI and may contribute to behavioral dysfunction. Formation of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex is an important mechanism for neurotransmitter exocytosis. We previously showed that lithium treatment attenuated hippocampal decreases in α -synuclein and VAMP2, enhanced SNARE complex formation, and improved cognitive performance after TBI. However, the effect of TBI on striatal SNARE complex formation is not known. We hypothesized lithium treatment would attenuate TBI-induced impairments in evoked dopamine release and increase the abundance of synaptic proteins associated with dopamine neurotransmission. The current study evaluated the effect of lithium (1 mmol/kg/day) administration on striatal evoked dopamine neurotransmission, SNARE complex formation, and proposed actions of lithium, including inhibition of GSK3 β , assessment of synaptic marker protein abundance, and synaptic proteins important for dopamine synthesis and transport following controlled cortical impact (CCI). Sprague-Dawley rats were subjected to CCI or sham injury and treated daily with lithium chloride or vehicle for 7 days post-injury. We provide novel evidence that CCI reduces SNARE protein and SNARE complex abundance in the striatum at 1 week post-injury. Lithium administration improved evoked dopamine release and increased the abundance of α -synuclein, D2 receptor, and phosphorylated tyrosine hydroxylase in striatal synaptosomes post-injury. These findings show that lithium treatment attenuated dopamine neurotransmission deficits and increased the abundance of synaptic proteins important for dopamine signaling after TBI.

Keywords: alpha synuclein; dopamine; neurotransmission; SNARE; striatum; traumatic brain injury

Introduction

CLINICAL AND EXPERIMENTAL STUDIES highlight that traumatic brain injury (TBI) results in impaired cognitive abilities, including difficulties with memory, learning, attention and executive function, that can persist for years following the injury.^{1–7} Multiple brain regions exhibit impaired neurotransmission that can contribute to changes in cognitive function including the cortex, hippocampus, and striatum in experimental models of TBI and in the striatum of TBI patients.^{8–14} Experimental TBI results in altered dopamine signaling, axonal injury, impaired mitochondrial function, and neuronal loss in the striatum or substantia nigra in the days and weeks post-injury.^{15–23} Experimental TBI results in acute increases in dopamine content within hours of injury,^{24,25} followed by reduced dopamine neurotransmission in the weeks following injury.^{13,14,26} TBI-induced changes in tyrosine hydroxylase (TH)

activity, dopamine transporter expression, and dopamine receptor have been described in the striatum during the weeks post-injury,^{13,26–28} which may contribute to impaired neurotransmission. Therapeutic interventions in pre-clinical models aimed at enhancing dopaminergic system activity have been shown to improve motor and cognitive performance.^{29–34}

Reductions in neurotransmitter release may be attributed to impairments in intrasynaptic vesicular docking and the machinery necessary for vesicular translocation in the synapse. The highly conserved soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex and associated proteins, including cysteine string protein α (CSP α) and α -synuclein (α -syn), are important for regulated fusion of a synaptic vesicle with the plasma membrane and the subsequent release of neurotransmitters into the synaptic cleft.^{35,36} Examination of genetically modified animals demonstrates the connection between reductions in

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SNARE complex formation and impaired neurotransmission.^{37–42} We previously reported that TBI significantly reduces the abundance of multiple monomer SNARE proteins and SNARE complexes in the hippocampus, and that these alterations occur concomitantly with alterations in synaptic vesicle density and distribution at 1 week following controlled cortical impact (CCI) injury.^{43,44} While neurotransmission impairments have been reported in the striatum, potential changes in SNARE protein abundance and SNARE complex formation have not been examined.

Lithium has been established as a therapy for psychiatric disorders, but the mechanism by which this beneficial effect is exerted is poorly understood. Published reports suggest that lithium may improve brain function by multiple mechanisms, including increasing synaptic density, inhibiting glycogen synthase kinase 3 β (GSK3 β), and modulating dopamine neurotransmission.^{45–51} While a function for GSK3 β was first identified in glycogen metabolism regulation,⁵² GSK3 β has now been shown to play important roles in signaling cascades, including WNT and Akt, and cellular functions of differentiation, proliferation, and apoptosis.^{53–57} Phosphorylation of GSK3 β at serine 9 inhibits GSK3 β and is associated with increased β -catenin expression, reduced cell loss, and improved neurobehavioral function after TBI.^{50,51,58} Cordiero and colleagues reported that treatment with lithium increased the expression of the SNARE complex chaperone protein CSP α *in vitro* and *in vivo*,^{59,60} highlighting a potential novel mechanism of lithium's actions in the brain. We recently demonstrated that treatment with lithium in the context of TBI increased the abundance of several SNARE proteins, including α -syn and VAMP2, and promoted SNARE complex formation in the hippocampus at multiple time-points post-injury.⁴³ Together, these findings suggest that lithium may improve synaptic function in the striatum after TBI.

In this study, we sought to examine the effect of lithium treatment on dopaminergic neurotransmission and the abundance of synaptic proteins in the striatum at 1 week post-injury, a time-point of established neurotransmission impairments following controlled cortical impact (CCI). Potassium-evoked dopamine release was evaluated by microdialysis and measured with high-performance liquid chromatography at 1 week post-injury. The effect of lithium on SNARE protein abundance, markers of synaptic density, GSK3 β abundance, and dopamine related proteins were evaluated in striatal synaptosomes at 1 week after TBI.

Methods

Animals

All experimental procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee in accordance with the guidelines established by the National Institutes of Health in the Guide for the Care and Use of Laboratory Animals. Animals were housed up to two rats per cage in the University of Pittsburgh vivarium with a 12:12 light/dark photoperiod (lights on at 7:00 A.M.) and provided food and water *ad libitum*. Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 325–425 g were utilized in this study.

CCI

Rats were randomized for assignment to injury and treatment status. Rats were anesthetized with 4% isoflurane in a 2:1 N₂O/O₂ mixture in a ventilated anesthesia chamber. Following endotracheal intubation, rats were mechanically ventilated and anesthesia was maintained with a 2% isoflurane mixture. Rats were placed in a stereotaxic frame in the supine position and body temperature was

monitored by rectal thermistor probe and maintained at 37°C with a heating pad. Following a midline incision, soft tissues were reflected and a craniotomy (7 mm diameter) was completed between bregma and lambda and centered 5 mm lateral of the sagittal suture to expose the dura mater over the right parietal cortex. Control sham-injury animals were subjected to anesthesia and surgical procedures but did not receive a TBI. The CCI injury was produced using a small bore (1.975 cm) double-acting stroked-constrained pneumatic cylinder with a 5.0 cm stroke.⁶¹ An impactor tip (6 mm in diameter) was set to produce a tissue deformation of 2.8 mm at a velocity of 4 m/sec with a dwell time of 150 msec. Following sham or CCI injury, the scalp was sutured, anesthesia stopped, and the righting time of each animal was recorded. Once ambulatory, the rats were returned to their home cage.

Administration of lithium

A therapeutic dose of 1 mmol/kg/day lithium chloride was selected based upon published work demonstrating that 1 mmol/kg/day lithium chloride increased SNARE protein abundance in the hippocampus⁴³ and was neuroprotective after TBI.^{50,51,62,63} Treatment with lithium chloride (Sigma, St. Louis, MO), or vehicle (U.S.P. saline) was initiated at 5 min post-injury by intraperitoneal injection and continued daily until date of euthanasia.

Measurement of evoked neurotransmission

A total of 26 rats ($n=6-7$ per group) were generated to evaluate the efficacy of lithium to improve evoked dopamine release in the striatum. Rats were randomly assigned to receive sham or CCI injury and treated with vehicle or lithium as described. At 1 week post-injury, the animals were anesthetized as described and placed in the stereotaxic frame for microdialysis probe insertion. The incision was opened and soft tissues reflected for the placement of two set screws and the completion a burr hole for probe insertion in the striatum (AP, +0.0 mm; ML, +2.8; DV, –5.0). A guide cannula (SciPro, Sanborn, NY) was inserted into the brain and secured with dental cement. Following curing of the cement, the microdialysis probe (3 mm length, 0.6 mm diameter, 35 kDa molecular weight cutoff; SciPro) was lowered and secured in the guide cannula. Following a 10-min equilibration period, anesthesia was stopped and the animals were placed in a Plexiglass chamber designed for continuous microdialysate collection in awake free-moving animals, as previously described.¹³ Artificial cerebrospinal fluid (aCSF; 126.5 mM NaCl, 2.4 mM KCl, 1.1 mM CaCl₂, 0.83 mM MgCl₂, 27.5 mM NaHCO₃, and 0.5 mM KH₂PO₄) was continuously infused at a rate of 2 μ L/min for 3 h to allow equilibration prior to collection of microdialysate for analysis. After the equilibration period, microdialysate was collected every 20 min for 60 min (–60, –40, –20 min). At the 0 min time-point, the dialysate was switched to high-potassium aCSF (48.9 mM NaCl, 80 mM KCl, 1.1 mM CaCl₂, 0.83 mM MgCl₂, 27.5 mM NaHCO₃, and 0.5 mM KH₂PO₄) in order to stimulate dopamine release for a period of 40 min (0 and 20 min), followed by a second switch for infusion of normal aCSF until the completion of the experiment (40, 60, 80, and 100 min).

After collection of the last sample, rats were euthanized by overdose of sodium pentobarbital (intraperitoneal, 100 mg/kg), and transcardially perfused with saline, followed by 10% neutral-neutral buffered formalin (Fisher Scientific, Waltham, MA); the brains removed for post-fixation for 24 h, and cryoprotected with 30% sucrose in 0.1 M phosphate-buffered saline. Brains were frozen, cut into 35 μ m thick coronal sections and stained with cresyl violet to verify the location of the probes. *In vitro* analysis of the probe was completed to verify probe function. The concentration of dopamine in the microdialysates was measured by high-performance liquid chromatography consisting of a CoulArray

FIG. 1. Lithium treatment improved striatal evoked dopamine release following controlled cortical impact (CCI). **(A)** High-potassium-evoked dopamine (DA) release in the striatum was assessed by microdialysis at 1 week after CCI. DA levels of sham animals from vehicle and lithium treatment groups were not significantly different, and were combined for comparisons to injured groups ($n=7$ Sham+vehicle [Veh]; $n=6$ Sham+lithium [Li]). Vehicle treatment following CCI ($n=6$) resulted in significantly reduced DA levels at 0, 20, and 40 min during high-potassium artificial cerebrospinal fluid (K aCSF)-evoked release (0–40 min, line), compared with sham animals ($*p<0.01$). Treatment with lithium after CCI ($n=7$) resulted in reduced DA levels at 0 and 20 min, but was not significantly different at the 40-min time-point, compared with sham ($\#p<0.05$). **(B,C)** Treatment with vehicle after CCI resulted in significantly reduced area under the curve (AUC) and peak DA concentrations, compared with sham ($*p<0.05$). AUC and peak DA concentrations from animals treated with lithium after CCI were not significantly different from sham. SEM, standard error of the mean.

Detector (ESA, Chelmsford, MA), eight coulometric electrodes using -120 to $+300$ mV potentials in 60 mV increments and a C₁₈ column. Dopamine levels of sham animals from the vehicle and lithium-treatment groups were not significantly different and were combined for statistical comparisons with injured groups.

Synaptosome isolation for Western blot analysis

A total of 24 rats ($n=6$ per group) were injured and treated with vehicle or lithium as described above. Rats received an overdose of sodium pentobarbital (intraperitoneal, 100 mg/kg) at 1 week post-injury, and the ipsilateral striatum was rapidly dissected on a chilled ice plate, immediately snap frozen in liquid nitrogen, and stored at -80°C . Tissue samples were processed to produce synaptosomal-enriched lysates as previously described.⁶⁴ The tissues were homogenized using a dounce homogenizer with SYN-PER isolation reagent (Thermo Scientific, Pittsburgh, PA) with a freshly prepared protease inhibitor cocktail (Sigma). The homogenized lysates were centrifuged at $1200\times g$ at 4°C for 10 min, and supernatants were collected and centrifuged at $15,000\times g$ at 4°C for 20 min. The pellet was suspended in isolation reagent and protein concentration was measured with a bicinchoninic acid protein assay kit (Thermo Scientific) using a 96-well microplate reader (Biotek, Winooski, VT).

Immunoblotting

To examine the abundance of synaptic proteins and monomeric SNARE proteins in the striatum, synaptosomal lysates were boiled for 10 min prior to SDS-PAGE. SNARE complex abundance was examined in unboiled lysates to retain high molecular weight complexes, defined as SNAP-25 immunoreactive material greater than 50 kDa that was absent from boiled samples, as previously described.^{39,43,44} Protein lysates (20 μ g per well) and molecular weight markers were separated by SDS-PAGE, and electrophoretically transferred to a polyvinylidene fluoride membrane (Invitrogen, Carlsbad, CA). Membranes were incubated in 5% nonfat dry milk in tris-buffered saline (TBS) containing 0.1% Tween 20 (blocking solution) for 1 h. Primary antibodies were diluted in blocking solution and incubated overnight at 4°C . Primary antibodies utilized were mouse anti-SNAP-25 (Biolegend, San Diego, CA), mouse anti- α -syn (BD Transduction, San Jose, CA), rabbit anti-CSP α (Thermo-Fisher, Rockford, IL), rabbit anti-VAMP2 (Millipore, Temecula, CA), rabbit anti-synaptophysin (Abcam, Cambridge, MA), rabbit anti-post-synaptic density protein 95 (PSD95: Abcam), rabbit anti-phospho-GSK3 β (Ser9; Cell Signaling, Danvers, MA), rabbit GSK3 β (Cell Signaling), rabbit anti-phospho-tyrosine hydroxylase (P-Ser40 TH; Millipore), mouse anti-dopamine transporter (Abcam), rabbit anti-dopamine D2 receptor (Abcam), and mouse anti- β -actin (Sigma-Aldrich). On the following day, the membranes were rinsed with $1\times$ TBS buffer and incubated in blocking solution containing horseradish peroxidase-conjugated secondary antibodies for 1 h. Proteins were visualized using a chemiluminescence detection system (Supersignal, Pierce) and imaged with a Chemidoc Imager (BioRad).

To normalize for protein content, the membranes were stripped and reprobed with mouse anti- β -actin antibody (Sigma-Aldrich). Lysates from all groups were loaded in the same gel for comparison. Optical density (OD) of each protein of interest was measured using ImageJ (National Institutes of Health). The OD of β -actin was measured for each lane and used for normalization as a loading control for each sample. Values are presented as the ratio of OD of samples as a percentage of Sham+Vehicle (100%). Sham+Vehicle and Sham+Lithium groups were not combined for immunoblot analysis to evaluate potential effects of lithium treatment on protein abundance in control sham rats. OD for proteins of interest were normalized to Sham+Vehicle rats to examine the potential effects of lithium treatment between groups.

Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical comparisons of evoked dopamine release were completed using a two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests, when appropriate. Statistical

comparisons of dopamine area under the curve and peak values were compared using a one-way ANOVA, followed by Tukey's multiple comparison tests, when appropriate. Immunoblot data were compared using a two-way ANOVA. For immunoblot data, no significant interactions were found for two-way ANOVA comparisons and as a result, no *post hoc* comparisons were completed. A *p* value less than 0.05 was considered statistically significant for all tests. Statistical tests were completed using Graphpad (Graphpad, La Jolla, CA).

Results

Lithium improves evoked striatal dopamine release after TBI

We sought to evaluate the efficacy of lithium to attenuate neurotransmitter release deficits in the striatum following CCI. We utilized microdialysis and high-performance liquid chromatography to measure the levels of dopamine during high-potassium-

FIG. 2. Lithium treatment increased α -synuclein abundance in striatal synaptosomes at 1 week following controlled cortical impact (CCI). Representative Western blot images of α -synuclein (α -syn), cysteine string protein α (CSP α), VAMP2, and SNAP-25 immunoreactive soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes in the striatum at 1 week post-injury. **(A)** Assessment of α -syn (18 kDa, marker is 14 kDa) revealed that sham and CCI-injured rats treated with lithium (S-L and I-L, respectively) exhibited significantly increased abundance of α -syn, compared with rats that received vehicle treatment (S-V, I-V), independent of injury status (treatment main effect, $p < 0.01$). **(B,C)** Analysis revealed that CCI injury significantly reduced CSP α (34 kDa, marker is 28 kDa) abundance (injury main effect, $p < 0.05$) and VAMP2 (18 kDa, marker is 14 kDa) abundance (injury main effect, $p < 0.05$), compared with sham injury, independent of treatment status. **(D)** Assessment of SNAP-25 immunoreactive SNARE complexes (markers are 38, 49, 198 kDa) revealed that CCI injury significantly reduced SNARE complex abundance, compared with sham injury, independent of treatment status (injury main effect, $p < 0.05$). Proteins of interest were normalized to actin (42 kDa, marker is 38 kDa; $n = 6$ /group).

evoked release in the striatum at 1 week post-injury. Sham animals exhibited an increase in dopamine during the infusion of high-potassium infusion (0–40 min; Fig. 1A), consistent with previous reports.¹³ Dopamine levels of sham animals from vehicle and lithium treatment groups were not significantly different and were combined for comparisons to injured groups. Animals treated with vehicle following CCI injury showed a blunted response that was significantly decreased at 0, 20, and 40 min, compared with sham animals (group effect $p < 0.001$; time effect $p < 0.0001$, interaction $p < 0.05$; *post hoc* $p < 0.01$). Rats treated with lithium following CCI injury exhibited a significant reduction in dopamine concentrations at 0 and 20 min (*post hoc* $p < 0.05$, $p < 0.05$, respectively), but was not significantly different from sham animals at the 40-min time-point. Vehicle treatment after CCI injury resulted in significant reductions in area under the curve (ANOVA $p < 0.05$; *post hoc* $p < 0.05$) and peak dopamine concentration (ANOVA $p < 0.05$; *post hoc* $p < 0.05$; Fig. 1B, 1C). Conversely, area under the curve and peak dopamine concentrations with lithium treatment after CCI were not significantly different from sham injury.

Lithium increases α -syn abundance after TBI

Previous work from our lab showed that treatment with lithium significantly increased SNARE protein and SNARE complex abundance in the hippocampus at 1 week following CCI.⁴³ We sought to examine the effect of lithium administration on SNARE protein abundance in striatal synaptosomes at 1 week post-injury. Treatment with lithium significantly increased α -syn abundance compared with vehicle treatment, independent of injury status (treatment main effect, $p < 0.01$; no injury main effect or interaction; Fig. 2A). The abundance of CSP α and VAMP2 were significantly reduced in CCI-injured rats, independent of treatment status, compared with sham injury (injury main effects, $p < 0.05$; $p < 0.05$, respectively; no treatment main effects or interactions; Fig. 2B, 2C). Assessment of SNAP-25 immunoreactive SNARE complexes revealed that CCI injury significantly reduced SNARE complex

formation, but treatment with lithium did not attenuate this reduction (injury main effect $p < 0.05$; no treatment main effect or interaction; Fig. 2D). CCI resulted in significant reductions in CSP α , VAMP2, and SNARE complex formation in the striatum. Treatment with lithium enhanced α -syn abundance but did not attenuate CCI-induced reductions in other SNARE proteins and SNARE complex formation.

Lithium does not increase synaptic density after TBI

We next sought to evaluate the abundance of synaptic marker proteins synaptophysin (pre-synaptic) and PSD95 (post-synaptic). CCI injury significantly reduced synaptophysin (injury main effect $p < 0.05$, no treatment main effect or interaction) and PSD95 (main injury effect $p < 0.05$; no treatment main effect or interaction), compared with sham injury (Fig. 3A, 3B). Treatment with lithium did not attenuate the CCI-induced reductions in synaptophysin or PSD95. Taken together, these data suggest that increased synaptic density with lithium treatment is not a primary mechanism underlying improved neurotransmission.

Lithium does not inhibit GSK3 β signaling after TBI

We next evaluated the effect of lithium treatment on phosphorylation of serine 9 of GSK3 β (P-Ser9 GSK3 β) in the striatum. Assessment of P-Ser9 GSK3 β and total GSK3 β revealed that both CCI injury and treatment with lithium did not significantly alter the abundance of either protein in striatal synaptosomes at 1 week post-injury (Fig. 4A, 4B). Analysis of the ratio of P-Ser9 GSK3 β / GSK3 β revealed a trend toward increased GSK3 β inhibition in CCI-injured lithium-treated rats, but this did not reach significance (main treatment effect, $p = 0.16$; Fig. 4C). Taken together, these data suggest that inhibition of GSK3 β is not a primary mechanism underlying improved neurotransmission in the striatum.

FIG. 3. Controlled cortical impact (CCI) reduced synaptophysin and PSD95 abundance in striatal synaptosomes at 1 week post-injury. Representative Western blot images of synaptophysin (Syp) and post-synaptic density protein 95 (PSD95) in the striatum at 1 week post-injury. (A,B) Analysis revealed that CCI injury significantly reduced Syp abundance (35 kDa, marker is 28 kDa; injury main effect, $p < 0.05$) and PSD95 abundance (80 kDa, marker is 69 kDa; injury main effect, $p < 0.05$), compared with sham injury, independent of treatment status. Proteins of interest were normalized to actin (42 kDa, marker is 38 kDa; $n = 6$ /group).

FIG. 4. Lithium did not increase GSK3 β phosphorylation in striatal synaptosomes at 1 week following controlled cortical impact (CCI). Representative Western blot images of phosphorylated serine 9 (P-Ser9) glycogen synthase kinase 3 β (GSK3 β) and total GSK3 β in the striatum at 1 week post-injury. (A) Assessment of P-Ser9 GSK3 β (46 kDa, marker is 49 kDa) revealed that CCI injury and lithium treatment did not significantly alter protein abundance. (B) Analysis revealed that CCI injury and lithium treatment did not significantly alter total GSK3 β abundance (46 kDa, marker is 49 kDa). Assessment of P-Ser9 GSK3 β normalized to total GSK3 β revealed that lithium treatment trended toward increased GSK3 β inhibition in CCI-injured rats, but this did not reach significance. Proteins of interest were normalized to actin (42 kDa, marker is 38 kDa; $n=6$ / group).

Lithium increases tyrosine hydroxylase phosphorylation and D2 receptor abundance after TBI

We sought to examine the effect of lithium treatment on the abundance of phosphorylated tyrosine hydroxylase, D2 receptor, and dopamine transporter (DAT) in the striatum at 1 week post-injury. Treatment with lithium significantly increased the abundance of P-Ser40 tyrosine hydroxylase (treatment main effect $p<0.05$; no injury main effect or interaction) and the D2 receptor (treatment main effect $p<0.05$; no injury main effect or interaction), compared with vehicle treatment, independent of injury status (Fig. 5A, 5B). CCI injury and treatment with lithium did not significantly alter the abundance of DAT in striatal synaptosomes at 1 week post-injury. These data show that lithium treatment increases the abundance of two proteins related to dopamine synthesis and neurotransmission.

Discussion

In the current study, we evaluated the efficacy of lithium to improve dopamine neurotransmission and increase synaptic protein

FIG. 5. Lithium treatment increased tyrosine hydroxylase phosphorylation and D2 receptor abundance in striatal synaptosomes at 1 week following controlled cortical impact (CCI). Representative Western blot images of phosphorylated serine 40 (P-Ser40) tyrosine hydroxylase (TH), dopamine D2 receptor, and dopamine transporter (DAT) in the striatum at 1 week post-injury. (A) Assessment revealed that treatment with lithium resulted in significantly increased abundance of P-Ser40 TH (60 kDa, marker is 49 kDa), compared with vehicle treatment, independent of injury status (treatment main effect, $p<0.05$). (B) Treatment with lithium significantly increased the abundance of D2 receptors (49 kDa, marker is 49 kDa), compared with vehicle treatment, independent of injury status (treatment main effect, $p<0.05$). Assessment of DAT (69 kDa, marker is 68 kDa) revealed that CCI injury and lithium treatment did not significantly alter protein abundance. Proteins of interest were normalized to actin (42 kDa, marker is 38 kDa; $n=6$ /group).

abundance in the striatum at 1 week following CCI. We show for the first time that SNARE proteins and SNARE complex formation are significantly reduced in striatal synapses after TBI. We provide novel findings that administration of lithium improved potassium-evoked dopamine neurotransmission in the striatum at 1 week post-injury. Additionally, lithium treatment increased the abundance of α -syn, phosphorylated tyrosine hydroxylase, and D2 receptors in striatal synaptosomes. Treatment with lithium did not significantly increase the abundance of CSP α , VAMP2, SNARE complexes, synaptophysin, PSD95, phosphorylated GSK3 β , or DAT. These findings contribute to a growing body of literature highlighting lithium treatment as a promising therapeutic to target synaptic dysfunction and improve neurobehavioral recovery after TBI.

We have previously shown that CCI results in significantly impaired evoked striatal dopamine neurotransmission in the weeks after experimental TBI.^{13,14} Wagner and colleagues (2005)²⁸ previously demonstrated that CCI reduces medial forebrain-stimulated release

and clearance of dopamine using fast scan cyclic voltammetry at 2 weeks post-injury. The current findings are in agreement with previous reports showing high-potassium-evoked dopamine release is significantly impaired at 1 week post-injury.^{13,26} We previously showed, using a similar microdialysis infusion strategy, TBI produces a significant reduction in dopamine release over a 40-min period of high-potassium aCSF infusion.^{26,33} Additionally, we have shown that this method can be utilized to assess the efficacy of therapeutics to improve potassium-evoked dopamine release.^{26,33} While microdialysis does not provide resolution of dopamine release on the time scale of action potentials, this method coupled with high-performance liquid chromatography can provide a direct measurement of dopamine and other analytes in the interstitial fluid, likely reflective of systemic changes in dopamine release and clearance. Additional studies utilizing enzyme-based microelectrode arrays and fast-scan cyclic voltammetry could provide important complementary insight into the actions of lithium treatment on dopamine neurotransmission after TBI.

We show for the first time that lithium treatment improves neurotransmission following TBI. In treatment regimens reflecting usage of lithium for bipolar affective disorder and depression, chronic treatment of lithium is associated with an inhibition of dopamine neurotransmission in the nucleus accumbens and ventral tegmental area.^{48,65,66} However, in the same study, an acute lithium treatment paradigm did not elicit the same reduction in dopamine amplitude following 60 Hz pulse train stimulation,⁴⁸ suggesting that the duration of lithium treatment may be an important consideration for determining an appropriate treatment regimen for synaptic dysfunction following TBI. Ferrie and colleagues previously showed that 25 days of lithium treatment influences potassium-evoked dopamine release in the nucleus accumbens and persists for days after withdrawal of lithium treatment.⁶⁶ This report suggests that the effect of lithium treatment of neurotransmission can extend beyond the treatment period and may be related to protein expression changes that persist after the cessation of treatment. To this point, Zigova and colleagues showed that lithium treatment for 5 days in a neuronal derived human cell line increased TH abundance at Day 10, five days following the cessation of lithium treatment.⁶⁷

In the context of TBI, previous therapeutic strategies aimed at enhancing dopaminergic activity have been shown to improve dopamine release and neurobehavioral function after TBI.^{14,27,29,32,33} Together with previous studies demonstrating the beneficial neurobehavioral effects of lithium in mice and rats after TBI,^{43,50,51,62,68} our current findings suggest that improved neurotransmission may contribute to the functional recovery of motor and cognitive abilities reported with lithium treatment following TBI. Our current findings showing that lithium treatment improved striatal evoked dopamine release highlight that the actions of lithium in the TBI brain may be different from the context of psychiatric disorders and warrant additional studies to understand the synaptic effects of lithium treatment after TBI.

Neurotransmission impairments may be attributed to alterations in intrasynaptic vesicular protein machinery important for translocation and docking of neurotransmitter containing vesicles at the active zone of the synapse.^{35,36} Our findings of CCI-induced reductions in CSP α , VAMP2, and SNARE complex formation show that impairments in vesicular docking and fusion may contribute to neurotransmitter release impairments in the striatum after TBI. Previous work from our lab showed that TBI significantly reduced hippocampal abundance of key SNARE proteins (including α -syn and VAMP2) and impaired SNARE complex formation in the

weeks post-injury following both CCI and lateral fluid percussion injury.^{43,44,64} However, prior to this study, TBI-induced changes in SNARE protein abundance in the striatum had not been examined. Synaptosomal lysates were utilized to enhance the detection of potential changes in SNARE proteins in striatal synapses after TBI.

Multiple reports highlight that reductions in CSP α , VAMP2, or SNAP-25 alone in genetically modified animal models are sufficient to impair SNARE complex formation in the synapse.^{37–39,41,42,69,70} The finding that SNARE complex formation is impaired in striatal synapses suggests that intrasynaptic deficits in vesicular docking and fusion may contribute to the development of neurotransmitter release deficits in the striatum. While we specifically measured impairments in evoked dopamine release, deficits in SNARE complex formation likely extend beyond dopaminergic neurons. Previous studies report that TBI results in reduced tyrosine hydroxylase activity and dopamine transporter expression in the striatum in the weeks post-injury.^{13,14,28} The observation of SNARE complex deficits in striatal synapses adds to the pathological responses that may contribute to impaired dopaminergic neurotransmission.

Published reports support an important physiological role for α -syn in SNARE complex formation and neurotransmission.^{37,39} In naïve rats, a 4-week treatment regimen of lithium and olanzapine, a medication used to treat bipolar disorder and schizophrenia, increased cortical α -syn abundance.⁷¹ In the current study, treatment with lithium alone for a period of 1 week significantly increased the abundance of α -syn in striatal synaptosomes. We previously demonstrated that post-traumatic lithium treatment attenuated CCI-induced reductions in hippocampal α -syn, CSP α , and VAMP2 abundance.⁴³ Interestingly, unlike in the hippocampus,⁴³ CCI did not significantly reduce α -syn in the striatum at 1 week post-injury. Despite the increase in striatal α -syn with lithium treatment, the abundance of CSP α , VAMP2, or SNARE complexes were not restored as previously observed in the hippocampus.⁴³ This difference may suggest that in order to restore SNARE complex formation, multiple SNARE proteins, in addition to α -syn, may need to be increased. It is plausible that a different treatment regimen or treatment window may be needed to promote SNARE protein expression in the striatum, compared with the hippocampus, following TBI. Alternatively, it also may suggest that lithium may improve striatal dopamine release in a mechanism independent of enhanced SNARE complex formation.

The mechanism underlying the beneficial effects of lithium in the brain is poorly understood. Proposed mechanisms of action include increased synaptic density, inhibition of GSK3 β , and modulating dopamine neurotransmission.^{45–49,72–78} While CCI significantly reduced striatal pre-synaptic and post-synaptic marker protein abundance, treatment with lithium did not increase the abundance of these synaptic proteins. It is possible that a treatment regimen of lithium beyond 7 days may promote synaptic plasticity. Our current findings suggest that lithium administered over a period of 1 week post-injury does not overtly enhance synaptic density in the striatum. However, a more quantitative approach, including ultrastructural analysis, may be needed to definitively evaluate the effect of lithium treatment on synaptic density and the potential influence of lithium treatment duration on synaptic plasticity in the striatum after injury.

The inhibitory effects of lithium on GSK3 β signaling have been well described.^{45,46,48} In the hippocampus, TBI-induced alterations in GSK3 β signaling develop as early as 30 min post-injury and persist for 3 days post-injury.⁵⁰ Inhibition of GSK3 β with lithium treatment attenuated neuronal loss and improved cognitive function following CCI; however, in the same study, treatment with a

selective inhibitor of GSK3 β only partially protected against TBI,⁵⁰ suggesting that lithium may exert multiple protective effects, in addition to GSK3 β inhibition after TBI. In the current study, there was a trend toward increased phosphorylation of GSK3 β in lithium-treated rats, but this trend did not reach significance. Increased P-Ser9 GSK3 β has been reported in hippocampal whole-cell lysates with the same dose of lithium,^{50,51} but this is the first evaluation of GSK3 β in synaptosomal lysates following TBI. It is possible that the limited effect of lithium on P-Ser9 GSK3 β in the current study is reflective of the utilization of synaptosomes for immunoblot analysis or potential regional differences after TBI. Examination of whole-cell lysate may have demonstrated a greater effect of lithium of GSK3 β inhibition, but additional studies are needed to understand the effects of lithium on subcellular locations of GSK3 β after TBI.

In two studies with small groups of TBI-injured patients, single-photon emission tomography revealed reduced striatal DAT and D2 receptor binding⁸ and the contribution of genetic variants in DAT on receptor binding after injury.⁷² Additionally, in a group of 63 patients, DAT polymorphisms were associated with dopamine and metabolite levels in the CSF following severe TBI.⁷³ Experimental TBI results in reduced TH activity at 1 and 4 weeks post-injury, reduced TH phosphorylation at serine 40 in the striatum at 1 week post-injury, and reduced dopamine transporter abundance at 15 days following CCI.^{13,28} In the current study, lithium treatment significantly increased P-Ser40 TH and D2 receptor abundance, suggesting that lithium may promote dopamine synthesis and neurotransmission. While DAT abundance has been shown to be reduced in the striatum at 2 weeks following CCI,¹⁴ in the current study we did not observe an effect of CCI or lithium treatment on DAT abundance. It is possible this difference could be related to the different time-points assessed between the studies or could originate from the assessment of whole-cell lysates versus synaptosomal preparations.

Phosphorylation at serine 40 increases the rate-limiting enzymatic activity of TH, which is an important step for the synthesis of dopamine.^{74,75} Multiple kinases, including the cAMP-dependent protein kinase A have been shown to phosphorylate serine 40.^{76–78} Rats fed lithium-supplemented chow for either 6 or 35 days exhibit increased cAMP-PKA catalytic subunit,⁷⁹ suggesting that increased PKA activity may play a role in the therapeutic benefits of lithium. In the current study, it is not possible to determine the causative reason for increased phosphorylation of TH as multiple signaling changes, including Akt, may alter the kinase and phosphatase regulation of P-Ser40 TH.^{79–81} Additionally, changes in total TH abundance could also contribute, as lithium has been shown to increase TH expression *in vitro* and *in vivo*.^{67,82} Future work is warranted to better understand the effects of lithium on signaling cascades that can modulate TH activity and expression to enhance dopamine synthesis and transmission.

A growing body of evidence highlights that α -syn can modulate the synthesis, storage, and clearance of dopamine.⁸³ We show that lithium treatment increased α -syn abundance and enhanced phosphorylation of serine 40 of TH. In contrast to these findings, previous reports show that overexpression of α -syn in cultured dopaminergic cells is associated with a reduced abundance of TH or reduced serine 40 phosphorylation of TH.^{84–86} It is plausible that lithium-induced increases in α -syn after TBI result in differential effects on the dopaminergic system than the actions of chronically elevated α -syn levels in models of Parkinson's disease. Additional work is warranted to better understand the chronic effects of post-traumatic lithium administration on the abundance of α -syn and the

potential interactions of increased α -syn on other synaptic proteins important for dopamine synthesis and neurotransmission.

In summary, CCI significantly reduced the abundance of the SNARE proteins CSP α and VAMP2, and impaired SNARE complex formation in the striatum. Treatment with lithium improved potassium-evoked dopamine release in the striatum at 1 week following CCI. In striatal synaptosomes, treatment with lithium increased the abundance of α -syn, phosphorylated TH, and dopamine D2 receptor, but did not increase the abundance of CSP α , VAMP2, SNAP-25 immunoreactive SNARE complexes, synaptophysin, PSD95, phosphorylated GSK3 β , or DAT at 1 week post-injury. These data demonstrate that lithium attenuates synaptic dysfunction and increases the abundance of synaptic proteins associated with dopamine synthesis, release, and receptor binding in the striatum. This study provides novel evidence that post-traumatic lithium treatment promotes recovery of synaptic function and warrant the need for additional studies to understand the effect of lithium in the synapse after TBI.

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Author Disclosure Statement

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