JOURNAL OF NEUROTRAUMA 29:2342–2351 (September 1, 2012)

DOI: 10.1089/neu.2012.2449

Lithium Reduces BACE1 Overexpression, Beta Amyloid Accumulation, and Spatial Learning Deficits in Mice with Traumatic Brain Injury

Fengshan Yu,[1–3] Yumin Zhang,[2,3] and De-Maw Chuang[1,2]

Abstract

Traumatic brain injury (TBI) leads to both acute injury and long-term neurodegeneration, and is a major risk factor for developing Alzheimer's disease (AD). Beta amyloid (Aβ) peptide deposits in the brain are one of the pathological hallmarks of AD. Aβ levels increase after TBI in animal models and in patients with head trauma, and reducing Aβ levels after TBI has beneficial effects. Lithium is known to be neuroprotective in various models of neurodegenerative disease, and can reduce Aβ generation by modulating glycogen synthase kinase-3 (GSK-3) activity. In this study we explored whether lithium would reduce Aβ load after TBI, and improve learning and memory in a mouse TBI model. Lithium chloride (1.5 mEq/kg, IP) was administered 15 min after TBI, and once daily thereafter for up to 3 weeks. At 3 days after injury, lithium attenuated TBI-induced Aβ load increases, amyloid precursor protein (APP) accumulation, and β-APP-cleaving enzyme-1 (BACE1) overexpression in the corpus callosum and hippocampus. Increased Tau protein phosphorylation in the thalamus was also attenuated after lithium treatment following TBI at the same time point. Notably, lithium treatment significantly improved spatial learning and memory in the Y-maze test conducted 10 days after TBI, and in the Morris water maze test performed 17–20 days post-TBI, in association with increased hippocampal preservation. Thus post-insult treatment with lithium appears to alleviate the TBI-induced Aβ load and consequently improves spatial memory. Our findings suggest that lithium is a potentially useful agent for managing memory impairments after TBI or other head trauma.

Key words: beta amyloid; β-APP-cleaving enzyme-1; learning and memory; lithium; traumatic brain injury

Introduction

Traumatic brain injury (TBI) causes devastating acute effects, often leads to long-term neurodegeneration, and increases the risk of developing Alzheimer's disease (AD). Beta amyloid (Aβ) deposits are one of the pathological hallmarks of AD. Furthermore, Aβ42, a 42-amino acid form of Aβ, is elevated in the cerebrospinal fluid (CSF) of patients with head trauma (Olsson et al., 2004; Raby et al., 1998), and post-mortem human studies have similarly found Aβ deposits in TBI patients (Roberts et al., 1991,1994; Uryu et al., 2007). Another study found both Aβ40 and Aβ42—the two primary types of Aβ associated with AD—in the temporal cortex of head trauma victims (Ikonomovic et al., 2004). Increased Aβ levels have also been observed in animal models of TBI (Abrahamson et al., 2009; Loane et al., 2009; Smith et al., 1998).

Both endogenous and exogenous elevated Aβ levels have been associated with neuronal death and behavioral dysfunction (Mattson, 2004). Increased Aβ load has also been linked to massive hippocampal neuronal death and cognitive impairment in a TBI model (Smith et al., 1998). Conversely, reducing Aβ levels appears to have beneficial effects against TBI. For example, inhibition of β- or γ-secretase enzymes, which play a major role in the generation of Aβ from amyloid precursor protein (APP), increases hippocampal tissue preservation and improves functional outcome (Loane et al., 2009). Decreasing Aβ levels by modulating ATP binding cassette protein A1 (ABCA1, which enhances Aβ clearance) also has beneficial effects (Loane et al., 2011). Interestingly, simvastatin, a drug used clinically to reduce high cholesterol levels, reduced hippocampal Aβ levels and improved Morris water maze performance after TBI in an animal model (Abrahamson et al., 2009).

[1]Section on Molecular Neurobiology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland.
[2]Center for Neuroscience and Regenerative Medicine, [3]Department of Anatomy, Physiology, and Genetics, Uniformed Services University of the Health Sciences, Bethesda, Maryland.

Accumulating evidence suggests that lithium, which has been used to treat bipolar disorder for more than half a century, has neuroprotective effects in various models of neurodegenerative disease, including AD, cerebral ischemia, spinal cord injury, and TBI (Chiu and Chuang, 2010; Dash et al., 2011; Yu et al., 2012). Lithium is also known to both directly and indirectly inhibit glycogen synthase kinase-3 (GSK-3), a constitutively active, master switch kinase that regulates a variety of cellular functions by serine/threonine phosphorylation; indeed, this may be the mechanism underlying most of lithium's effects (Chiu and Chuang, 2010; Li and Jope, 2010). Interestingly, GSK-3 increases Aβ production by regulating APP cleavage (Hooper et al., 2008; Phiel et al., 2003), and lithium treatment has been shown to reduce Aβ levels *in vitro* and *in vivo* through GSK-3 inhibition (Phiel et al., 2003; Su et al., 2004). In a transgenic AD model, lithium treatment increased microtubule-associated protein-2 (MAP-2)-positive neurons and improved Morris water maze performance in association with reduced Aβ load (Rockenstein et al., 2007). Taken together, the extant findings suggest that both pre- and post-insult treatment with lithium reduce neurodegeneration, attenuate neuroinflammation, and improve behavioral performance after TBI (Dash et al., 2011; Yu et al., 2012; Zhu et al., 2010). Thus the present study was designed to investigate the effects of lithium on Aβ levels and on learning and memory in a mouse model of TBI.

Materials and Methods

Animals and surgery

Seven-week-old male C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed in standard cages in a 12-h/12-h light-dark cycle with free access to food and water. Mice were randomly assigned to three experimental groups: sham-injured, TBI, and TBI with lithium treatment. All animals were treated in accordance with Uniformed Services University of the Health Sciences guidelines and the National Research Council's *Guide for the Care and Use of Laboratory Animals*. TBI was induced in these mice about 1 week after arrival using a controlled cortical impact (CCI) device, as previously described (Yu et al., 2012). Briefly, mice were anesthetized with 2.0% isoflurane in O_2 and placed in a stereotaxic frame with an adaptor (Kopf Instruments, Tujunga, CA). A 4-mm-diameter craniotomy was performed over the left parietal cortex between the bregma and the lambda, 1 mm lateral to the midline. The point of impact was identified midway between the lambda and bregma sutures, as well as midway between the central suture and the left temporalis muscle. CCI injury was performed using a 3-mm-diameter convex tip set to compress the brain 1.5 or 2.0 mm at a speed of 5.0 m/sec. The 1.5-mm condition was used for the biochemical studies, and the 2.0-mm condition was used for behavioral tests and histology studies. The craniotomy was then closed with the initially removed bone flap. Sham-injured mice underwent identical craniotomy procedures without CCI injury. Body temperature was maintained at 37 ± 0.5°C with a heating pad coupled to a rectal probe.

Lithium chloride (1.5 mEq/kg dissolved in normal saline; Sigma-Aldrich, St. Louis, MO), or an equal volume of saline, was injected intraperitoneally (IP) 15 min after TBI. The same dose of lithium or saline was given once daily thereafter for up to 3 weeks.

Enzyme-linked immunosorbent assay (ELISA)

Mice ($n = 12$/group) were sacrificed 3 days after surgery. Ipsilateral hippocampi (including surrounding white matter tracts) were collected as previously described (Tran et al., 2011). Brain tissues were homogenized in 1:5 w/v 5 M guanidine hydrochloride containing protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany), and phosphatase inhibitors I and II (Sigma-Aldrich); the homogenates were rotated at room temperature for 3–4 h, and then centrifuged at 13,300 *g* for 20 min at 4°C. Protein concentrations were determined via the BCA method (Pierce Biotech, Rockford, IL), and the Aβ contents in tissue lysates were determined using an $A\beta_{1-42}$ ELISA kit (Invitrogen, Carlsbad, CA) per the manufacturer's instructions.

Immunohistochemical analysis

For immunohistochemical analysis, animals ($n = 6$/group) were deeply anesthetized and then transcardially perfused with heparin saline followed by 4% formaldehyde at 3 days after injury. Brains were collected and fixed in the same fixative for 24–48 h, and then in 30% sucrose for an additional 24–48 h. Sections (30 μm thick) were cut using a cryostat (Leica, Wetzlar, Germany) starting at 600 μm anterior to the bregma. To assess the expression levels of APP, Aβ, and phospho-Tau protein after injury, coronal sections between bregma −1.5 and −2.5 mm were first blocked with 5% normal donkey serum, then incubated overnight at 4°C with polyclonal rabbit anti-β-APP (1:100; Zymed, San Francisco, CA), biotin-conjugated mouse monoclonal anti-Aβ (4G8, 1:100; Covance Research Products, Inc., Richmond, CA), or polyclonal rabbit anti-phospho-Tau (p-Tau, T205, Invitrogen) antibody. The sections were then reacted for 1 h at room temperature with a Cy 3-conjugated donkey anti-rabbit antibody (for β-APP and p-Tau, 1:100; Jackson ImmunoResearch Laboratories, West Grove, PA), or a Cy 3-conjugated anti-biotin antibody (for 4G8; 1:100; Jackson ImmunoResearch Laboratories). Finally, the sections were stained with 4,6-diamino-2-phenylindole (DAPI; Sigma-Aldrich), mounted with Vector-shield mounting medium (Vector Laboratories, Burlingame, CA), and visualized with fluorescence microscopy (Olympus, Center Valley, PA). Phospho-Tau-positive cell density was measured according to a method previously described (Shein et al., 2009; Yu et al., 2012) with slight modifications. Briefly, immunostaining of three sections approximately 50 μm apart between bregma −1.5 and −2.5 mm from a given animal was performed, and three high-power (40×) photomicrographs of each section were taken in the ipsilateral thalamus. Immuno-positive cells were identified and counted using Adobe Photoshop (Mountain View, CA) and averaged for a given animal. Cell counting was performed by an observer blind to treatment status. Area fractions of APP and 4G8 were evaluated with NIH ImageJ software (National Institutes of Health, Bethesda, MD) as previously described (Tran et al., 2012). Briefly, the images were first converted to 8-bit gray scale, and the image threshold was adjusted to highlight stained objects. The percentage area of positive staining was quantified by the Analyze Particles function in the ipsilateral corpus callosum and hippocampus.

Western blotting analysis

Western blotting analysis with 6E10 and β-APP-cleaving enzyme-1 (BACE1) antibodies was performed to assess the levels of Aβ and β-secretase, respectively. Three days after TBI, the ipsilateral hippocampi (including surrounding white matter tracts; $n = 5$/group) were collected and sonicated for 30 sec in RIPA buffer (Cell Signaling, Danvers, MA), containing phosphatase inhibitors I and II (Sigma-Aldrich), and protease inhibitor cocktail (Roche Diagnostics). The lysates were centrifuged at 12,000 rpm for 20 min and protein concentrations were determined using the BCA method. An aliquot containing 30 μg of protein was loaded to each lane, and proteins were separated by electrophoresis on SDS-polyacrylamide gels of 4–12% (Invitrogen), then transferred to a polyvinylidene difluoride membrane. The membranes were incubated overnight at 4°C with a monoclonal mouse anti-Aβ (6E10, 1:1000; Covance Research Products, Inc.), or a monoclonal mouse anti-BACE1 (1:1000; Millipore, Bedford, MA) antibody. The membranes were then incubated with a goat anti-mouse IRDye 800CW-conjugated secondary antibody, and scanned with an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE).

Y-maze test

Ten days after CCI, mice ($n = 12$/group) were tested in a Y-maze apparatus consisting of three enclosed arms, each 50 cm long, 11 cm wide, and 10 cm high, made of black acrylic glass, set at an angle of 120° to each other, in the shape of a Y (Yau et al., 2007). The floor of the maze was covered with mouse-soiled sawdust to eliminate olfactory cues, and visual cues were placed inside and around the maze in the testing room and kept constant throughout the testing sessions. The test consisted of two trials, conducted 2 h apart. For the first trial (the acquisition trial), one arm (the novel arm) was blocked. Mice were placed at the end of a pseudorandomly chosen arm (the start arm), allowed to explore the maze for 5 min, and then returned to their home cages for 2 h. For the second trial (the retention trial), mice were allowed to explore all three arms for 5 min. The time spent exploring each arm was video recorded and analyzed using ANY-Maze software (Stoelting Co., Wood Dale, IL) by an observer blind to the treatment condition. The percentage of time spent in the novel arm versus the total time spent in all three arms during the first 2 min of the retention trial was analyzed. This corresponds to the maximal exploratory activity in the novel arm, which typically declines in the subsequent 2 min (Yau et al., 2007).

Morris water maze test

The cognitive function of the mice ($n = 17$/group) was assessed using the standard Morris water maze test, as previously described (Loane et al., 2009). The apparatus consisted of a large, white circular 1.2-meter diameter pool with a 12-cm diameter acrylic glass platform submerged 1 cm below the surface of the water. During training, the platform was placed in one quadrant of the pool. The animal was gently and randomly placed in the water facing the wall at one of the remaining quadrants, and allowed to swim to find the platform. The animals underwent four training trials per day from day 17 to day 20 post-TBI. Each animal was given 60 sec to find the platform, and could remain on the platform for 15 sec to become familiar with visual cues from the surroundings before being removed from the platform. If the animal failed to locate the platform within 60 sec on any given trial, it was led by the experimenter to the platform, and allowed to remain there for 15 sec. One hour after the last training session on day 20, the platform was removed and the animals were tested in a probe trial to measure quadrant preference and platform localization. Movement within the maze was monitored using a video camera and analyzed using ANY-Maze software.

Evaluation of hippocampal tissue preservation

Hippocampal tissue preservation was measured 3 weeks after CCI injury. Animals ($n = 6$/group) were sacrificed and the brains were collected and sectioned as described above in the immunohistochemical analysis section. Serial sections at 270-μm intervals were stained with hematoxylin and eosin (H&E) and scanned with an Epson scanner; the area of the ipsilateral and contralateral hippocampus was measured with ImageJ software (NIH). The volume of preserved ipsilateral hippocampus and contralateral normal hippocampus was calculated as previously described (Dash et al., 2010; Yu et al., 2012):

$$\{0.5A_1 + 0.5(A_1 + A_2) + \ldots + 0.5(A_{n-1} + A_n) + 0.5A_n\}X$$

where A stands for the lesion area (mm^2) for each slice, and X stands for the distance (mm) between two sequential slices. Hippocampal tissue preservation was calculated and expressed as percentage of the ipsilateral hippocampal volume versus that of the contralateral hippocampus.

Statistical analysis

All data analyses were carried out using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA). Two-way repeated measures analysis of variance (ANOVA) with *post-hoc* Bonferroni comparison was used to analyze results of the Morris water maze test. One-way ANOVA was used to compare multiple groups, and the Student's *t*-test was used to compare two groups. The results were quantified and expressed as mean ± standard error of the mean (SEM). Statistical significance was defined as $p < 0.05$.

Results

Post-insult lithium treatment reduces TBI-induced Aβ levels

Evidence suggests that in animal models of TBI, Aβ levels increase from the first to the seventh day, peaking on day 3 (Loane et al., 2009); therefore, the present study focused predominantly on day 3 post-injury. Western blot with the 6E10 antibody showed that a 27-kDa Aβ oligomer band was increased approximately threefold after TBI (Fig. 1A; $p < 0.01$), and that this increase was robustly reduced by lithium treatment (Fig. 1A; $p < 0.05$).

Because Aβ oligomers are toxic in animal models of AD, and because some low-n Aβ oligomers are SDS resistant (Walsh and Selkoe, 2007), we postulated that lithium treatment would significantly reduce Aβ oligomer levels and provide neuroprotection. Levels of Aβ42, which is more toxic than Aβ40, were evaluated by ELISA and found to be significantly increased in the hippocampus after TBI (Fig. 1B; $p < 0.01$); lithium treatment significantly reduced this TBI-induced increase in Aβ42 levels (Fig. 1B; $p < 0.05$).

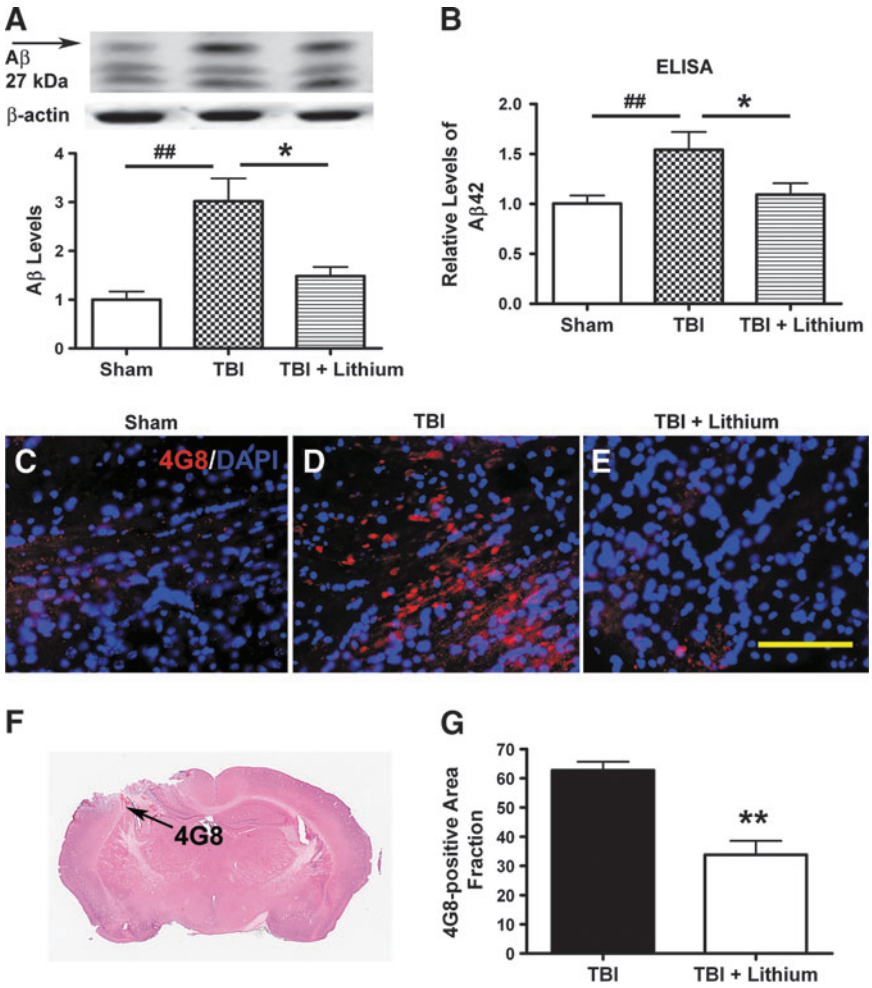

FIG. 1. Lithium reduces beta amyloid (Aβ) levels in the hippocampus 3 days post-TBI. (**A**) Representative Western blots of Aβ peptide are shown here. Three days post-TBI, several bands of Aβ oligomers were detected using the 6E10 antibody. A 27-kDa Aβ oligomer band (arrow) increased after TBI, and this increase was suppressed by lithium treatment ($n=5$/group; $^{\#\#}p<0.01$; $^{*}p<0.05$). (**B**) ELISA was performed 3 days after TBI to evaluate changes in Aβ42 levels. Aβ42 levels increased in the hippocampus after TBI, and this increase was attenuated by lithium treatment ($n=12$/group; $^{\#\#}p<0.01$; $^{*}p<0.05$). (**C**, **D**, and **E**) Representative microphotographs of Aβ staining with the 4G8 antibody in the corpus callosum 3 days after TBI are shown here. (**F**) The area of Aβ staining is indicated by an arrow in the H&E micrograph. (**C**) No Aβ-positive signal was found in the corpus callosum of the sham-injured animals. (**D**) Three days post-injury, a robust increase in Aβ accumulation in axonal bulbs was seen in the corpus callosum in the TBI model group. (**E**) Aβ accumulation was reduced in this area following lithium treatment. (**G**) Quantitative data showed a significant reduction in the area fraction of 4G8-positive staining in the lithium-treated group compared to the TBI group in the corpus callosum ($n=6$/group; $^{**}p<0.01$; scale bar = 50 μm). Nuclei were stained with DAPI as shown with blue fluorescence. Data are represented as mean ± SEM. Levels of β-actin were used as loading control (H&E, hematoxylin and eosin; TBI, traumatic brain injury; SEM, standard error of the mean; ELISA, enzyme-linked immunosorbent assay; DAPI, 4,6-diamino-2-phenylindole).

Immunostaining with the 4G8 antibody found that Aβ accumulation was increased in the axonal bulbs in the corpus callosum 3 days after TBI, and that this accumulation was similarly reduced by lithium (Fig. 1C–E and G; $p<0.01$). In addition, Aβ accumulation in the hippocampus was detected in more than one-third of the animals in the TBI model group, but this was not observed in the lithium-treated group (data not shown).

Lithium treatment reduces APP and BACE1 levels following TBI

When APP and BACE1 expression was examined 3 days after TBI, axonal bulbs positive for APP were observed in the corpus callosum and hippocampus (Fig. 2C and G), but not in sham-injured controls (Fig. 2B and F). Lithium treatment significantly reduced APP accumulation in axonal bulbs in both the corpus callosum and hippocampus (Fig. 2D, H, I, and J; $p<0.05$). In addition, Western blot analysis showed that TBI markedly increased BACE1 levels in the hippocampus 3 days after TBI (Fig. 3; $p<0.01$), and that this increase was completely blocked by lithium treatment (Fig. 3; $p<0.01$).

Lithium reduces Tau phosphorylation after TBI

To evaluate Tau phosphorylation, immunostaining was performed 3 days after TBI. Tau phosphorylation at Thr205 was

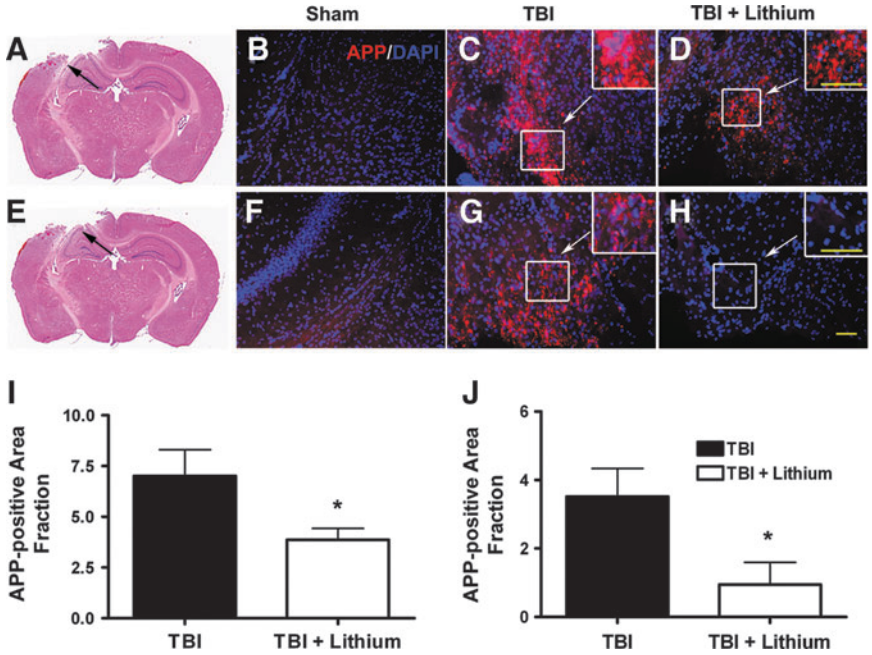

FIG. 2. Lithium reduces amyloid precursor protein (APP) accumulation in the corpus callosum and hippocampus 3 days post-TBI. Representative microphotographs of APP staining in the corpus callosum (**B**–**D**) and hippocampus (**F**–**H**) 3 days after injury are shown. Areas of APP staining are indicated by arrows in H&E micrographs (**A** and **E**). No APP-positive signal was found in the corpus callosum or hippocampus of the sham-injured animals (**B** and **F**). Three days post-TBI, enlarged axonal bulbs were dramatically increased in both areas in the TBI model group (**C** and **G**), and this was suppressed by lithium treatment (**D** and **H**). The inserts in photomicrographs **C**, **D**, **G**, and **H** are higher-magnification photographs of the arrow-pointed boxes. Quantitative data showed a significant reduction in the area fraction of APP accumulation in the lithium-treated group compared to the TBI model group for both the corpus callosum and hippocampus (**I** and **J**, respectively; $n = 6$/group; $^*p < 0.05$; scale bar = 50 μm). Nuclei were stained with DAPI as shown with blue fluorescence. Data are represented as mean ± standard error of the mean (TBI, traumatic brain injury; DAPI, 4,6-diamino-2-phenylindole).

dramatically increased in the thalamus (Fig. 4B) of the TBI model mice, and this increase was robustly reduced by lithium treatment (Fig. 4C); in contrast, virtually no signal was observed in the sham-injured mice (Fig. 4A). Quantified data demonstrated that lithium treatment significantly reduced the number of p-Tau-positive cells following TBI (Fig. 4D; $p < 0.01$).

Lithium treatment improves spatial learning after TBI

In the Morris water maze test, a classic test for spatial learning and memory, TBI model mice had significantly longer escape latencies during days 18–20 in the hidden platform training sessions (Fig. 5A; $p < 0.001$) than the sham-injured controls. These escape latencies were significantly reduced by lithium treatment, notably on day 20 (Fig. 5A; $p < 0.01$). In the probe trial conducted on day 20 post-injury, TBI model mice spent less time in the target quadrant in search of the platform than sham-injured animals (Fig. 5B; $p < 0.05$); lithium treatment significantly increased the time spent searching in the correct quadrant (Fig. 5B; $p < 0.05$). Treatment condition did not affect average swim speed, indicating that differences in time spent in the target quadrant were unrelated to changes in swimming speed (Fig. 5C).

In the Y-maze test, which evaluates hippocampal-dependent learning and memory (Yau et al., 2007), TBI model mice spent less time in the novel arm than sham-injured animals, indicating impaired spatial learning and memory (Fig. 5D; $p < 0.01$). Lithium treatment significantly increased the time that TBI model mice spent in the novel arm (Fig. 5D; $p < 0.05$).

Lithium treatment increases hippocampal preservation after TBI

TBI-induced increases in Aβ levels are known to correlate with massive neuronal death in the mouse hippocampus (Smith et al., 1998). H&E staining showed that TBI did indeed result in massive hippocampal damage, but that lithium treatment significantly increased hippocampal preservation at 3 weeks post-TBI (Fig. 6; $p < 0.05$).

Discussion

In the present study we found that post-insult treatment with lithium attenuated TBI-induced Aβ load increases, APP accumulation in axonal bulbs, and BACE1 overexpression in the hippocampus and corpus callosum, areas enriched in APP and Aβ. TBI-induced increases in Tau phosphorylation in the thalamus were also suppressed by lithium treatment 3 days post-injury. In addition, long-term lithium treatment (3 weeks) improved spatial learning and memory as assessed by the Morris water maze and Y-maze tests. Finally, lithium also increased hippocampal tissue preservation in TBI model mice. To our knowledge, this is the first report to show that lithium has the ability to suppress TBI-induced β-secretase overexpression, and to reduce Aβ load through β-secretase inhibition.

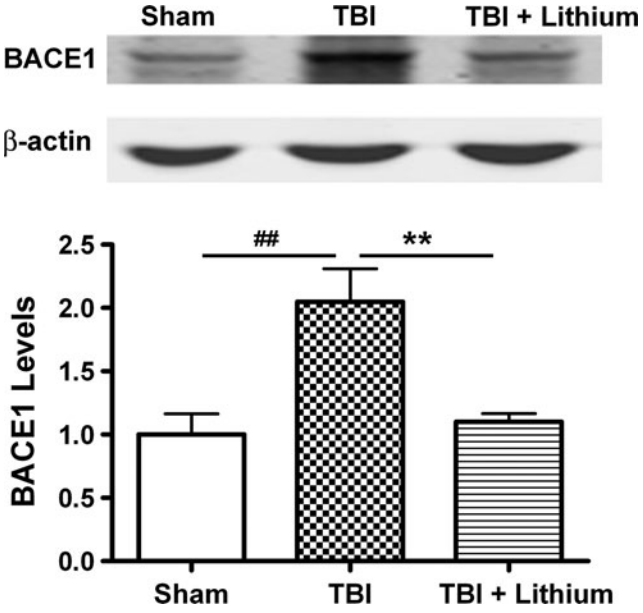

FIG. 3. Lithium blocks TBI-induced BACE1 increases 3 days post-TBI. Representative Western blots showing that lithium blocked TBI-induced BACE1 increases 3 days after TBI. BACE1 was visualized as a band of around 70 kDa, and its levels were markedly increased in the ipsilateral hippocampus in the TBI model group compared with the sham-injured group ($n = 5$/group; $^{\#\#}p < 0.01$), and this increase was completely blocked by lithium treatment ($n = 5$/group; $^{**}p < 0.01$). Data are represented as mean ± standard error of the mean. Levels of β-actin were used as loading control (TBI, traumatic brain injury; BACE1, β-APP-cleaving enzyme-1).

Aβ is the primary component of amyloid plaques, and Aβ deposits in the brain are thought to play a central role in the progression of AD (Carter et al., 2010; Walsh and Selkoe, 2007). Reducing brain Aβ load has beneficial effects in animal models of AD, and remains an important strategy for halting the progression of AD (Carter et al., 2010). In animal models of TBI, Aβ load reductions in the brain have similarly been shown to be neuroprotective (Abrahamson et al., 2009; Loane et al., 2009). The oligomeric forms of Aβ are toxic, while the monomer forms are thought to be neuroprotective (Carter et al., 2010; Giuffrida et al., 2010). One study found that the 27-kDa band of the Aβ oligomers was toxic (Lambert et al., 1998), and another study found that reducing the 21-kDa band of the Aβ oligomers was neuroprotective in an animal model of AD (Tchantchou et al., 2007). The present study found that a 27-kDa Aβ oligomer was robustly elevated after TBI, and that treatment with lithium attenuated this increase. ELISA analysis further confirmed that lithium attenuated the Aβ42 increases induced by TBI. The immunostaining results indicated that TBI-induced Aβ accumulated in the axonal bulbs, and that this accumulation was markedly suppressed by lithium treatment. Taken together, this evidence suggests that lithium reduced the TBI-induced Aβ load increase, thus mitigating its potentially damaging downstream effects.

Diffuse axonal injury is one of pathological features of TBI (Johnson et al., 2010, 2012). Dynamic axonal deformation and the subsequent interruption of axonal transport results in the accumulation of transported material in the axonal bulbs (Johnson et al., 2012). APP, which has been shown to accumulate in these axonal bulbs after TBI, has been widely used as a marker for axonal injury (Bramlett et al., 1997; Johnson et al., 2010; Tran et al., 2011). Reducing APP levels with an infusion of anti-APP

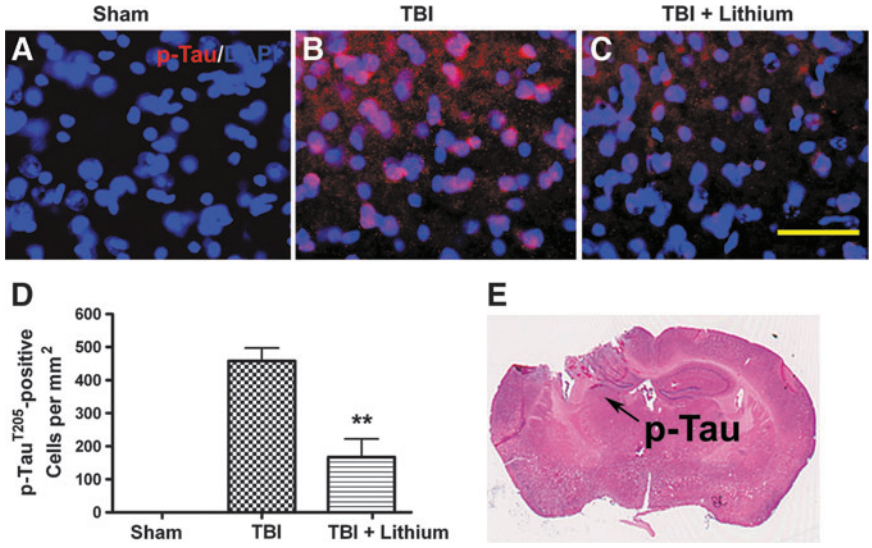

FIG. 4. Lithium reduces Tau phosphorylation in the thalamus after TBI. Representative microphotographs of phospho-Tau (p-Tau) staining (**A**–**C**) in the thalamus 3 days after injury are shown. The area of p-Tau staining is indicated by an arrow in the H&E micrograph (**E**). No p-Tau-positive signal was detected in the thalamus of the sham-injured animals (**A**). A robust increase in p-Tau-positive cells was observed in the thalamus 3 days after TBI (**B**), which was significantly suppressed by lithium treatment (**C**). Quantitative data showing that lithium reduced the number of TBI-induced p-Tau-positive cells compared with the TBI model group (**D**; $n = 5$/group; $^{**}p < 0.01$; scale bar = 50 μm). Nuclei were stained with DAPI as shown with blue fluorescence. Data are represented as mean ± standard error of the mean (TBI, traumatic brain injury; DAPI, 4,6-diamino-2-phenylindole; H&E, hematoxylin and eosin).

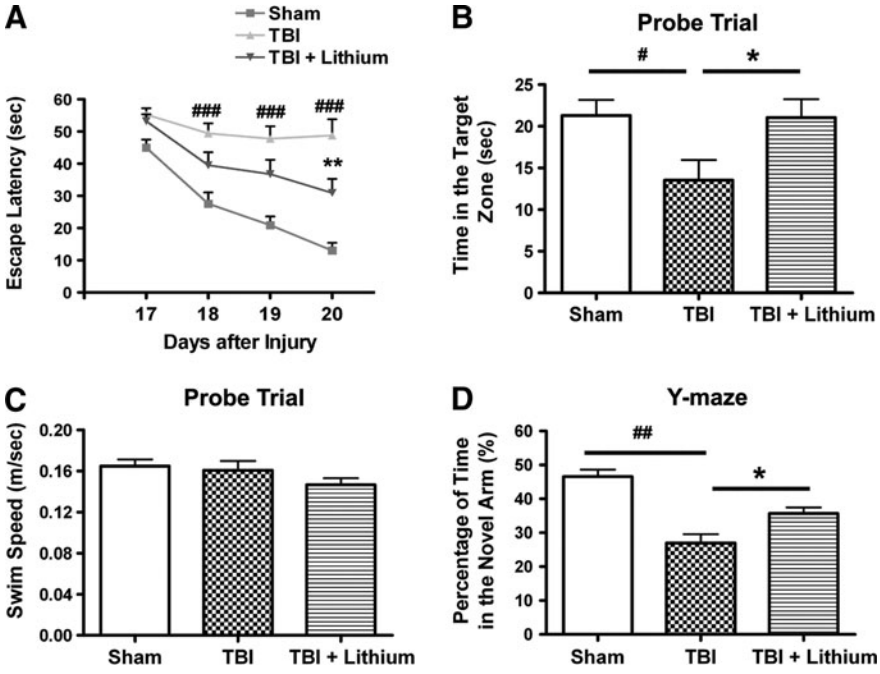

FIG. 5. Lithium improves spatial learning in the Morris water maze and Y-maze tests. The Morris water maze test was conducted 17–20 days after TBI. During the hidden platform training portion of the test, the TBI group took significantly more time to locate the platform, indicating a deficit in spatial learning (**A**; $n = 17$/group; $^{###}p < 0.001$). Lithium treatment significantly improved this learning deficit, especially on day 20 ($n = 17$/group; $^{**}p < 0.01$), compared with the TBI group. In the probe trial of the Morris water maze test performed 20 days following injury, TBI model mice spent less time in the target quadrant than sham-injured mice (**B**; $n = 10$/group; $^{\#}p < 0.05$). Lithium treatment reversed this ($n = 10$/group, $^{*}p < 0.05$). The average swim speed did not differ between groups (**C**). The Y-maze test was conducted 10 days post-TBI to evaluate spatial learning. Ten days after TBI, the TBI model group spent significantly less time than sham-injured mice in the novel arm versus the total time spent in all three arms (**D**; $n = 12$/group; $^{\#\#}p < 0.01$). In contrast, lithium treatment significantly attenuated this decline in the lithium-treated group ($n = 12$/group; $^{*}p < 0.05$). Data are represented as mean ± standard error of the mean (TBI, traumatic brain injury).

antibodies reduces tissue damage, increases MAP-2 immunoreactivity, and improves functional outcome (Itoh et al., 2009). Consistent with these findings, the present study demonstrated that APP accumulation in axonal bulbs increased after TBI, and notably, that lithium treatment reduced APP accumulation. Because APP can be sequentially cleaved by β- and γ-secretase to generate toxic Aβ, we hypothesize that in the present study, lithium treatment reduced APP accumulation, thereby decreasing the availability of substrate needed to generate Aβ.

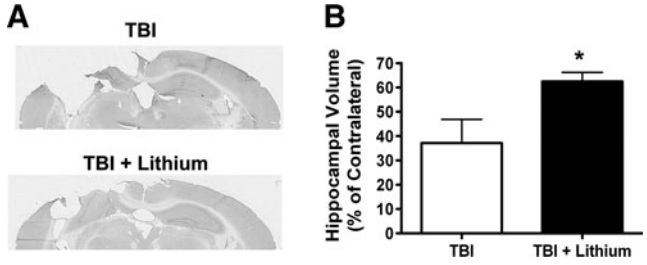

FIG. 6. Lithium increases hippocampal tissue preservation after TBI. TBI caused massive hippocampal tissue loss 3 weeks after injury, and this tissue loss was reduced by lithium treatment (**A**). Quantified data showing that lithium significantly increased hippocampal tissue preservation (**B**; $n = 6$; $^{*}p < 0.05$). Data are represented as mean ± standard error of the mean (TBI, traumatic brain injury).

This study also examined BACE1, which is the major β-secretase involved in producing Aβ in neurons (Cai et al., 2001). BACE1 levels are increased after TBI in human patients with head trauma, as well as in TBI rodent models (Blasko et al., 2004; Loane et al., 2009; Uryu et al., 2007). Knockdown of β-secretase in BACE1$^{-/-}$ mice improves behavioral and histological outcomes after TBI (Loane et al., 2009). Consistent with these reports, we found that TBI robustly increased BACE1 expression, and that lithium treatment completely blocked this increase. Because β-secretase catalyzes the initial and rate-limiting step of Aβ generation (Roggo, 2002), we believe that the lithium-induced blockade of BACE1 expression observed in this study accounted for most of the reduction in Aβ load, with substantial subsequent beneficial effects. However, it is possible that the lithium-induced decrease in levels of APP also contributed to reduced Aβ production because of limited availability of Aβ precursor.

Memory impairments are frequently seen in head trauma patients, as well as in animal models of TBI (Dash et al., 2011; Loane et al., 2009; Spikman et al., 2012). Consistent with these reports, in the present study we noted that TBI impaired performance in the Morris water maze and Y-maze tests. Notably, however, lithium improved spatial learning and memory, as indicated by shortened escape latencies in the Morris water maze test, increased time spent searching for the platform in the probe trial, and increased time spent in the novel arm in the Y-maze test. Interestingly, a previous study

found that TBI in transgenic mice overexpressing mutant APP led to increased Aβ load; this resulted in massive hippocampal neuronal death, which correlated with cognitive impairment (Smith et al., 1998). Another study found that lithium reduced Aβ load and improved Morris water maze performance in an AD model (Rockenstein et al., 2007).

Taken together, the results of the present study strongly suggest that lithium improved spatial learning and memory by reducing Aβ load. Because it is well established that the hippocampus is involved in learning and memory, and because lithium increased hippocampal preservation in this study, we conclude that reducing hippocampal damage improves memory function. It is also well recognized that synaptic plasticity is key to regulating learning and memory. Chronic lithium treatment has been shown to increase brain-derived neurotrophic factor protein levels in the brain (Fukumoto et al., 2001; Hashimoto et al., 2002; Jacobsen and Mork, 2004), a neurotrophin that modulates synaptic plasticity (Autry and Monteggia, 2012). Thus, long-term lithium treatment was employed in this study to potentially maximize its beneficial effects on learning and memory. It should be noted that the behavioral benefits of lithium in various models of CNS disorders, including Alzheimer's disease, also require long-term drug treatment (Chiu and Chuang, 2010). Because high levels of Aβ have been shown to inhibit hippocampal long-term potentiation and cause synaptic dysfunction and memory deficits (Barry et al., 2011; Shankar et al., 2008), further studies are warranted to address the effects of lithium on synaptic plasticity in the TBI model animals.

The thalamus is also involved with memory. For example, patients with thalamic lesions were found to have executive memory impairments, especially with regard to spatio-temporal relations (Weiler et al., 2011). In addition, increased thalamic inflammation has been correlated with severity of cognitive function in head trauma patients (Ramlackhansingh et al., 2011). In the current study, lithium treatment attenuated TBI-induced Tau phosphorylation at Thr205 in the thalamus. GSK-3 is a major kinase for Tau phosphorylation, and hyperphosphorylation of Tau has been linked to the pathology of AD (Liu et al., 2012). Thus the effects of lithium on TBI-induced Tau phosphorylation observed in the present study may also protect against neurodegeneration and cognitive deficits.

It should be noted that in animal models of AD, Aβ has been shown to occur upstream of Tau phosphorylation (Oddo et al., 2004); however, in a TBI model, Aβ accumulation and Tau phosphorylation were shown to be separate events (Tran et al., 2011). The present study did not address the relationship between Aβ accumulation and Tau phosphorylation. Nevertheless, both Aβ accumulation and Tau phosphorylation can induce neuronal damage and result in additional long-term functional deficits. Thus, by reducing both Aβ accumulation and Tau phosphorylation, lithium reduces hippocampal tissue damage and subsequently improves spatial learning and memory. In this context, *in vitro* studies have shown that lithium reduces Aβ load and Tau phosphorylation through GSK-3 inhibition (Hong et al., 1997; Phiel et al., 2003; Su et al., 2004). Recently, we and others reported that lithium reduces hippocampal neuronal death after TBI by increasing GSK-3β Ser9 phosphorylation, thereby inhibiting its enzymatic activity (Dash et al., 2011; Yu et al., 2012).

Taken together, the existing evidence suggests that the Aβ load reductions and beneficial behavioral effects associated with lithium in this study are likely mediated via GSK-3 inhibition. Lithium has a long history of safe and therapeutic use in bipolar disorder; its clinical features are well-known and safety is not a concern. The results of the present study suggest that lithium could potentially be used to manage memory impairment in head trauma victims and warrant further investigation.

Acknowledgments

Support for this work included funding from the Department of Defense in the Center for Neuroscience and Regenerative Medicine (CNRM), the Blast Lethality Injury and Research Program, and the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH). We are grateful to Drs. Oz Malkesman and Amanda Fu, and to Ms. Laura Tucker from the CNRM for assistance with the behavioral tests and CCI surgery. We would like to thank Drs. Chi-Tso Chiu and Zhifei Wang of the NIMH, and Dr. Flaubert Tchantchou from the Uniformed Services University of the Health Sciences, for their helpful discussions. The authors also wish to gratefully acknowledge the editorial assistance of Peter Leeds and Ioline Henter of the NIMH.

Author Disclosure Statement

No conflicting financial interests exist.

References

Abrahamson, E.E., Ikonomovic, M.D., Dixon, C.E., and DeKosky, S.T. (2009). Simvastatin therapy prevents brain trauma-induced increases in beta-amyloid peptide levels. Ann. Neurol. 66, 407–414.

Autry, A.E., and Monteggia, L.M. (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol. Rev. 64, 238–258.

Barry, A.E., Klyubin, I., McDonald, J.M., Mably, A.J., Farrell, M.A., Scott, M., Walsh, D.M., and Rowan, M.J. (2011). Alzheimer's disease brain-derived amyloid-beta-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. J. Neurosci. 31, 7259–7263.

Blasko, I., Beer, R., Bigl, M., Apelt, J., Franz, G., Rudzki, D., Ransmayr, G., Kampfl, A., and Schliebs, R. (2004). Experimental traumatic brain injury in rats stimulates the expression, production and activity of Alzheimer's disease beta-secretase (BACE-1). J. Neural Transm. 111, 523–536.

Bramlett, H.M., Kraydieh, S., Green, E.J., and Dietrich, W.D. (1997). Temporal and regional patterns of axonal damage following traumatic brain injury: a beta-amyloid precursor protein immunocytochemical study in rats. J. Neuropathol. Exp. Neurol. 56, 1132–1141.

Cai, H., Wang, Y., McCarthy, D., Wen, H., Borchelt, D.R., Price, D.L., and Wong, P.C. (2001). BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. Nat. Neurosci. 4, 233–234.

Carter, M.D., Simms, G.A., and Weaver, D.F. (2010). The development of new therapeutics for Alzheimer's disease. Clin. Pharmacol. Ther. 88, 475–486.

Chiu, C.T., and Chuang, D.M. (2010). Molecular actions and therapeutic potential of lithium in preclinical and clinical studies of CNS disorders. Pharmacol. Ther. 128, 281–304.

Dash, P.K., Johnson, D., Clark, J., Orsi, S.A., Zhang, M., Zhao, J., Grill, R.J., Moore, A.N., and Pati, S. (2011). Involvement of the

glycogen synthase kinase-3 signaling pathway in TBI pathology and neurocognitive outcome. PLoS One 6, e24648.

Dash, P.K., Orsi, S.A., Zhang, M., Grill, R.J., Pati, S., Zhao, J., and Moore, A.N. (2010). Valproate administered after traumatic brain injury provides neuroprotection and improves cognitive function in rats. PLoS One 5, e11383.

Fukumoto, T., Morinobu, S., Okamoto, Y., Kagaya, A., and Yamawaki, S. (2001). Chronic lithium treatment increases the expression of brain-derived neurotrophic factor in the rat brain. Psychopharmacology (Berl.) 158, 100–106.

Giuffrida, M.L., Caraci, F., De Bona, P., Pappalardo, G., Nicoletti, F., Rizzarelli, E., and Copani, A. (2010). The monomer state of beta-amyloid: where the Alzheimer's disease protein meets physiology. Rev. Neurosci. 21, 83–93.

Hashimoto, R., Takei, N., Shimazu, K., Christ, L., Lu, B., and Chuang, D.M. (2002). Lithium induces brain-derived neurotrophic factor and activates TrkB in rodent cortical neurons: an essential step for neuroprotection against glutamate excitotoxicity. Neuropharmacology 43, 1173–1179.

Hong, M., Chen, D.C., Klein, P.S., and Lee, V.M. (1997). Lithium reduces tau phosphorylation by inhibition of glycogen synthase kinase-3. J. Biol. Chem. 272, 25326–25332.

Hooper, C., Killick, R., and Lovestone, S. (2008). The GSK3 hypothesis of Alzheimer's disease. J. Neurochem. 104, 1433–1439.

Ikonomovic, M.D., Uryu, K., Abrahamson, E.E., Ciallella, J.R., Trojanowski, J.Q., Lee, V.M., Clark, R.S., Marion, D.W., Wisniewski, S.R., and DeKosky, S.T. (2004). Alzheimer's pathology in human temporal cortex surgically excised after severe brain injury. Exp. Neurol. 190, 192–203.

Itoh, T., Satou, T., Nishida, S., Tsubaki, M., Hashimoto, S., and Ito, H. (2009). Improvement of cerebral function by anti-amyloid precursor protein antibody infusion after traumatic brain injury in rats. Mol. Cell Biochem. 324, 191–199.

Jacobsen, J.P., and Mork, A. (2004). The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. Brain Res. 1024, 183–192.

Johnson, V.E., Stewart, W., and Smith, D.H. (2012). Axonal pathology in traumatic brain injury. Exp. Neurol. [Epub ahead of print].

Johnson, V.E., Stewart, W., and Smith, D.H. (2010). Traumatic brain injury and amyloid-beta pathology: a link to Alzheimer's disease? Nat. Rev. Neurosci. 11, 361–370.

Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., Wals, P., Zhang, C., Finch, C.E., Krafft, G.A., and Klein, W.L. (1998). Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. USA 95, 6448–6453.

Liu, H.C., Leu, S.J., and Chuang, D.M. (2012). Roles of glycogen synthase kinase-3 in Alzheimer disease: from pathology to treatment target. J. Exp. Clin. Med. 4, 135–139.

Li, X., and Jope, R.S. (2010). Is glycogen synthase kinase-3 a central modulator in mood regulation? Neuropsychopharmacology 35, 2143–2154.

Loane, D.J., Pocivavsek, A., Moussa, C.E., Thompson, R., Matsuoka, Y., Faden, A.I., Rebeck, G.W., and Burns, M.P. (2009). Amyloid precursor protein secretases as therapeutic targets for traumatic brain injury. Nat. Med. 15, 377–379.

Loane, D.J., Washington, P.M., Vardanian, L., Pocivavsek, A., Hoe, H.S., Duff, K.E., Cernak, I., Rebeck, G.W., Faden, A.I., and Burns, M.P. (2011). Modulation of ABCA1 by an LXR agonist reduces beta-amyloid levels and improves outcome after traumatic brain injury. J. Neurotrauma 28, 225–236.

Mattson, M.P. (2004). Pathways towards and away from Alzheimer's disease. Nature 430, 631–639.

Oddo, S., Billings, L., Kesslak, J.P., Cribbs, D.H., and LaFerla, F.M. (2004). Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 43, 321–332.

Olsson, A., Csajbok, L., Ost, M., Hoglund, K., Nylen, K., Rosengren, L., Nellgard, B., and Blennow, K. (2004). Marked increase of beta-amyloid(1–42) and amyloid precursor protein in ventricular cerebrospinal fluid after severe traumatic brain injury. J. Neurol. 251, 870–876.

Phiel, C.J., Wilson, C.A., Lee, V.M., and Klein, P.S. (2003). GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature 423, 435–439.

Raby, C.A., Morganti-Kossmann, M.C., Kossmann, T., Stahel, P.F., Watson, M.D., Evans, L.M., Mehta, P.D., Spiegel, K., Kuo, Y.M., Roher, A.E., and Emmerling, M.R. (1998). Traumatic brain injury increases beta-amyloid peptide 1–42 in cerebrospinal fluid. J. Neurochem. 71, 2505–2509.

Ramlackhansingh, A.F., Brooks, D.J., Greenwood, R.J., Bose, S.K., Turkheimer, F.E., Kinnunen, K.M., Gentleman, S., Heckemann, R.A., Gunanayagam, K., Gelosa, G., and Sharp, D.J. (2011). Inflammation after trauma: microglial activation and traumatic brain injury. Ann. Neurol. 70, 374–383.

Roberts, G.W., Gentleman, S.M., Lynch, A., and Graham, D.I. (1991). Beta A4 amyloid protein deposition in brain after head trauma. Lancet 338, 1422–1423.

Roberts, G.W., Gentleman, S.M., Lynch, A., Murray, L., Landon, M., and Graham, D.I. (1994). Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry 57, 419–425.

Rockenstein, E., Torrance, M., Adame, A., Mante, M., Bar-on, P., Rose, J.B., Crews, L., and Masliah, E. (2007). Neuroprotective effects of regulators of the glycogen synthase kinase-3beta signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. J. Neurosci. 27, 1981–1991.

Roggo, S. (2002). Inhibition of BACE, a promising approach to Alzheimer's disease therapy. Curr. Top. Med. Chem. 2, 359–370.

Shankar, G.M., Li, S., Mehta, T.H., Garcia-Munoz, A., Shepardson, N.E., Smith, I., Brett, F.M., Farrell, M.A., Rowan, M.J., Lemere, C.A., Regan, C.M., Walsh, D.M., Sabatini, B.L., and Selkoe, D.J. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat. Med. 14, 837–842.

Shein, N.A., Grigoriadis, N., Alexandrovich, A.G., Simeonidou, C., Lourbopoulos, A., Polyzoidou, E., Trembovler, V., Mascagni, P., Dinarello, C.A., and Shohami, E. (2009). Histone deacetylase inhibitor ITF2357 is neuroprotective, improves functional recovery, and induces glial apoptosis following experimental traumatic brain injury. FASEB J. 23, 4266–4275.

Smith, D.H., Nakamura, M., McIntosh, T.K., Wang, J., Rodriguez, A., Chen, X.H., Raghupathi, R., Saatman, K.E., Clemens, J., Schmidt, M.L., Lee, V.M., and Trojanowski, J.Q. (1998). Brain trauma induces massive hippocampal neuron death linked to a surge in beta-amyloid levels in mice overexpressing mutant amyloid precursor protein. Am. J. Pathol. 153, 1005–1010.

Spikman, J.M., Timmerman, M.E., Milders, M.V., Veenstra, W.S., and van der Naalt, J. (2012). Social cognition impairments in relation to general cognitive deficits, injury severity, and prefrontal lesions in traumatic brain injury patients. J. Neurotrauma 29, 101–111.

Su, Y., Ryder, J., Li, B., Wu, X., Fox, N., Solenberg, P., Brune, K., Paul, S., Zhou, Y., Liu, F., and Ni, B. (2004). Lithium, a common drug for bipolar disorder treatment, regulates amyloid-beta precursor protein processing. Biochemistry 43, 6899–6908.

Tchantchou, F., Xu, Y., Wu, Y., Christen, Y., and Luo, Y. (2007). EGb 761 enhances adult hippocampal neurogenesis and phosphorylation of CREB in transgenic mouse model of Alzheimer's disease. FASEB J. 21, 2400–2408.

Tran, H.T., LaFerla, F.M., Holtzman, D.M., and Brody, D.L. (2011). Controlled cortical impact traumatic brain injury in 3xTg-AD mice causes acute intra-axonal amyloid-beta accumulation and independently accelerates the development of tau abnormalities. J. Neurosci. 31, 9513–9525.

Tran, H.T., Sanchez, L., and Brody, D.L. (2012). Inhibition of JNK by a peptide inhibitor reduces traumatic brain injury-induced tauopathy in transgenic mice. J. Neuropathol. Exp. Neurol. 71, 116–129.

Uryu, K., Chen, X.H., Martinez, D., Browne, K.D., Johnson, V.E., Graham, D.I., Lee, V.M., Trojanowski, J.Q., and Smith, D.H. (2007). Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans. Exp. Neurol. 208, 185–192.

Walsh, D.M., and Selkoe, D.J. (2007). A beta oligomers—a decade of discovery. J. Neurochem. 101, 1172–1184.

Weiler, J., Suchan, B., Koch, B., Schwarz, M., and Daum, I. (2011). Differential impairment of remembering the past and imagining novel events after thalamic lesions. J. Cogn. Neurosci. 23, 3037–3051.

Yau, J.L., McNair, K.M., Noble, J., Brownstein, D., Hibberd, C., Morton, N., Mullins, J.J., Morris, R.G., Cobb, S., and Seckl, J.R. (2007). Enhanced hippocampal long-term potentiation and spatial learning in aged 11beta-hydroxysteroid dehydrogenase type 1 knock-out mice. J. Neurosci. 27, 10487–10496.

Yu, F., Wang, Z., Tchantchou, F., Chiu, C.T., Zhang, Y., and Chuang, D.M. (2012). Lithium ameliorates neurodegeneration, suppresses neuroinflammation, and improves behavioral performance in a mouse model of traumatic brain injury. J. Neurotrauma 29, 362–374.

Zhu, Z.F., Wang, Q.G., Han, B.J., and William, C.P. (2010). Neuroprotective effect and cognitive outcome of chronic lithium on traumatic brain injury in mice. Brain Res. Bull. 83, 272–277.

Address correspondence to:
De-Maw Chuang, Ph.D.
Section on Molecular Neurobiology
National Institute of Mental Health–National Institutes of Health
10 Center Drive, MSC-1363
Bethesda, MD 20892-1363

E-mail: chuang@mail.nih.gov