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The dopamine and cAMP regulated phosphoprotein, 32 kDa (DARPP-32) signaling pathway: A novel therapeutic target in traumatic brain injury

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Abstract

Traumatic brain injury (TBI) causes persistent neurologic deficits. Current therapies, predominantly focused upon cortical and hippocampal cellular survival, have limited benefit on cognitive outcomes. Striatal damage is associated with deficits in executive function, learning, and memory. Dopamine and cAMP regulated phosphoprotein 32 (DARPP-32) is expressed within striatal medium spiny neurons and regulates striatal function. We found that controlled cortical impact injury in rats produces a chronic decrease in DARPP-32 phosphorylation at threonine-34 and an increase in protein phosphatase-1 activity. There is no effect of injury on threonine-75 phosphorylation or on DARPP-32 protein. Amantadine, shown to be efficacious in treating post-TBI cognitive deficits, given daily for two weeks is able to restore the loss of DARPP-32 phosphorylation and reduce protein phosphatase-1 activity. Amantadine also decreases the phosphorylation of threonine-75 consistent with activity as a partial N-methyl-D-aspartate (NMDA) receptor antagonist and partial dopamine agonist. These data demonstrate that targeting the DARPP-32 signaling cascade represents a promising novel therapeutic approach in the treatment of persistent deficits following a TBI.

Keywords

DARPP-32; TBI; Striatum; Amantadine; Dopamine

Introduction

Traumatic brain injury (TBI) causes persistent deficits in cognitive function. A complex etiology makes the identification of therapeutic targets difficult in TBI (Kochanek, 1993; DeKosky et al., 1998). Subcortical regions, the thalami and basal ganglia, represent novel

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areas for therapeutic targeting in TBI given their importance to functional deficits and integration with other brain regions (Donnemiller et al., 2000; Vertes, 2006).

The basal ganglia are involved in executive function, emotional control, attention, and memory and learning (Ridley et al., 2006; Baldo and Kelley, 2007). The striatum represents the major entry into the basal ganglia. Striatal medium spiny neurons (MSN) receive glutamate inputs from the cerebral cortex and dopamine (DA) innervation from the midbrain (Pennartz and Kitai, 1991). The interaction of glutamate and DA within MSNs modulate the plasticity of corticostriatal synapses (Calabresi et al., 2007).

In TBI there is an increase in glutamatergic release leading to excitotoxicity and oxidative stress (Palmer et al., 1993). Alterations in glutamate receptor subunit composition and expression contribute to persistent dysfunction in plasticity (Osteen et al., 2004). Dysfunctions in DAergic signaling after TBI include increases in DA acutely (Massucci et al., 2004; Kobori et al., 2006), alterations in dopamine transporter (DAT), chronic reductions in evoked DA release (Wagner et al., 2005, 2009), and alterations in tyrosine hydroxylase (Yan et al., 2007). Pharmacotherapies targeting glutamate and DA have clinical (McAllister et al., 2004; Warden et al., 2006) and experimental (Kline et al., 2000; Dixon et al., 1999) benefits in TBI. Striatal signaling has been proposed to be of particular interest in persistent deficits in multiple cognitive modalities post TBI (Bales et al., 2009).

The dopamine and cAMP regulated phosphoprotein-32 (DARPP-32) is a cytoplasmic phosphoprotein found in 95% of MSNs. Two phosphorylation sites, threonine-34 (Thr34) and threonine-75 (Thr75), make DARPP-32 a bifunctional signal transduction molecule that controls the activities of protein phosphatase-1 (PP-1) and protein kinase A (PKA) (Greengard et al., 1999; Nishi et al., 2002; Valjent et al., 2005).

DA acting at D1 receptors promotes DARPP-32 phosphorylation at Thr34, leading to inhibition of PP-1. Glutamate, through calcium (Ca^{2+}) activation of protein phosphatase 2B (PP-2B) and protein phosphatase 2A (PP-2A), decreases phosphorylation at Thr34. A reduction in phosphorylation at Thr34 removes the inhibitory affect of DARPP-32 on PP-1 (Nairn et al., 2004; Svenningsson et al., 2004). The regulation of PP-1 and PKA by DARPP-32 allows DA and glutamate to regulate cellular signaling and protein transcription (Fienberg et al., 1998; Hakansson et al., 2004).

DARPP-32 has been proposed as a therapeutic target in Parkinson's disease (PD) (Coccurello et al., 2004). In addition to DA agonists, NMDA antagonists, such as amantadine hydrochloride (AMH), are potential mediators of DARPP-32 signaling in PD (Greenamyre and O'Brien, 1991).

This study represents the first to examine DARPP-32 phosphorylation in TBI. We hypothesize that, given known alterations in glutamate and DA signaling post TBI, there will be a decrease in the phosphorylation of DARPP-32 at Thr34, an increase in DARPP-32 at Thr75, and a subsequent increase in PP-1 activity within the striatum that persists chronically. This study is also the first to examine the effect of AMH on DARPP-32 phosphorylation. We hypothesize that the administration of AMH following a TBI will prevent the loss of DARPP-32 phosphorylation at Thr34 and the increase in PP-1 activity due to AMHs activity as a partial NMDA antagonist.

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Materials and methods

Animals

Adult male Sprague-Dawley rats (*N* = *172*) were used in the study. Rats (300–325 g) were purchased from Hilltop Laboratories (Scottsdale, PA, USA) and housed in pairs under a 12:12 light/dark cycle. Rats were given food and water with *ad libitum* throughout the study. All experiments were carried out in accordance with the University of Pittsburgh's guidelines for the Care and Use of Laboratory Animals. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Surgical procedures for TBI and drug regimen

On the day of surgery anesthesia was initiated with 4% isoflurane (IsoFlo; Abbott Laboratories, North Chicago, IL, USA) and 2:1 N_2O/O_2. Rats were intubated and maintained on 1.5-2% isofluorane during the surgical procedure. Following intubation, rats were placed on a thermal blanket to regulate body temperature (37 °C) and the animals head placed in a stereotaxic frame. An incision was made down the midline of the skull and the soft tissues and periosteum deflected. A craniotomy was then performed over the right parietal bone to expose the dura. Controlled cortical injury (CCI, Pittsburgh Precision Instruments, Inc.) at a depth of 2.6 mm-2.8 mm at 4 m/s was carried out as previously reported (Dixon et al., 1991; Yan et al., 2000). A total of 91 rats were injured, and the remaining 97 rats were shams. Righting reflex (Dixon et al., 1991) was monitored in the immediate postsurgical period to assess acute recovery.

AMH treated animals

Beginning one day post CCI or sham surgery animals received daily i.p. injections of AMH (10 mg/kg; Sigma, St. Louis, MO) dissolved in physiological saline (n = 6) or saline alone (n = 12). AMH was made fresh daily.

Tissue preparation, immunohistochemistry, and immunofluorescence staining

Animals (*N* = 3 in each group for each time point for a total *N* = *30*) *were* given an overdose of sodium pentobarbital (100 mg/kg; i.p), and perfused intra-aortically with 0.1 M heparinized PBS in 4% PFA/0.1 M PBS. Brains were dissected, submerged in increasing concentrations of sucrose, and stored at −80 °C. Brains were then sectioned at 35 μm in a cryostat, and free floating sections collected in tissue plate wells containing 0.1 M TBS (pH 7.5).

All immunohistochemical procedures and incubations were carried out with agitation with the exception of the chromogen step. All treatment groups were stained together within each immunohistochemical session. Sections were matched by region, rinsed 3 × 5 min in washing buffer (0.1%Triton-X in 0.1 M TBS) and blocked in a mixture of 10% normal donkey serum in washing buffer for 2 h at room temperature. Sections were then incubated overnight at 4 °C in a primary rabbit antibody specific to DARPP-32 (1:1000; Cell Signaling) diluted in washing buffer/5% normal donkey serum. Following incubation, sections were then washed 3 × 8 min in washing buffer and endogenous peroxidase activity was quenched with 0.3% H_2O_2 in methanol for 10 min. Following 3 × 5 min washing in washing buffer, sections were incubated for 2 h at RT in biotin conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch, PA) at 1:400 in 5% normal donkey serum in washing buffer. Sections were then washed 3 × 5 min in washing buffer and incubated in ABC reagent (company name here) for 30 min. Immunoreactivity (IR) was then visualized using 0.01% 3′ 3′ diaminobenzidine (DAB) following 5 × 5 min washing in washing buffer. The DAB reaction was terminated with dH_2O and sections were rinsed in 0.1 M TBS,

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mounted onto slides, air dried and cover-slipped for light microscope analysis. All sections within the reaction were exposed to each of the reagents for the same time period.

For double labeling, sections (*N = 4* in each group for each timepoint for a total *N = 40*) were incubated with primary antibody (Cell Signaling; p-DARPP-32-Thr34 1:50) in 10% normal donkey serum in washing buffer with mouse anti-NeuN (1:2000) at 4 °C for 16–24 h. Alexa fluorescent dyes (488, 594) conjugated to goat anti-rabbit or donkey anti-mouse secondaries, respectively, were used for double labeling of p-DARPP-32-Thr34 and NeuN. At least 3 sections of brain tissue through the striatum were processed for each experiment. Control experiments omitting primary antibodies were run in parallel to confirm antibody specificity. Images were captured using a confocal microscope (LSM 510; Zeiss, Jena, Germany).

Western blot analysis

Animals (*N* = 6 in each timepoint for each group for a total *N = 60* for DARPP-32 and p-DARPP-32 timecourse analysis; *N = 6* for each group for a total of *N = 18* for amantadine hydrochloride studies). After deeply anesthetized with pentobarbital (Nembutal, 80–100 mg/kg; Abbott Laboratories, North Chicago, IL), animals were decapitated and the brains quickly removed and chilled on ice. Tissue from the striatum was excised and frozen in liquid nitrogen. Tissue was stored at −70 °C until used for analysis. Striatal tissue was homogenized in 400 μl of lysis buffer containing 0.1 M NaCl, 0.01 M Tris–Cl (pH 7.6), 0.001 M EDTA (pH 8.0), 1 μg/ml aprotinin, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF) and phosphatase inhibitor cocktail 1 (Sigma, St. Louis, MO) was added to samples used for western blot. Protein concentrations were determined using a BCA protein Assay Kit (PIERCE, Rockford, IL). Samples containing 20–100 μg of protein were subjected to SDS-polyacrylamide gel electrophoresis through a 10% acrylamide gel, and after were transferred to nitrocellulose membranes. Membranes were blocked with 5% bovine serum albumin (Sigma, St. Louis, MO) in tris-buffered saline (TBS-T). Following block membranes were washed 3 × 10 min in TBS-T and immunolabeled with antibodies (1:10,000 DARPP-32; 1:2000 p-DARPP-32-Thr34; 1:2000 p-DARPP-32-Thr75; Cell Signaling, Danvers, MA) in 5% BSA in TBST for 18–24 h. Following primary incubation membranes were washed 3 × 10 min in TBS-T and incubated for 2 h in by goat anti-rabbit immunoglobulin G conjugated to peroxidase (1:5,000; PIERCE, Rockford, IL). Proteins were visualized with a chemiluminescence detection system (SuperSignal, PIERCE, Rockford, IL). To assure equal loading, all membranes were striped and re-blotted with rabbit anti-actin antibody (1:20,000, Sigma, St. Louis, MO). Blots were exposed to autoradiographic X-ray film for 10 s to 2 min and bands were semi-quantified using ImageJ (NIH, USA) software. Values are given as a ratio (percentage change) of optical density of injured samples versus sham control within individual blots. All optical densities were normalized to β-actin prior to analysis.

Data are expressed as the group means ± standard error (S.E.) of the mean. Statistical evaluations were performed according to ANOVA followed by Scheffé test as a post hoc comparison. A significance level of $p \leq 0.05$ was used for all tests.

Protein phosphatase-1 activity assay

Animals (*N* = 6 in each timepoint for each group for a total *N = 24; N = 18* for amantadine hydrochloride PP-1 study). Striatal tissue was prepared following the same protocol as used for western blots, with the exception of the use of the phosphatase inhibitor cocktail in the lysis buffer. The following protocol utilized a modified PP-2A activity assay kit (Millipore, Temecula, CA) (Supplemental Fig. 1). 100 μg of protein, determined via BCA assay, was added to an Eppendorf tube with 25 μl of agarose A/G beads and 6 μl of anti-PP-1δ rabbit

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antibody (Millipore, Temecula, CA). Following overnight incubation in 4 °C and 3 min spin down at 10,000 *g*, beads were washed 3 times with ice cold TBS and once with Ser/Thr phosphopeptide buffer. Beads were spun down in between each wash for 1 min in 4 °C. Following the final wash, 30 μl of phosphopeptide and 20 μl of Ser/Thr phosphopeptide buffer was added to the Eppendorf tube. The Eppendorf tube was then incubated for 10 min in a water bath at 30 °C. 25 μl of supernatant was placed in a 96 well plate (in duplicates) and malachite green detection assay was utilized to visualize free phosphates. The plate was read on a plate-reader at 650 nm. A standard curve was calculated and the level of phosphatase activity is reported in picomoles of phosphate released per 25 μl of supernatant. Negative controls (antibody omitted and rabbit DARPP-32 antibody, Cell signaling) showed no reaction in malachite detection, with phosphate levels comparable to background (lysis buffer alone). 10 μl of TBS and 10 μl of 2× loading buffer were then added to the Eppendorf. The Eppendorf tube was then heated to 95 °C for 5 min and western blots were run to determine the relative amount of PP-1 protein in each tube. Furthermore, given the homology between PP-2A and PP-1, western blots were run to verify that the PP-1 antibody did not pull down PP-2A (Supplemental Fig. 1). Final values are normalized to western data to ensure equal protein for each sample.

Data are expressed as the group means ± S.E.M. Statistical evaluations were performed according to ANOVA. A significance level of $p \leq 0.05$ was used for all tests.

Results

Traumatic brain injury causes a specific decrease in the phosphorylation of DARPP-32 at threonine-34 within the striatum

Following a TBI there is an acute decrease in DA tissue levels and chronic increase (Huger and Patrick, 1979; Dunn-Meynell et al., 1994; McIntosh et al., 1994; Massucci et al., 2004). Alterations in DA have been shown to affect the phosphorylation state of DARPP-32 in MSNs (Halpain et al., 1990; Nishi et al., 2002). We therefore examined whether, following a TBI, there was a change in DARPP-32 phosphorylation state at the Thr34 and Thr75 phosphorylation sites (Fig. 1). TBI caused an acute decrease in the phosphorylation of DARPP-32 at Thr34 (p-DARPP-32-Thr34) as assessed utilizing western blots (Fig. 1D). This decrease in p-DARPP-32-Thr34 persisted through all timepoints examined in both the ipislateral and contralateral, relevant to injury, striatum (Fig. 1D). The decrease in p-DARPP-32-Thr34 was not due to changes in DARPP-32 expression (Fig. 1B). Furthermore, this was a site specific alteration that was not accompanied by any change in the phosphorylation state of the Thr75 site (p-DARPP-32-Thr75) (Fig. 1C), indicating that the loss of p-DARPP-32-Thr34 was not due to non-specific increases in phosphatase activity upon DARPP-32. To assess the regionality of this alteration we also examined the frontal cortex. Examination of the frontal cortex demonstrated decreases in p-DARPP-32-Thr34 at 1 week and 2 weeks post injury, however the decrease in p-DARPP-32-Thr34 was only identified in the ipsilateral cortex, relative to injury, did not appear acutely, and did not persist past 2 weeks. No alterations in DARPP-32 protein expression or p-DARPP-32-Thr75 were appreciated in the frontal cortex (data not shown).

Visual analysis of DARPP-32 phosphorylation following traumatic brain injury

Utilizing DAB immunohistochemistry of free-floating rat striatal slices we confirmed that there was no difference in DARPP-32 expression within the MSNs in sham versus injury (Fig. 2) Furthermore, immuno-fluorescence co-localization visually confirmed the decreases in p-DARPP-32-Thr34 identified by western blot analysis (Fig. 3) within the striatum and qualitatively showed decreases in both the cell soma and surrounding neuropil (Fig. 3).

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Protein phosphatase-1 activity is increased following traumatic brain injury

The phosphorylation state of DARPP-32 directly regulates protein phosphatase-1 (PP-1) activity (Hemmings and Greengard, 1986). Increases in p-DARPP-32-Thr34 lead to a decrease in PP-1 activity while decreases in p-DARPP-32-Thr34 lead to an increase in PP-1 activity (Hemmings et al., 1990). Given the decrease in p-DARPP-32-Thr34 seen following injury we analyzed PP-1 activity utilizing a modified protein phosphatase 2A activity assay (Millipore) to determine the effect of decreased p-DARPP-32-Thr34 on PP-1 activity following TBI (Fig. 4). There was a significant increase in PP-1 activity in injured ipsilateral striatum compared to sham at both 1 day and 2 weeks following injury (Fig. 4). At 1 day following injury there was also a significant increase in PP-1 activity in the contralateral injured striatum compared to sham that was not seen at 2 weeks post injury (Fig. 4). Increases in PP-1 activity are consistent with the loss of p-DARPP-32-Thr34 seen following TBI indicating that decreases in p-DARPP-32-Thr34 after TBI are part of a persistent dysfunction in intracellular signaling cascades.

Chronic amantadine treatment post traumatic brain injury attenuates the alteration in the striatal DARPP-32 signaling cascade

Clinically AMH has shown benefit in both Parkinson's disease (Brenner et al., 1989) and TBI (Kraus et al., 2005). AMH has also shown beneficial effects in the CCI model of TBI (Dixon et al., 1999), showing an improvement in Morris water maze following 2 weeks of treatment. However, it remains unclear what the exact mechanisms of AMH benefit in TBI is. Given AMH's effects on DA and glutamate DARPP-32 represents a potential signaling molecule for AMH action. We therefore analyzed the effect of AMH on DARPP-32 following TBI (Fig. 5). 2 weeks of AMH given daily (10 mg/kg i.p.), as described previously (Dixon et al., 1999), demonstrated an increase in p-DARPP-32-Thr34 over the level in sham animals in both the ipsilateral and contralateral striatum (Fig. 5B). There was also a significant reduction in p-DARPP-32-Thr75 with chronic AMH treatment compared to both sham and injured striatum (Fig. 5C). This is consistent with the proposed mechanisms of AMH activity as both a partial DAergic agonist and partial NMDA receptor antagonist. In particular, partial inhibition of NMDA receptors should reduce Ca^{2+} signaling within the MSN thus leading to an increase in DARPP-32 phosphorylation at Thr34 while leading to a decrease in DARPP-32 phosphorylation at Thr75. To determine whether the attenuation of p-DARPP-32-Thr34 decreases was functionally significant the effect of daily AMH treatment for 2 weeks post injury on PP-1 activity was assessed. Rats treated with AMH daily (10 mg/kg i.p.) for 2 weeks showed significantly reduced PP-1 activity compared to animals receiving vehicle (saline injection) (Fig. 5D). There was no effect of AMH treatment on DARPP-32 protein expression.

Discussion

Multiple lines of evidence have previously implicated DA as an important player in persistent cognitive dysfunction following TBI (Bales et al., 2009). Animal studies have consistently demonstrated benefits in post-TBI cognitive tasks including learning and memory following the administration of DAergic agonists (Dixon et al., 1999; Kline et al., 2004; Wagner et al., 2005). Striatal dysfunction contributes to executive function, memory, and learning difficulties in a number of disorders including Parkinson's disease (Sawamoto et al., 2007), Huntington's disease (Peinemann et al., 2005), and schizophrenia (Merims and Freedman, 2008; Koch et al., 2008). DARPP-32 has been implicated in each of these disorders (Hakansson, et al., 2004; Lang et al., 2007).

We showed that a CCI model of TBI induces a decrease in the phosphorylation of DARPP-32 at Thr34 within striatal MSN that occurs acutely and persists for all examined

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timepoints. The decrease in phosphorylation at Thr34 is not due to downregulation or loss of DARPP-32 protein (Fig. 1), nor is it indicative of non-specific dephosphorylation of DARPP-32 as indicated by the lack of change in Thr75 phosphorylation (Fig. 1). While loss of Thr34 phosphorylation is primarily observed in the striatum, it does become apparent in the FC at later timepoints indicating that the affect of TBI on the phosphorylation state of DARPP-32 is ubiquitous, with the striatum being the most profoundly affected.

We had predicted that a decrease in the phosphorylation state of DARPP-32 at Thr34 would be coupled with an increase in phosphorylation at Thr75. TBI is associated with increased glutamate signaling acutely (Dekosky et al., 1998; Palmer et al., 1993), and deficits in DA signaling chronically (Wagner et al., 2005, 2009). DARPP-32 phosphorylation is dictated by glutamatergic and DAergic alterations (Fernandez et al., 2006; Valjent et al., 2005). Increased glutamate typically leads to decreased Thr34 phosphorylation and increased Thr75 phosphorylation through its effects on $Ca2^{+}$ influx (Fernandez et al., 2006). DA, acting on the D1 receptor, causes the opposite response in the DARPP-32 system compared to glutamate (Bateup et al., 2008, Bertran-Gonzalez et al., 2008). A decrease in p-DARPP-32-Thr34 is consistent with an increase in glutamate acutely and a chronic decrease in DA. However, we did not see an increase in p-DARPP-32-Thr75 as would also be predicted. This is likely a consequence of alterations in multiple signaling systems affected by a TBI including GABAergic and adrenergic signaling pathways. Assessing the contributions of these separate systems is an important goal for future research.

The loss of p-DARPP-32-Thr34 seen with western blot analysis (Fig. 1) appears to be due to losses in phosphorylation throughout the striatal architecture. Visual analysis of confocal co-localization of p-DARPP-Thr34 with NeuN shows that there is a decrease in p-DARPP-32-Thr34 in both the cell soma and throughout the neuropil (Fig. 3). The loss in p-DARPP-32-Thr34 immunostaining does not appear to be due to a loss in DARPP-32 neurons (Fig. 2). Future analysis of cellular compartments including nuclear and cytoplasmic fractions will need to be done to quantitatively confirm that the alteration in DARPP-32 phosphorylation is not localized to a particular cellular compartment.

Consistent with the alteration in DARPP-32 phosphorylation there is a significant increase in PP-1 activity at examined timepoints (Fig. 4), indicating that the loss of Thr34 phosphorylation is associated with an alteration in relevant downstream DARPP-32 signaling. Increased PP-1 activity leads to a decrease in p-CREB and subsequent reduction in nuclear transcription of numerous proteins including neurotrophic factors and cell survival signals (Genoux et al., 2002; Choe et al., 2004). Furthermore, PP-1 activity is important to the regulation of glutamatergic signaling and synaptic plasticity (Centonze, et al., 2001; Kopnisky et al., 2003). Our data indicates that though contralateral effects on DARPP-32 phosphorylation persist until chronic timepoints (Fig. 1D), the increase in PP-1 activity recovers at later timepoints in contralateral tissue (Fig. 4). There are a number of potential explanations for this including changes in kinase activity. In particular, alterations in protein kinase A activity upstream may be able to alter PP-1 signaling in a relatively intact contralateral striatum compared to the ipsilateral striatum. Initial analysis of protein kinase A activity in the CCI model suggests that at chronic timepoints there is an increase in activity that is not seen acutely (data not shown). Furthermore, evoked DA release in the ipsilateral striatum, post TBI, is chronically impaired when compared to the contralateral striatum (Wagner et al., 2005).

One limitation to our analysis of PP-1 activity was the use of only one PP-1 isoform, PP-1δ. While the PP-1δ, also known as PP-1β, isoform is expressed within the rat brain (Shima et al., 1993), there are multiple isoforms which demonstrate significant striatal expression (da Cruz e Silva et al., 1995). However antibodies directed against multiple isoforms of PP-1

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were unable to pull down PP-1 exclusively without also binding PP-2a. Furthermore, antibodies against PP-1(α and γ), while being specific for PP-1 did not show any activity in our assay even under baseline conditions. To our knowledge there is no research demonstrating differences in isoform activity within the striatum relevant to DARPP-32 signaling and examination of their structure shows nearly identical catalytic domains (Andreassen et al., 1998). However, there may be specific alterations in isoform activity that we cannot account for given our examination.

DARPP-32 acts as a signal transduction molecule with that responds to multiple neurotransmitter systems. DA, glutamate, acetylcholine, and calcium signaling all play a role in modulating DARPP-32 within the striatum (Hamada et al., 2005; Valjent et al., 2005; Ahn et al., 2007). Following TBI there are well documented increases in glutamate release and calcium influx that occur almost immediately following injury and contribute to persistent cellular dysfunction (Hovda et al., 1990; Shapira et al., 1989; Fineman et al., 1993).

However, a number of possible mechanisms exist that could explain the alteration in DARPP-32 signaling including specific changes in neurotransmitter systems, such as a decrease in DA signaling or increases in glutamatergic release. It is also possible that neurotransmitter independent intracellular events such as calcium release or changes in phosphatase and kinase activity can contribute to this alteration. Given the complexities of TBI, the identification of acute and persistent DARPP-32 alterations after TBI is most likely indicative of multiple ongoing disease processes. Treatments aimed at molecular targets that are integrative, such as DARPP-32, may allow for therapeutic interventions that target multiple mechanisms of cognitive dysfunction following a TBI.

We have shown that AMH hydrochloride, a partial NMDA antagonist with catecholaminergic effects, can effectively restore sham levels of p-DARPP-32-Thr34 in injured animals (Fig. 5). AMH improves cognitive function in CCI injured animals (Dixon et al., 1999). The exact mechanism of AMH's action following a TBI is unclear. Here we demonstrate that chronically (Fig. 5B) AMH is able to prevent the loss of p-DARPP-32-Thr34 following a TBI. Furthermore, the increase in p-DARPP-32-Thr34 over vehicle treated animals, with 2 weeks of (10 mg/kg i.p.) AMH corresponds with significantly less PP-1 activity compared to injury (Fig. 5D). Chronic AMH treatment also showed significantly less p-DARPP-32-Thr75, compared to vehicle treated sham and injured animals (Fig. 5C). There is no effect of AMH treatment on DARPP-32 protein levels. Our results suggest that the effect of AMH on striatal signaling post TBI is most likely a combination of DAergic effects and NMDA antagonism. A shift of p-DARPP-32 towards Thr34 phosphorylation and concurrent decrease in Thr75 phosphorylation is consistent with reduced Ca^{2+} signaling due to NMDA antagonism and activation of adenylyl cyclase via DA receptor D1 (Valjent et al., 2005; Nishi et al., 2002). We have demonstrated that reversing the TBI induced p-DARPP-32-Thr34 decrease is associated with a reversal of the increase in PP-1 activity. However, we cannot be certain that AMH benefits when given post TBI are acting through DARPP-32. Future experiments examining DARPP-32 knockout animals will need to be done to verify that the alterations in DARPP-32 signaling seen with AMH treatment after a TBI lead to functionally relevant outcomes.

Conclusion

This study provides the first evidence of a TBI induced alteration in DARPP-32 signaling in the striatum of injured animals. The data presented indicate that the decrease in p-DARPP-32-Thr34 following TBI is associated with a functionally relevant alteration in downstream PP-1 activity and is relatively striatal specific. AMH, associated with

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improvements in cognitive and motor performance when given following a TBI, reduces the loss of p-DARPP-32-Thr34 seen with injury and leads to a decrease in PP-1 activity compared to untreated injured animals. The present results further implicate persistent striatal cellular dysfunction following TBI. This study is the first to provide an initial analysis of the feasibility of targeting DARPP-32 striatal dysfunction with clinically relevant therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Ahn JH, Sung JY, McAvoy T, Nishi A, Janssens V, Goris J, Greengard P, Nairn AC. The B′/PR72 subunit mediates $Ca2^{+}$–dependent dephosphorylation of DARPP-32 by protein phosphatase 2A. Proc Natl Acad Sci USA. 2007; 104:9876–9881. [PubMed: 17535922]

Andreassen PR, Lacroix FB, Villa-Moruzzi E, Margolis RL. Differential subcellular localization of protein phosphatase-1 alpha, gamma1, and delta isoforms during both interphase and mitosis in mammalian cells. J Cell Biol. 1998; 141:1207–1215. [PubMed: 9606212]

Baldo BA, Kelley AE. Discrete neurochemical coding of distinguishable motivational processes: insights from nucleus accumbens control of feeding. Psychopharmacology (Berl). 2007; 191:439–459. [PubMed: 17318502]

Bales JW, Wagner AK, Kline AE, Dixon CE. Persistent cognitive dysfunction after traumatic brain injury: a dopamine hypothesis. Neurosci Biobehav Rev. 2009; 33:981–1003. [PubMed: 19580914]

Bateup HS, Svenningsson P, Kuroiwa M, Gong S, Nishi A, Heintz N, Greengard P. Cell type-specific regulation of DARPP-32 phosphorylation by psychostimulant and antipsychotic drugs. Nat Neurosci. 2008; 11:932–939. [PubMed: 18622401]

Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamales M, Herve D, Valjent E, Girault JA. Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J Neurosci. 2008; 28:5671–5685. [PubMed: 18509028]

Brenner M, Haass A, Jacobi P, Schimrigk K. Amantadine sulphate in treating Parkinson's disease: clinical effects, psychometric tests and serum concentrations. J Neurol. 1989; 236:153–156. [PubMed: 2709063]

Calabresi P, Picconi B, Tozzi A, Di Filippo M. Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci. 2007; 30:211–219. [PubMed: 17367873]

Centonze D, Picconi B, Gubellini P, Bernardi G, Calabresi P. Dopaminergic control of synaptic plasticity in the dorsal striatum. Eur J Neurosci. 2001; 13:1071–1077. [PubMed: 11285003]

Choe ES, Parelkar NK, Kim JY, Cho HW, Kang HS, Mao L, Wang JQ. The protein phosphatase 1/2A inhibitor okadaic acid increases CREB and Elk-1 phosphorylation and c-fos expression in the rat striatum in vivo. J Neurochem. 2004; 89:383–390. [PubMed: 15056282]

Coccurello R, Breysse N, Amalric M. Simultaneous blockade of adenosine A2A and metabotropic glutamate mGlu5 receptors increase their efficacy in reversing Parkinsonian deficits in rats. Neuropsychopharmacology. 2004; 29:1451–1461. [PubMed: 15039773]

da Cruz e Silva EF, Fox CA, Ouimet CC, Gustafson E, Watson SJ, Greengard P. Differential expression of protein phosphatase 1 isoforms in mammalian brain. J Neurosci. 1995; 15:3375–3389. [PubMed: 7751917]

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DeKosky ST, Kochanek PM, Clark RS, Ciallella JR, Dixon CE. Secondary injury after head trauma: subacute and long-term mechanisms. Semin Clin Neuropsychiatry. 1998; 3:176–185. [PubMed: 10085205]

Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL. Acontrolled cortical impact model of traumatic brain injury in the rat. J Neurosci Methods. 1991; 39:253–262. [PubMed: 1787745]

Dixon CE, Kraus MF, Kline AE, Ma X, Yan HQ, Griffith RG, Wolfson BM, Marion DW. Amantadine improves water maze performance without affecting motor behavior following traumatic brain injury in rats. Restor Neurol Neurosci. 1999; 14:285–294. [PubMed: 12671249]

Donnemiller E, Brenneis C, Wissel J, Scherfler C, Poewe W, Riccabona G, Wenning GK. Impaired dopaminergic neurotransmission in patients with traumatic brain injury: a SPECT study using 123I-beta-CIT and 123I-IBZM. Eur J Nucl Med. 2000; 27:1410–1414. [PubMed: 11007526]

Dunn-Meynell A, Pan S, Levin BE. Focal traumatic brain injury causes widespread reductions in rat brain norepinephrine turnover from 6 to 24 h. Brain Res. 1994; 660:88–95. [PubMed: 7828006]

Fernandez E, Schiappa R, Girault JA, Le Novere N. DARPP-32 is a robust integrator of dopamine and glutamate signals. PLoS Comput Biol. 2006; 2:1619–1633.

Fienberg AA, et al. DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science. 1998; 281:838–842. [PubMed: 9694658]

Fineman I, Hovda DA, Smith M, Yoshino A, Becker DP. Concussive brain injury is associated with a prolonged accumulation of calcium: a 45Ca autoradiographic study. Brain Res. 1993; 624:94–102. [PubMed: 8252419]

Genoux D, Haditsch U, Knobloch M, Michalon A, Storm D, Mansuy IM. Protein phosphatase 1 is a molecular constraint on learning and memory. Nature. 2002; 418:970–975. [PubMed: 12198546]

Greenamyre JT, O'Brien CF. N-methyl-D-aspartate antagonists in the treatment of Parkinson's disease. Arch Neurol. 1991; 48:977–981. [PubMed: 1835370]

Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron. 1999; 23:435–447. [PubMed: 10433257]

Hakansson K, Lindskog M, Pozzi L, Usiello A, Fisone G. DARPP-32 and modulation of cAMP signaling: involvement in motor control and levodopa-induced dyskinesia. Parkinsonism Relat Disord. 2004; 10:281–286. [PubMed: 15196506]

Halpain S, Girault JA, Greengard P. Activation of NMDA receptors induces dephosphorylation of DARPP-32 in rat striatal slices. Nature. 1990; 343:369–372. [PubMed: 2153935]

Hamada M, Hendrick JP, Ryan GR, Kuroiwa M, Higashi H, Tanaka M, Nairn AC, Greengard P, Nishi A. Nicotine regulates DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa) phosphorylation at multiple sites in neostriatal neurons. J Pharmacol Exp Ther. 2005; 315:872–878. [PubMed: 16040813]

Hemmings HC Jr, Greengard P. DARPP-32, a dopamine-regulated phosphoprotein. Prog Brain Res. 1986; 69:149–159. [PubMed: 3328873]

Hemmings HC Jr, Nairn AC, Elliott JI, Greengard P. Synthetic peptide analogs of DARPP-32 (Mr 32,000 dopamine- and cAMP-regulated phosphoprotein), an inhibitor of protein phosphatase-1. Phosphorylation, dephosphorylation, and inhibitory activity. J Biol Chem. 1990; 265:20369–20376. [PubMed: 2173704]

Hovda DA, Yoshino A, Kawamata T, Katayama Y, Fineman I, Becker DP. The increase in local cerebral glucose utilization following fluid percussion brain injury is prevented with kynurenic acid and is associated with an increase in calcium. Acta Neurochir Suppl (Wien). 1990; 51:331–333. [PubMed: 2089930]

Huger F, Patrick G. Effect of concussive head injury on central catecholamine levels and synthesis rates in rat brain regions. J Neurochem. 1979; 33:89–95. [PubMed: 458474]

Kline AE, Yan HQ, Bao J, Marion DW, Dixon CE. Chronic methylphenidate treatment enhances water maze performance following traumatic brain injury in rats. Neurosci Lett. 2000; 280:163–166. [PubMed: 10675786]

Kline AE, Massucci JL, Ma X, Zafonte RD, Dixon CE. Bromocriptine reduces lipid peroxidation and enhances spatial learning and hippocampal neuron survival in a rodent model of focal brain trauma. J Neurotrauma. 2004; 21:1712–1722. [PubMed: 15684763]

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Kobori N, Clifton GL, Dash PK. Enhanced catecholamine synthesis in the prefrontal cortex after traumatic brain injury: implications for prefrontal dysfunction. J Neurotrauma. 2006; 23:1094–1102. [PubMed: 16866622]

Koch K, Wagner G, Nenadic I, Schachtzabel C, Schultz C, Roebel M, Reichenbach JR, Sauer H, Schlösser RG. Fronto-striatal hypoactivation during correct information retrieval in patients with schizophrenia: an fMRI study. Neuroscience. 2008; 153:54–62. 22. [PubMed: 18359576]

Kochanek PM. Ischemic and traumatic brain injury: pathobiology and cellular mechanisms. Crit Care Med. 1993; 21:S333–S335. [PubMed: 8395991]

Kopnisky KL, Chalecka-Franaszek E, Gonzalez-Zulueta M, Chuang DM. Chronic lithium treatment antagonizes glutamate-induced decrease of phosphorylated CREB in neurons via reducing protein phosphatase 1 and increasing MEK activities. Neuroscience. 2003; 116:425–435. [PubMed: 12559097]

Kraus MF, Smith GS, Butters M, Donnell AJ, Dixon E, Yilong C, Marion D. Effects of the dopaminergic agent and NMDA receptor antagonist amantadine on cognitive function, cerebral glucose metabolism and D2 receptor availability in chronic traumatic brain injury: a study using positron emission tomography (PET). Brain Inj. 2005; 19:471–479. [PubMed: 16134735]

Lang UE, Puls I, Muller DJ, Strutz-Seebohm N, Gallinat J. Molecular mechanisms of schizophrenia. Cell Physiol Biochem. 2007; 20(6):687–702. [PubMed: 17982252]

Massucci JL, Kline AE, Ma X, Zafonte RD, Dixon CE. Time dependent alterations in dopamine tissue levels and metabolism after experimental traumatic brain injury in rats. Neurosci Lett. 2004; 372:127–131. [PubMed: 15531102]

McAllister TW, Flashman LA, Sparling MB, Saykin AJ. Working memory deficits after traumatic brain injury: catecholaminergic mechanisms and prospects for treatment – a review. Brain Inj. 2004; 18:331–350. [PubMed: 14742148]

McIntosh TK, Yu T, Gennarelli TA. Alterations in regional brain catecholamine concentrations after experimental brain injury in the rat. J Neurochem. 1994; 63:1426–1433. [PubMed: 7931293]

Merims D, Freedman M. Cognitive and behavioural impairment in Parkinson's disease. Int Rev Psychiatry. 2008; 20(4):364–373. [PubMed: 18925485]

Nairn AC, Svenningsson P, Nishi A, Fisone G, Girault JA, Greengard P. The role of DARPP-32 in the actions of drugs of abuse. Neuropharmacology. 2004; 47(Suppl 1):14–23. [PubMed: 15464122]

Nishi A, Bibb JA, Matsuyama S, Hamada M, Higashi H, Nairn AC, Greengard P. Regulation of DARPP-32 dephosphorylation at PKA- and Cdk5-sites by NMDA and AMPA receptors: distinct roles of calcineurin and protein phosphatase-2A. J Neurochem. 2002; 81:832–841. [PubMed: 12065642]

Osteen CL, Giza CC, Hovda DA. Injury-induced alterations in N-methyl-D-aspartate receptor subunit composition contribute to prolonged 45calcium accumulation following lateral fluid percussion. Neuroscience. 2004; 128:305–322. [PubMed: 15350643]

Palmer AM, Marion DW, Botscheller ML, Swedlow PE, Styren SD, DeKosky ST. Traumatic brain injury-induced excitotoxicity assessed in a controlled cortical impact model. J Neurochem. 1993; 61:2015–2024. [PubMed: 7504079]

Peinemann A, Schuller S, Pohl C, Jahn T, Weindl A, Kassubek J. Executive dysfunction in early stages of Huntington's disease is associated with striatal and insular atrophy: a neuropsychological and voxel-based morphometric study. J Neurol Sci. 2005; 239:11–19. 15. [PubMed: 16185716]

Pennartz CM, Kitai ST. Hippocampal inputs to identified neurons in an in vitro slice preparation of the rat nucleus accumbens: evidence for feed-forward inhibition. J Neurosci. 1991; 11:2838–2847. [PubMed: 1679123]

Ridley RM, Cummings RM, Leow-Dyke A, Baker HF. Neglect of memory after dopaminergic lesions in monkeys. Behav Brain Res. 2006; 166:253–262. [PubMed: 16198007]

Sawamoto N, Honda M, Hanakawa T, Aso T, Inoue M, Toyoda H, Ishizu K, Fukuyama H, Shibasaki H. Cognitive slowing in Parkinson disease is accompanied by hypofunctioning of the striatum. Neurology. 2007; 68:1062–1068. [PubMed: 17389313]

Shapira Y, Yadid G, Cotev S, Shohami E. Accumulation of calcium in the brain following head trauma. Neurol Res. 1989; 11:169–172. [PubMed: 2573853]

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Shima H, Hatano Y, Chun YS, Sugimura T, Zhang Z, Lee EY, Nagao M. Identification of PP1 catalytic subunit isotypes PP1 gamma 1, PP1 delta and PP1 alpha in various rat tissues. Biochem Biophys Res Commun. 1993; 192:1289–1296. [PubMed: 7685164]

Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P. DARPP-32: an integrator of neurotransmission. Annu Rev Pharmacol Toxicol. 2004; 44:269–296. [PubMed: 14744247]

Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, Greengard P, Herve D, Girault JA. Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc Natl Acad Sci USA. 2005; 102:491–496. [PubMed: 15608059]

Vertes RP. Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience. 2006; 142:1–20. [PubMed: 16887277]

Wagner AK, Chen X, Kline AE, Li Y, Zafonte RD, Dixon CE. Gender and environmental enrichment impact dopamine transporter expression after experimental traumatic brain injury. Exp Neurol. 2005; 195:475–483. [PubMed: 16023635]

Wagner AK, Drewencki LL, Chen X, Santos FR, Khan AS, Harun R, Torres GE, Michael AC, Dixon CE. Chronic methylphenidate treatment enhances striatal dopamine neurotransmission after experimental traumatic brain injury. J Neurochem. 2009; 108:986–997. [PubMed: 19077052]

Warden DL, Gordon B, McAllister TW, Silver JM, Barth JT, Bruns J, Drake A, Gentry T, Jagoda A, Katz DI, Kraus J, Labbate LA, Ryan LM, Sparling MB, Walters B, Whyte J, Zapata A, Zitnay G. Guidelines for the pharmacologic treatment of neurobehavioral sequelae of traumatic brain injury. J Neurotrauma. 2006; 23:1468–1501. [PubMed: 17020483]

Yan HQ, Yu J, Kline AE, Letart P, Jenkins LW, Marion DW, Dixon CE. Evaluation of combined fibroblast growth factor-2 and moderate hypothermia therapy in traumatically brain injured rats. Brain Res. 2000; 887:134–143. [PubMed: 11134598]

Yan HQ, Ma X, Chen X, Li Y, Shao L, Dixon CE. Delayed increase of tyrosine hydroxylase expression in rat nigrostriatal system after traumatic brain injury. Brain Res. 2007; 1134:171–179. [PubMed: 17196177]

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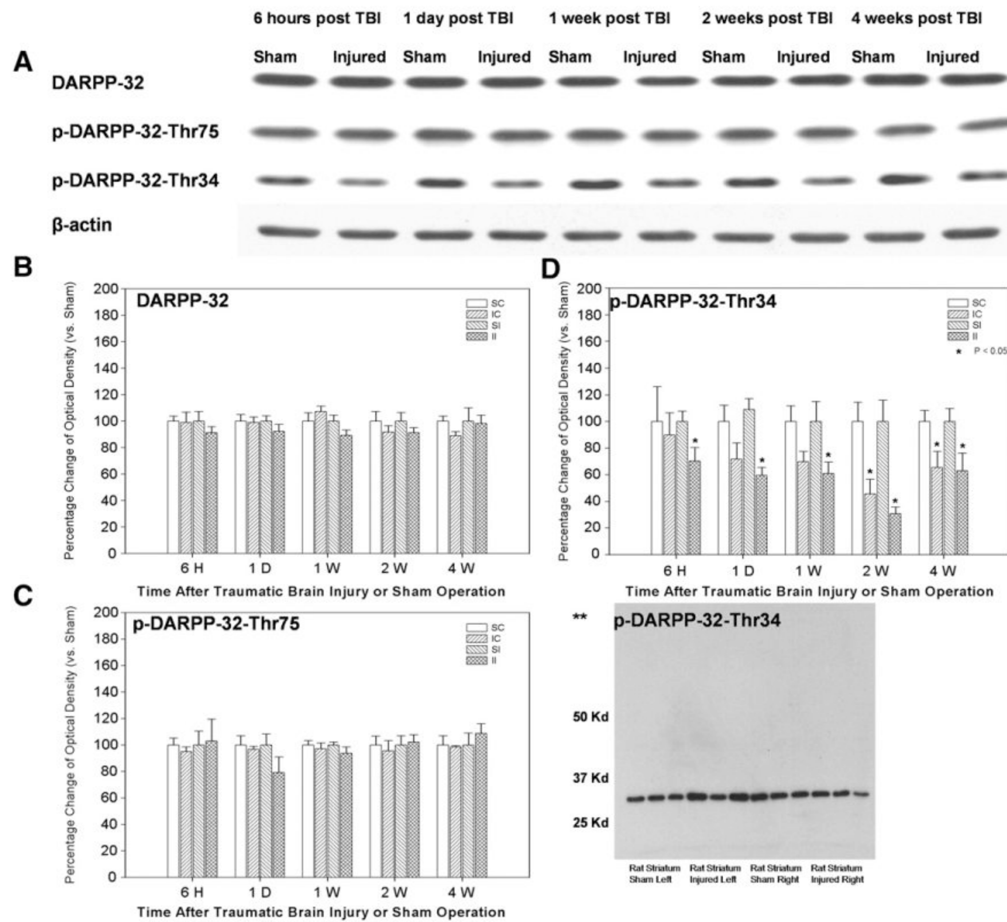

Fig. 1.

Timecourse of TBI effect on DARPP-32 protein expression and phosphorylation state in rat striatum (N = 6 per group at each timepoint). A, representative western blot timecourse of DARPP-32, p-DARPP-32-Thr75, and p-DARPP-32-Thr34 protein expression. B, optical density of DARPP-32 showing no change in DARPP-32 protein expression at any examined timepoint post TBI. C, optical density of p-DARPP-32-T75 showing no significant change in p-DARPP-32-T75 following TBI. D, optical density of p-DARPP-32-Thr34 showing a significant reduction in p-DARPP-32-T34 in the striatum ipsilateral to injury at all timepoints examined and in the striatum contralateral to injury at 1 day-4 weeks post injury. **Inset image: control western with p-DARPP-32-Thr34 antibody demonstrating a single band on rat striatal tissue. *$p \leq 0.05$ normalized to β-actin and compared to sham; ANOVA. Data represented as a percentage of sham following normalization to β-actin ± S.E.M. Abbreviations: SC = sham contralateral to injury; IC = injured contralateral; SI = sham ipisilateral to injury; II = injured ipsilateral; S = sham; I = injured.

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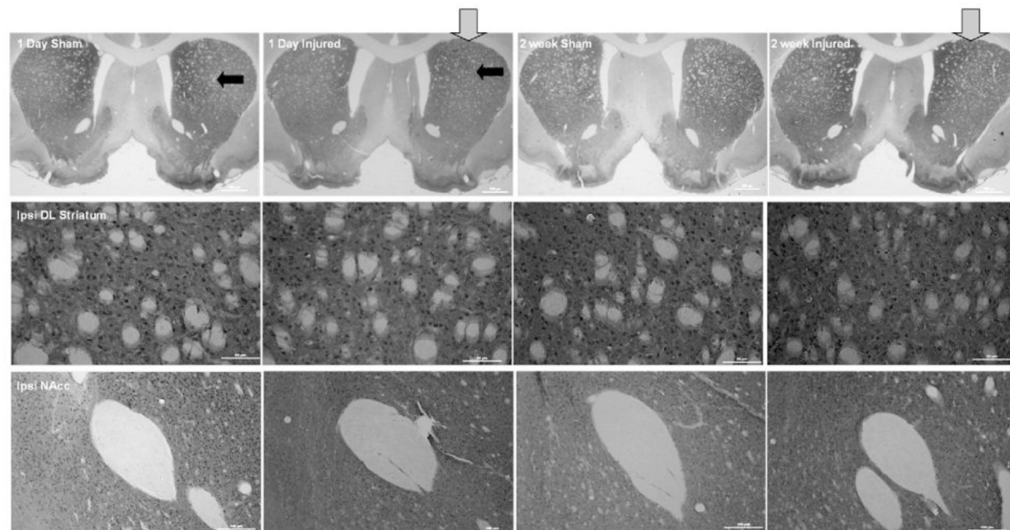

Fig. 2.
Timecourse of TBI effect on DARPP-32 DAB immunoreactivity in rat striatum (N = 3 per group at each timepoint; boxes indicate area of higher power view shown below). Representative sections of rat striatum at 1 day and 2 weeks post injury showing no effect of TBI on DARPP-32 expression in either the DLStr or Nacc. Abbreviations: Ipsi = ipsilateral to injury; DL = dorsolateral; NAcc = nucleus accumbens.

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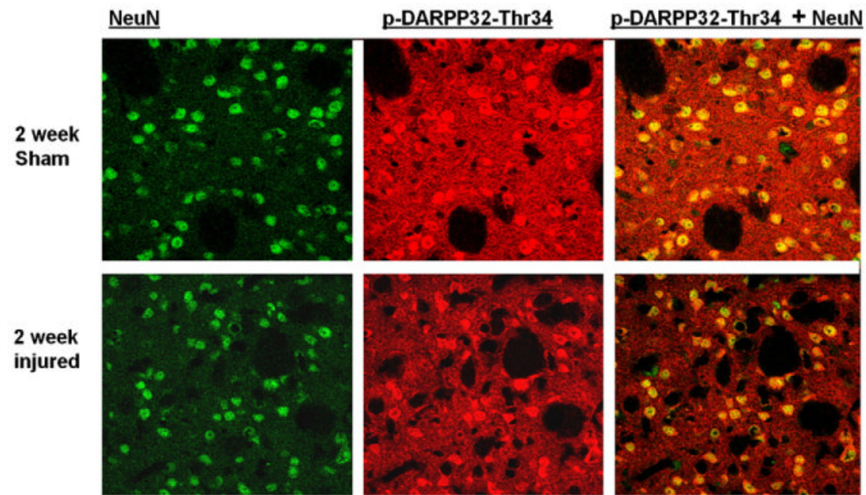

Fig. 3.

Representative microscopic photos (N = 4 per group) of double-labeled immunofluorescent staining for p-DARPP-32-Thr34 (red, Alexa Fluor 594), and neuronal marker NeuN (green, Alexa 488) visually illustrates that TBI causes a decreased expression of p-DARPP-32-Thr34 in the ipsilateral dorsolateral striatum compared to the sham controls in rats. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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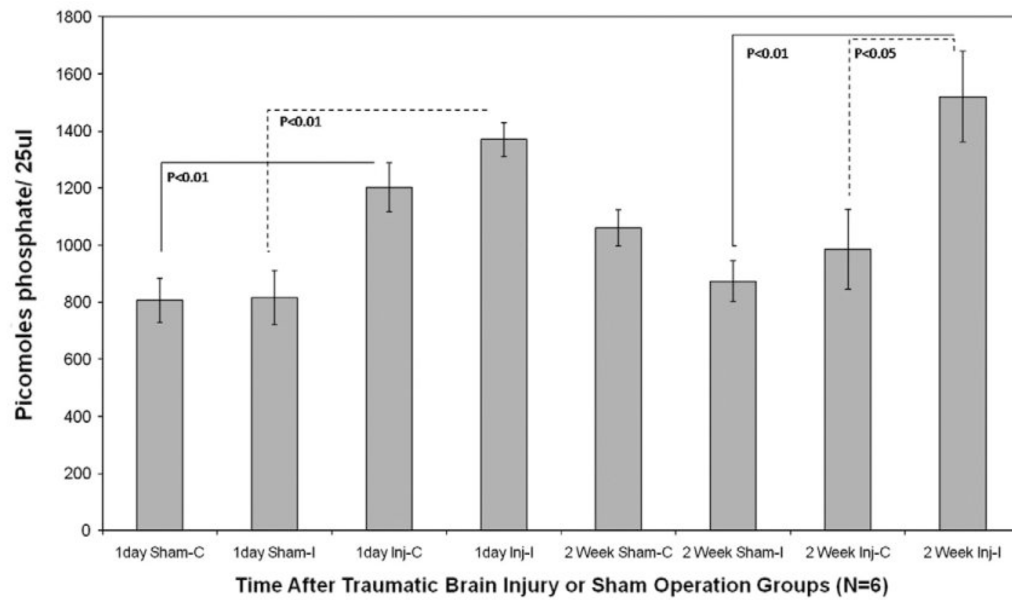

Fig. 4.

Analysis of protein phosphatase-1 (PP-1) activity in homogenized rat striatum. There is a significant increase in PP-1 activity in both the contralateral (N = 6/group; $p < 0.01$; ANOVA) and ipsilateral (N = 6/group; $p < 0.01$; ANOVA) striatum compared to sham at 1 day post injury. By 2 weeks post injury there remains a significant increase in PP-1 activity in the ipsilateral (N = 6/group; $p < 0.01$; ANOVA) striatum compared to sham. Increases in PP-1 activity are consistent with decreases in p-DARPP-32-T34. Data represents means ± S.E.M.

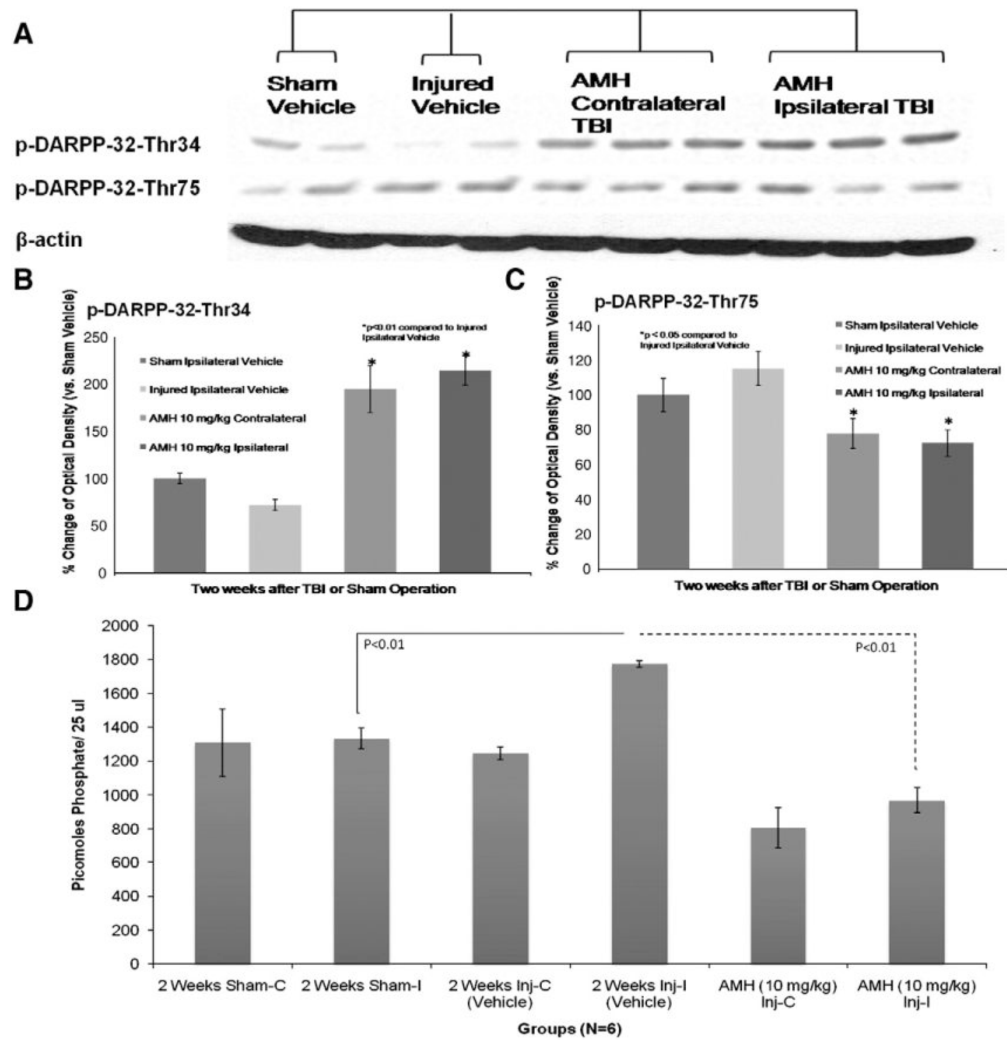

Fig. 5.

Analysis of AMH effects on DARPP-32 signaling post TBI. A, representative western blot of ipsilateral striatum following 2 weeks of 10 mg/kg i.p. AMH on p-DARPP-32-Thr34, p-DARPP-32-Thr75, and β-actin control. B, C, optical density of p-DARPP-32-Thr34 and p-DARPP-32-Thr75. (N = 6/group; $p < 0.01$ following normalization to β-actin compared to TBI with vehicle; ANOVA). Data represented as a percentage of sham following normalization to β-actin ± S.E.M. D, Effect of chronic AMH (10 mg/kg i.p. daily) on striatal PP-1 activity. Consistent with higher p-DARPP-32-Thr34 levels, following chronic AMH administration there is a relative reduction in PP-1 activity compared to TBI with vehicle. (N = 6/group; $p < 0.01$; ANOVA). Data represents means ± standard deviation. Abbreviations: AMH = amantadine hydrochloride, DARPP-32 = dopamine and cAMP regulated phosphoprotein 32; TBI = traumatic brain injury.

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