HHS Public Access

Author manuscript

Mol Neurobiol. Author manuscript; available in PMC 2022 January 01.

Published in final edited form as:

Mol Neurobiol. 2021 January ; 58(1): 362–374. doi:10.1007/s12035-020-02123-w.

Differential regional responses in soluble monomeric alpha synuclein abundance following traumatic brain injury

SW Carlson[1,*], **HQ Yan**[1,*], **Y Li**[1], **J Henchir**[1], **X Ma**[1], **MS Young**[1], **MD Ikonomovic**[2,3], **C.E. Dixon**[1,3,#]

[1]Neurological Surgery, University of Pittsburgh, 4401 Penn Avenue, Pittsburgh, PA 15224, United States of America

[2]Neurology, University of Pittsburgh, 200 Lothrop Street, Pittsburgh, PA 15261, USA

[3]VA Pittsburgh Healthcare System, Pittsburgh, PA, US

Abstract

Alpha synuclein (α-synuclein) is a neuronal protein found predominately in pre-synaptic terminals. While the pathological effects of α-synuclein aggregates has been a topic of intense study in several neurodegenerative conditions, less attention has been placed on changes in monomeric α-synuclein and related physiological consequences on neuronal function. A growing body of evidence supports an important physiological role of α-synuclein in neurotransmission. In the context of traumatic brain injury (TBI), we hypothesized that the regional abundance of soluble monomeric α-synuclein is altered over a chronic time period post-injury. To this end, we evaluated α-synuclein in the cortex, hippocampus and striatum of adult rats at 6 hours, 1 day, 1, 2, 4, and 8 weeks after controlled cortical impact (CCI) injury. Western blot analysis demonstrated decreased levels of monomer α-synuclein protein in the ipsilateral hippocampus at 6 hours, 1 day, 1, 2 and 8 weeks, as well as in the ipsilateral cortex at 1 and 2 weeks and in the ipsilateral striatum at 6 hours after CCI compared to sham animals. Immunohistochemical analysis revealed lower α-synuclein and a modest reduction in synaptophysin staining in the ipsilateral hippocampus at 1 week after CCI compared to sham animals, with no evidence of intracellular or extracellular a-synuclein aggregates. Collectively, these findings demonstrate that monomeric α-synuclein protein abundance in the hippocampus is reduced over an extensive (acute-to-chronic) post-injury interval.

Corresponding Author: C. Edward Dixon, Ph.D., Neurological Surgery, University of Pittsburgh, 4401 Penn Avenue, Pittsburgh, PA 15224 dixonec@upmc.edu; Telephone (412) 692-6471; Fax (412) 624-0943.

*Co-authors

Authors' Contributions

S.W.C., H.Y., Y.L., J.H., X.M. completed the investigation; S.W.C, M.D.I., C.E.D. completed data curation and analysis; S.W.C., H.Y., M.Y., M.D.I, and C.E.D drafted, edited, and revised the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of interest: No COI exist.

Declaration of Competing Interest: There are none.

Data availability: The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

This deficit may contribute to the chronically impaired neurotransmission known to occur after TBI.

Keywords

traumatic brain injury; α-synuclein; neurodegeneration; synapse

Introduction

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality with an estimated incidence of approximately 10 million worldwide [1]. TBI can result in memory and cognition impairment, altered states of consciousness, psychiatric disturbances including depression, and physical impairments that may be temporary or progress to permanent disability and contribute to reduced quality of life [2–4]. TBI initiates a complex cascade of pathophysiological changes that includes, among others, excitotoxicity, impaired mitochondrial function, axonal injury, and delayed long-lasting alterations in neurotransmitter release and synaptic function [5–14]. The rat model of controlled cortical impact (CCI) is a widely-utilized and well-established TBI model that produces a progressive cortical contusion and hippocampal synaptic and cell loss, in the dentate gyrus, CA1, CA3, and hilus, that contributes to altered synaptic function [14, 54, 71, 72] and the manifestation of motor and cognitive impairments, including in executive function and spatial learning and memory, in the days and weeks following CCI injury [6, 25, 73, 74, 75].

Synucleins are a family of small, abundant, presynaptic proteins including α-, β-, and γ-synuclein. The physiological role of synucleins in normal neuronal function has been a topic of ongoing investigation. Studies evaluating the structure, presynaptic location, and protein-protein interactions suggest a regulatory and modulatory role in synaptic vesicle trafficking and neurotransmitter release [15–19]. The formation of the N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex is an important step for synaptic vesicle docking and the release of neurotransmitters into the synaptic cleft [20–22]. A critical role for α-synuclein involvement in neurotransmitter release was identified in α-synuclein knockout mice exhibiting impaired SNARE complex formation [18,23], highlighting that a reduction in soluble monomeric α-synuclein may be associated with impaired synaptic function. We have previously demonstrated that SNARE complex formation is reduced in the hippocampus, in two experimental models of TBI, including CCI, in the weeks post-injury [24–26]. Collectively, these reports draw a strong link between α-synuclein abundance and the SNARE protein machinery important for neurotransmission and maintenance of the vesicle pool, providing support that changes in monomeric α-synuclein levels at the synapse contribute to impaired neuron function following TBI.

Accumulating evidence links chronic neurodegenerative proteinopathies including Parkinson disease (PD), Alzheimer's disease (AD), and chronic traumatic encephalopathy (CTE) to TBI-induced altered metabolism of proteins including α-synuclein, amyloid-β, tau, and TDP-43 [27, 28]. In particular for α-synuclein, prior efforts have been focused on understanding pathological forms, with a focus on the striatum and substantia nigra as these

regions are typically associated with PD. Further evidence comes from findings of pathological α-synuclein aggregates in neurons and axons following TBI in humans [31–33]. However little work has been completed to understand the effect of TBI on monomeric levels of α-synuclein. Elevated monomeric α-synuclein was observed in the cerebrospinal fluid (CSF) of adults [29] and infants and children [30] in the week following a severe TBI, highlighting the utility as a biomarker. This knowledge gap highlights the need for studies in experimental TBI to define alterations in monomeric α-synuclein, as these changes can have direct implications in impaired neurotransmission and chronic neurodegeneration.

In the current study, we sought to determine changes in soluble monomeric α-synuclein protein over an extensive time course (6 hours, 1 day, 1, 2, 4 and 8 weeks) after CCI injury in adult rats. The abundance of α-synuclein was assessed by western blot in the cortex hippocampus and striatum, and immunohistochemistry was assessed in the dorsal hippocampus, including CA3, a region of established alterations in SNARE protein abundance following CCI [26].

Materials and methods

Animals

All experimental procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee in accordance with the guidelines established by the National Institutes of Health in the Guide for the Care and Use of Laboratory Animals. A total of 144 adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–300 grams were used for this study. Rats were group-housed (2 per cage) in standard housing cages with 12-h light/dark cycles (light on at 07:00 AM) with access to food and water *ad libitum*.

Surgical Procedures for Controlled Cortical Impact

Animals were randomly assigned to receive either control sham or CCI injury as previously described [34,25]. All rats were anesthetized initially with 4% isoflurane with a 2:1 N_2O/O_2 mixture in a vented anesthesia chamber. Following endotracheal intubation, rats were ventilated mechanically with a 1–1.5% isoflurane mixture. Animals were mounted in a stereotaxic frame on the injury device and secured by ear and incisor bars. The head was held in a horizontal plane with respect to the interaural line. A midline incision was made, the soft tissues reflected, and a 7 mm craniotomy was made between lambda and bregma and centered 5 mm lateral of the central suture. The animals received an impact with a 6 mm diameter flat impactor tip to the exposed dura through the right craniotomy. The injury device was set to produce a tissue deformation of 2.8 mm at a velocity of 4 meters/sec with a dwell time of 150 msec. Control sham rats underwent identical surgical procedures but did not receive a TBI. Core body temperature was monitored continuously throughout surgical procedures by a rectal thermistor probe and maintained at 37 ± 0.5° C with a heating pad. Following sham or CCI injury, the scalp was sutured, anesthesia stopped, and the righting time of each animal was monitored and recorded. Once ambulatory, the animals were returned to their home cage.

Brain tissue preparation for Western blot analysis

A total of 72 rats (n=6 per injury group at each time point) received an overdose of sodium pentobarbital (i.p., 100 mg/kg; Fatal-plus, Vortech Pharmaceuticals, Dearborn, MI) and were euthanized at 6 hours, 1 day, 1, 2, 4, or 8 weeks following sham or CCI injury. The time course of 6 hours to 8 weeks post-injury was selected to assess α-synuclein protein abundance at time points preceding and subsequent to changes in hippocampal SNARE protein abundance described after CCI [25,26]. At the specified time points, brains were quickly removed and placed on a chilled ice plate for regional dissection. Tissues from the ipsilateral and contralateral cortex, hippocampus and striatum were rapidly dissected and immediately frozen in liquid nitrogen and stored at −80°C. Samples were washed with ice cold 0.1 M PBS then homogenized and sonicated in the lysis buffer (0.1M NaCl, 0.01M tris-Cl, 0.001M EDTA, pH 7.6) with protease inhibitor cocktail kit (Pierce, Rockford, IL), and centrifuged at 15,000 ×g for 30 minutes. The supernatant was collected, frozen and used for Western blot assays. The protein concentration was determined by a BCA protein assay kit (Thermo Scientific, Pittsburgh, PA) using a 96-well microplate reader (Biotek, Winooski, VT).

Brain tissue preparation for immunofluorescence and histology

Tissue preparation and immunohistochemistry procedures were completed as previously reported [26]. Briefly, a total of 72 rats (n=6 per injury group for each time point) received an overdose of sodium pentobarbital (i.p., 100mg/kgFatal-plus) at the designated time points between 6 hours to 8 weeks post-injury. Animals were transcardially perfused with USP saline, followed by 10% neutral buffered formalin (Fisher Scientific, Waltham, MA). The brains were post-fixed for an additional 24 hours in 10% neutral buffered formalin and transferred to 15% sucrose in 0.1 M phosphate buffer pH 7.4 at 4°C for 24 hours, switched to 30% sucrose in 0.1 M phosphate buffer pH 7.4 at 4 °C for an additional 24hrs, and once the brain were cryoprotected, frozen for sectioning. The frozen brains were embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA), and 35 μm thick coronal sections were cut using a cryostat (Leica Microsystems Inc., Buffalo Grove, IL). Sections between -−3.24mm and −3.60mm bregma were selected and processed for immunofluorescence and Cresyl violet staining.

Western blot analysis

To evaluate α-synuclein abundance in the ipsilateral and contralateral cortical, hippocampal and striatal tissues from injured or sham rats, samples containing 20 μg of protein were boiled for 10 minutes prior to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) through a 12% gel to separate protein samples and molecular weight markers (Bio-Rad, Hercules, CA). Resolved proteins were electrophoretically transferred to a nitrocellulose membrane. The membrane was blocked in 5% non-fat dry milk in 0.1 M PBS with 0.05% Tween-20 (PBST) at room temperature for one hour, and immunolabeled using a commercially available antibody recognizing monomeric α-synuclein at aa15–123 (mouse monoclonal clone 42/α-synuclein, 1:2,000, BD Biosciences) [18,35–37] with a representative immunoblot staining pattern shown at 6 hours post injury in Figure 1. Primary antibody was incubated at 4°C overnight followed by goat anti-mouse immunoglobulin G

conjugated to peroxidase (1:10,000; PIERCE, Rockford, IL) at room temperature for one hour. Proteins were visualized with a chemiluminescence detection system (SuperSignal, PIERCE, Rockford, IL). To normalize for protein loading, the membranes were stripped and re-blotted with mouse anti-β-actin monoclonal antibody (1:10,000, Sigma, St. Louis, MO). The injured tissue and the sham control were loaded together in the same gel for comparison. Blots were exposed to autoradiographic X-ray film for 10 sec to 1 min and bands were quantitated using SCION ImagePC (Frederick, MD) software. Values are reported as the ratio of optical densities of injured samples, normalized to actin, as a percentage of ipsilateral or contralateral sham control for each hemisphere at every time point.

Cresyl violet and immunofluorescence staining and double labeling

Immunofluorescence staining was completed in free floating coronal hippocampal sections in 24 well plates. Sections were rinsed in 1X PBS and blocked with 10% normal horse serum (NHS; Cat# S-2000,Vector Laboratories, Burlingame, CA) and 0.1% Triton X-100 in 0.1 M PBS. Sections ranging from −3.24mm to −3.60mm bregma were selected and incubated with the same commercially available α-synuclein antibody utilized for immunoblot analysis (mouse monoclonal anti-α-synuclein, 1:1,500, BD Biosciences) with 5% NHS and 0.1% Triton X-100 in 0.1 M PBS overnight at 4°C. For double labeling, sections were incubated with primary anti-α-synuclein antibody, and either rabbit polyclonal anti-NeuN (1:2,500, Millipore) or rabbit polyclonal anti-synaptophysin (1:500, Abcam) antibodies. On the following day, sections were rinsed in 1X PBS and incubated with biotin-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) for 1 hour at room temp, rinsed with 1X PBS, and subsequently incubated with Streptavidin conjugated Alexa Fluor 488 or 594 fluorophores (Molecular Probes) with 5% NHS and 0.1% Triton X-100 in 0.1 M PBS at 4°C for 2 hours on a rocker. Tissues were rinsed between all steps with 0.1% Triton X-100 in 0.1 M PBS three times for at least 10 min in each time. Stained sections were mounted on gelatin subbed slides, cover slipped and stored at 4°C until imaged. To corroborate the immunoblot findings, in the stained immunohistochemical sections hippocampal α-synuclein and synaptophysin immunoreactivity were quantified independently using mean pixel intensity measures with ImageJ (NIH). Briefly, the hippocampus was outlined to designate the region for analysis, and mean pixel value was measured for each animal (n=1 section/animal; n=6/group/time point). To normalize the hippocampal mean pixel intensity measures, at each time point, the measured intensity value for each section was normalized to the calculated mean value for the sham ipsilateral group. The reported values represent sham ipsilateral as 100% and percent difference for each time point. Cresyl violet staining was completed in coronal brain sections at −3.48mm (Sham control) and −3.60mm (CCI) bregma as previously described [24].

Confocal microscopy was performed to visualize changes in hippocampal α-synuclein protein immunoreactivity after CCI, as compared to NeuN and synaptophysin to assess neuronal localization. The 1 week time point was selected for double labeling immunofluorescence staining as α-synuclein protein levels were maximally lower in the hippocampus at this time point by immunoblot. Control experiments, in which primary antibodies were omitted, were completed in parallel to confirm antibody specificity. Images

were acquired on a C2 confocal microscope (Nikon, Melville, NY). Representative images in the figures are maximum intensity projection images collected as a z stack (1.5 μm step size) at 4X or 20X magnification.

Statistical analyses

All quantification was completed by an investigator blinded to the injury conditions of each animal. Western blot data for α-synuclein abundance are presented as the group means ± standard error of the mean (SEM). Western blot data for each hemisphere and region were compared by two-way analysis of variance (ANOVA). When appropriate, a Sidak post hoc multiple comparisons t-test was completed. Mean pixel intensity measures of immunohistochemical staining were analyzed by one-way ANOVA at each time point. When appropriate, a Sidak post hoc multiple comparisons t-test was completed. Statistical tests were completed using Graphpad (Graphpad, La Jolla, CA). A p value less than 0.05 was considered statistically significant for all comparisons.

Results

Western blot analyses of α-synuclein protein in cortex, hippocampus, and striatum of rats subject to CCI

In the ipsilateral cortex, α-synuclein abundance was significantly lower at 1 and 2 weeks following CCI compared to shams (Two-way ANOVA: main injury effect, $p<0.001$ $F(1, 60)=15.21$; main time effect, $p<0.05$ $F(5, 60)=2.559$; interaction, $p<0.05$ $F(5, 60)=2.559$; post hoc t-test, $p<0.05$; Figure 1A). In the contralateral cortex, there were no significant changes in α-synuclein protein except for a non-significant elevation at 8 weeks post-injury (main injury effect $p=0.22$ $F(1, 60)=1.532$; Figure 1B). In the ipsilateral hippocampus, CCI resulted in significantly lower α-synuclein protein abundance at 6 hours, 1 day, 1, 2, and 8 weeks, but not 4 weeks post-injury, as compared to sham injury (Two-way ANOVA: main injury effect, $p<0.0001$ $F(1, 60)=111.5$; post-hoc t-test $p<0.05$; Figure 2A). No significant changes in α-synuclein were observed in the contralateral hippocampus at any time point assessed (Two-way ANOVA: main injury effect, $p=0.07$ $F(1,60)=3.323$; main time effect, $p=0.18$ $F(5, 60)=1.591$; interaction, $p=0.18$ $F(5, 60)=1.592$; Figure 2B). In the ipsilateral striatum, CCI resulted in significantly lower α-synuclein protein levels at 6 hours post-injury, as compared to sham injury (Two-way ANOVA: main injury effect, $p<0.05$ $F(1, 60)=6.34$; post-hoc t-test $p<0.05$; Figure 3A). No significant changes in α-synuclein protein were observed in the contralateral striatum at any time point examined (Figure 3B).

Immunohistochemical analyses of α-synuclein in the hippocampus of rats subject to CCI

Cresyl-violet staining demonstrated a cortical lesion and cell loss in the hippocampal dentate gyrus, CA3 and hilus at 1 week following CCI compared to sham control surgery (Figure 4), as established previously in the unilateral CCI experimental model of TBI. Immunohistochemistry analysis of monomeric α-synuclein showed no evidence of α-synuclein aggregates at time points examined. Our analysis of monomeric α-synuclein immunolabeling in the hippocampus was performed at 1 week following CCI, the time point when α-synuclein protein levels were lowest and reduced SNARE complex formation was observed by immunoblot in our previous studies [25,26]. Consistent with the

immunoblotting results obtained in the entire hippocampus homogenate (described in Figure 2), α-synuclein immunoreactivity was significantly lower in the ipsilateral CCI hippocampus at 1 week after CCI when compared to contralateral CCI hippocampus and sham control (Figure 5A, B; one-way ANOVA; main effect, $p<0.005$ $F(3,20)=9.275$; post-hoc t-test $p<0.05$). In the same sections co-labeled with α-synuclein, immunoreactivity for synaptophysin (marker for pre-synaptic terminals) was also lower in the ipsilateral CCI hippocampus, but this was not significant (one-way ANOVA; main effect $p=0.26$ $F(3,20)=1.451$; Figure 5C). Mean pixel intensity analysis at each time point, shown in Table 1, showed no significant differences between CCI groups at 6 hr (one-way ANOVA: $p=0.1867$, $F(3, 20)=1.762$) or 24 hr (one-way ANOVA: $p<0.05$, $F(3, 20)=3.401$), but did reveal significant reductions in α-synuclein immunoreactivity in the ipsilateral hippocampus after CCI at 1 week (one-way ANOVA: $p<0.005$, $F(3, 20) = 9.275$), 2 weeks (one-way ANOVA: $p<0.001$, $F(3, 20) = 15.18$), 4 weeks (one-way ANOVA: $p<0.001$, $F(3, 20) = 4.972$) and 8 weeks (one-way ANOVA: $p<0.05$, $F(3, 20) = 4.671$) post-injury. Assessment of double-labeled α-synuclein and synaptophysin immunohistochemistry (Figure 6A, B) in the hippocampal CA3 at 1 week post-injury, showed reduced α-synuclein immunoreactive punctate structures and lower immunoreactivity in the CA3 radiatum layer. In the molecular layer, synaptophysin-immunoreactive punctate structures were also reduced. In addition to changes in synaptophysin, reduced NeuN-positive pyramidal neurons, suggestive of neuronal loss, was observed in CA3 (Figure 7A, B) at 1 week post-injury, supporting the observation of histological changes observed in Figure 4.

Discussion

The objective of the current study was to determine the effect of a unilateral TBI on the abundance of soluble monomeric α-synuclein protein over an extensive time course from 6 hours to 8 weeks following CCI injury. While aggregated α-synuclein have been a major focus of interest after TBI, little is known about changes in monomeric α-synuclein forms which play an important role in neurotransmission, and are the precursors of oligomeric and fibrillar pathological aggregates. The findings presented here provide new evidence of deficits in soluble monomeric α-synuclein protein in the ipsilateral cortex, hippocampus and striatum regions which are affected differentially during the course of 8-week survival following TBI.

In the ipsilateral cortex, CCI resulted in lower α-synuclein protein abundance at 1 and 2 weeks post-injury while in the ipsilateral hippocampal lower abundance was seen as early as 6 hours and up to 8 weeks post-injury. Only a handful of studies have evaluated the effect of TBI on α-synuclein in the hippocampus and cortex post-injury. In wild type mice subjected to CCI at 24 months of age, Uryu et al. (2003) reported age related transient increases in α-synuclein immunoreactivity in CA3 and fimbria at 1 and 9 weeks post-injury. Liu et al. [38] reported reduced hippocampal monomeric α-synuclein levels as early as 1 hour and sustained reductions at 1, 3, 5 and 7 days in rats subjected to CCI. Additionally, Liu et al. showed the post-translational regulator of α-synuclein, mR-153, was elevated at each of the test points between 1 hour and 7 days post-injury, suggesting that mR-153 could influence post-translational modifications or the expression of α-synuclein protein. In frontal cortical post-mortem tissue collected from a small group of retired boxers, decreased α-synuclein

concentration was measured in the gray matter by enzyme-linked immunoabsorbance assay [39]. The current immunohistochemical analysis shows lower α-synuclein and synaptophysin immunoreactivity throughout the ipsilateral hippocampus, particularly in the CA3. The findings in this study corroborate and expand the temporal assessments of previous reports to show lower cortical α-synuclein abundance at 1 and 2 weeks post-injury and a more extensive time course of protein loss in the hippocampus, during hours and up to 8 weeks following TBI. Interestingly, our Western blot analysis showed a transient recovery in hippocampal α-synuclein abundance at 4 weeks post-injury. While additional work is warranted to better understand the mechanisms leading to reduced monomeric α-synuclein, this finding could represent a biphasic curve and the combination of synaptic loss and synaptogenesis shown to occur in the hippocampus by ultrastructural analysis completed at time points assessed in the current study [54]. Our current findings also show lower α-synuclein abundance in the ipsilateral striatum, but this change was restricted to the most acute time point measured (6 hours post-injury). The current study design did not specifically address the mechanism underlying the transient striatal reduction in α-synuclein; however, it is possible that this reduction could be the result of post-translational changes in the species of α-synuclein from monomeric to another state that is not detected by the antibody utilized. It may also be necessary to utilize synaptosomal enriched lysates to enhance detection of striatal changes in α-synuclein abundance, as we have previously shown this approach to be sensitive to synaptic protein changes at 1 week post-injury [56]. Previous studies using CCI model reported increased α-synuclein abundance in the substantia nigra and tissue homogenates encompassing multiple midbrain regions at 1 and 2 months after injury in adult wild type CD1 mice [40] and adult Sprague-Dawley rats [41]. The current study did not focus on the substantia nigra, but together with the previous reports our findings in the striatum suggest that components of the nigrostriatal pathway may have differing responses to TBI.

Reductions in the abundance of soluble monomeric α-synuclein have important implications for neuronal function as a growing body of literature supports the physiological role of α-synuclein in presynaptic neurotransmitter release and the maintenance and trafficking of the synaptic vesicle pool [19,18,42,23,43,44]. A-synuclein promotes synaptic vesicle clustering and restricting the mobility of vesicles, which influences the kinetics of vesicular docking and neurotransmitter release [45,19,18]. α-synuclein has also been shown to promote the formation of the SNARE complex, the protein machinery critical for the docking of vesicles at the synaptic cleft [18], through its association with synaptobrevin-2 [46,18,47]. Highlighting the complex role of α-synuclein, there is some debate about the direct effect of α-synuclein in neurotransmitter release, as published studies utilizing multiple *in vitro* and *in vivo* models have shown that genetic modulation of α-synuclein protein levels results in decreased release [42,48–50], enhanced release [44,43,51,52], or no change in release [19,18,23,53]. Provided the intricate role α-synuclein plays in vesicular docking and neurotransmitter release, an improved understanding of the temporal and regional changes in soluble monomeric α-synuclein is essential to understanding and targeting α-synuclein related neurotransmission impairments in the context of neurodegeneration.

We have previously shown that lower soluble monomeric α-synuclein protein levels are associated with reductions in SNARE complex formation in the hippocampus in the days

following CCI or fluid percussion injury [24,25]. Collectively with our current study, these findings suggest that changes in synaptic proteins occur in multiple brains regions and in multiple experimental TBI models, highlighting the need for additional work to understand changes in α-synuclein across the spectrum of injury. We have previously reported CA3 of the dorsal hippocampus as a region of observable alterations in SNARE protein abundance [26]. In the current study, at 1 week post-injury the reduction of α-synuclein immunoreactivity in CA3 of the dorsal hippocampus was more pronounced than the loss of synaptophysin. In comparison to previous reports in the rat CCI model detailing the temporal changes in synaptophysin protein levels of the entire hippocampus [24,25] and a time course ultrastructural assessment of synaptic density in CA1 [54], α-synuclein displays greater reductions in the days following CCI compared to reductions in synaptic density, suggesting that loss of neurons and synapses may not fully account for the reduction in α-synuclein acutely after CCI. However, additional more direct methods may be required, beyond synaptophysin intensity assessments, to determine the contribution of cell loss to changes in α-synuclein abundance. While we did not exclusively assess CA1, as studied by Scheff et al. (2005), the observed changes in hippocampal α-synuclein immunoreactivity may be the result of neuronal loss in CA3 and other hippocampal subregions, and subsequent changes in Schaffer collaterals or other parts of the hippocampal trisynaptic circuit after CCI. Alternatively, considering the magnitude of acute synaptophysin immunoreactivity loss was less than that observed with α-synuclein, the current findings suggest that α-synuclein protein may be more vulnerable or it reflects a change in its conformational and aggregation state [32,55]. It is possible that acute reductions in α-synuclein protein abundance can be attenuated by therapeutic interventions, with the goal of improving neurotransmission. We have previously shown that daily posttraumatic administration of 1.0mmol/kg/d lithium increased hippocampal and striatal monomeric α-synuclein abundance, compared to vehicle treatment, and this was associated with improvements in both striatal dopamine neurotransmission and cognitive performance in the weeks following CCI [56,25]. Additional work is warranted to expand our understanding of the mechanism/s contributing to α-synuclein reductions after TBI, and the identification of therapeutic interventions that can restore α-synuclein abundance to promote recovery of synaptic and neurobehavioral function.

The role of pathologically altered synucleins has been increasingly recognized in the pathogenesis of several chronic neurodegenerative diseases. The spectrum of neurodegenerative disorders associated with aggregation of α-synuclein, which includes but is not limited to PD, dementia with Lewy bodies (DLB), and Lewy body variant of Alzheimer's disease, is collectively referred to as synucleinopathies [57–60]. Insoluble aggregates of α-synuclein are due to α-synuclein gene mutations in familial Parkinson's disease (PD) [61,62], and have pathogenic role as the primary component of Lewy bodies and Lewy neurites in sporadic PD and DLB [63–68].

α-synuclein positive Lewy bodies are also present in approximately 20 percent of autopsy brains diagnosed with chronic traumatic encephalopathy (CTE) [69] and chronic increases in α-synuclein immunoreactivity were reported in the dorsal raphe nucleus of professional athletes diagnosed with CTE [70]. Increased oxidized α-synuclein immunoreactivity and α,β,γ isoform immunoreactivity was observed in a subset of cases who had temporal

cortical biopsy tissue resected acutely (hours to days) after severe TBI, in addition to axonal accumulation of Aβ precursor protein (APP) and diffuse Aβ plaques [32,27]. Increased oxidized α-synuclein immunoreactivity was also observed in damaged neurons in survival times as early as 4 hours and up to 4 weeks in patients with a TBI [33,31]. At 1 week after CCI injury in mice, pathological forms of nitrated, oxidized and conformationally-altered α-synuclein were observed in the cortex and axon tracks of the striatum and corpus callosum in aged animals, but limited immunoreactivity was present in young mice [55]. While the current study was focused on examining TBI-induced changes in the soluble monomeric form of α-synuclein, these previous reports of post-translationally modified α-synuclein forms demonstrate that acute and sustained alterations in α-synuclein occur in human TBI and are recapitulated in experimental TBI models. An improved understanding of TBI induced changes in monomeric α-synuclein, as well as β,γ-synuclein isoforms, may shed light on the pathways leading to development of pathological forms of α-synuclein and their role in chronic neurodegeneration after TBI.

Conclusion

Our results demonstrate that soluble monomeric α-synuclein protein levels are differentially altered in the hippocampus, cortex and striatum in the days to months post-injury in the rat controlled cortical impact model of experimental TBI. These changes may contribute to the disruption of physiological processes in the synapse, impair neuronal transmission, and contribute to chronic neurodegeneration after TBI.

Acknowledgments

Funding: This work was supported by the National Institutes of Health NIH-NS091062 (CED), NIH-NS079061 (CED), NIH-NS111099 (SWC), 1-F32NS090748 (SWC), Veterans Administration Healthcare System VAI01RX001127 (CED) and VAI01RX001778 (MDI/CED), and the Walter L. Copeland Fund of The Pittsburgh Foundation (SWC).

Bibliography

1. Blennow K, Brody DL, Kochanek PM, Levin H, McKee A, Ribbers GM, Yaffe K, Zetterberg H (2016) Traumatic brain injuries. Nature reviews Disease primers 2:16084. doi:10.1038/nrdp.2016.84
2. Truelle JL, Koskinen S, Hawthorne G, Sarajuuri J, Formisano R, Von Wild K, Neugebauer E, Wilson L, Gibbons H, Powell J, Bullinger M, Hofer S, Maas A, Zitnay G, Von Steinbuechel N, Qolibri Task F (2010) Quality of life after traumatic brain injury: the clinical use of the QOLIBRI, a novel disease-specific instrument. Brain injury : [BI] 24 (11):1272–1291. doi:10.3109/02699052.2010.506865
3. Bodnar CN, Morganti JM, Bachstetter AD (2018) Depression following a traumatic brain injury: uncovering cytokine dysregulation as a pathogenic mechanism. Neural regeneration research 13 (10):1693–1704. doi:10.4103/1673-5374.238604 [PubMed: 30136679]
4. Lundin A, de Boussard C, Edman G, Borg J (2006) Symptoms and disability until 3 months after mild TBI. Brain injury : [BI] 20 (8):799–806. doi:10.1080/02699050600744327
5. McIntosh TK, Saatman KE, Raghupathi R, Graham DI, Smith DH, Lee VM, Trojanowski JQ (1998) The Dorothy Russell Memorial Lecture. The molecular and cellular sequelae of experimental traumatic brain injury: pathogenetic mechanisms. Neuropathology and applied neurobiology 24 (4):251–267 [PubMed: 9775390]
6. Xiong Y, Mahmood A, Chopp M (2013) Animal models of traumatic brain injury. Nature reviews Neuroscience 14 (2):128–142. doi:10.1038/nrn3407 [PubMed: 23329160]

7. Kochanek PM (1993) Ischemic and traumatic brain injury: pathobiology and cellular mechanisms. Critical care medicine 21 (9 Suppl):S333–335 [PubMed: 8395991]
8. Bales JW, Kline AE, Wagner AK, Dixon CE (2010) Targeting Dopamine in Acute Traumatic Brain Injury. The open drug discovery journal 2:119–128. doi:10.2174/1877381801002010119 [PubMed: 22308176]
9. Hill RL, Kulbe JR, Singh IN, Wang JA, Hall ED (2018) Synaptic Mitochondria are More Susceptible to Traumatic Brain Injury-induced Oxidative Damage and Respiratory Dysfunction than Non-synaptic Mitochondria. Neuroscience 386:265–283. doi:10.1016/j.neuroscience.2018.06.028 [PubMed: 29960045]
10. Povlishock JT, Becker DP, Cheng CL, Vaughan GW (1983) Axonal change in minor head injury. Journal of neuropathology and experimental neurology 42 (3):225–242 [PubMed: 6188807]
11. Reeves TM, Lyeth BG, Phillips LL, Hamm RJ, Povlishock JT (1997) The effects of traumatic brain injury on inhibition in the hippocampus and dentate gyrus. Brain research 757 (1):119–132 [PubMed: 9200506]
12. Witgen BM, Lifshitz J, Smith ML, Schwarzbach E, Liang SL, Grady MS, Cohen AS (2005) Regional hippocampal alteration associated with cognitive deficit following experimental brain injury: a systems, network and cellular evaluation. Neuroscience 133 (1):1–15. doi:10.1016/j.neuroscience.2005.01.052 [PubMed: 15893627]
13. Titus DJ, Furones C, Kang Y, Atkins CM (2013) Age-dependent alterations in cAMP signaling contribute to synaptic plasticity deficits following traumatic brain injury. Neuroscience 231:182–194. doi:10.1016/j.neuroscience.2012.12.002 [PubMed: 23238576]
14. Hunt RF, Scheff SW, Smith BN (2010) Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy. Journal of neurophysiology 103 (3):1490–1500. doi:10.1152/jn.00957.2009 [PubMed: 20089815]
15. Kanaan NM, Manfredsson FP (2012) Loss of functional alpha-synuclein: a toxic event in Parkinson's disease? Journal of Parkinson's disease 2 (4):249–267. doi:10.3233/JPD-012138
16. Stefanis L (2012) alpha-Synuclein in Parkinson's disease. Cold Spring Harbor perspectives in medicine 2 (2):a009399. doi:10.1101/cshperspect.a009399 [PubMed: 22355802]
17. Surguchov A (2013) Synucleins: are they two-edged swords? Journal of neuroscience research 91 (2):161–166. doi:10.1002/jnr.23149 [PubMed: 23150342]
18. Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC (2010) Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 329 (5999):1663–1667. doi:10.1126/science.1195227 [PubMed: 20798282]
19. Burre J, Sharma M, Sudhof TC (2014) alpha-Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. Proceedings of the National Academy of Sciences of the United States of America 111 (40):E4274–4283. doi:10.1073/pnas.1416598111 [PubMed: 25246573]
20. Sudhof TC, Rothman JE (2009) Membrane fusion: grappling with SNARE and SM proteins. Science 323 (5913):474–477. doi:10.1126/science.1161748 [PubMed: 19164740]
21. Sudhof TC, Rizo J (2011) Synaptic vesicle exocytosis. Cold Spring Harbor perspectives in biology 3 (12). doi:10.1101/cshperspect.a005637
22. Sollner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE (1993) A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75 (3):409–418 [PubMed: 8221884]
23. Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC (2005) Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Cell 123 (3):383–396. doi:10.1016/j.cell.2005.09.028 [PubMed: 16269331]
24. Carlson SW, Henchir J, Dixon CE (2017) Lateral Fluid Percussion Injury Impairs Hippocampal Synaptic Soluble N-Ethylmaleimide Sensitive Factor Attachment Protein Receptor Complex Formation. Frontiers in neurology 8 (532). doi:10.3389/fneur.2017.00532
25. Carlson SW, Yan H, Dixon CE (2017) Lithium increases hippocampal SNARE protein abundance after traumatic brain injury. Experimental neurology 289:55–63. doi:10.1016/j.expneurol.2016.12.006 [PubMed: 28011122]

26. Carlson SW, Yan H, Ma M, Li Y, Henchir J, Dixon CE (2016) Traumatic Brain Injury Impairs Soluble N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptor Complex Formation and Alters Synaptic Vesicle Distribution in the Hippocampus. Journal of neurotrauma 33 (1):113–121. doi:10.1089/neu.2014.3839 [PubMed: 25923735]
27. Ikonomovic MD, Abrahamson EE, Carlson SW, Graham SH, Dixon CE (2019) Novel therapies for combating chronic neuropathological sequelae of TBI. Neuropharmacology 145 (Pt B):160–176. doi:10.1016/j.neuropharm.2018.06.021 [PubMed: 29933008]
28. DeKosky ST, Ikonomovic MD, Gandy S (2010) Traumatic brain injury--football, warfare, and long-term effects. The New England journal of medicine 363 (14):1293–1296. doi:10.1056/NEJMp1007051 [PubMed: 20879875]
29. Mondello S, Buki A, Italiano D, Jeromin A (2013) alpha-Synuclein in CSF of patients with severe traumatic brain injury. Neurology 80 (18):1662–1668. doi:10.1212/WNL.0b013e3182904d43 [PubMed: 23553480]
30. Su E, Bell MJ, Wisniewski SR, Adelson PD, Janesko-Feldman KL, Salonia R, Clark RS, Kochanek PM, Kagan VE, Bayir H (2010) alpha-Synuclein levels are elevated in cerebrospinal fluid following traumatic brain injury in infants and children: the effect of therapeutic hypothermia. Developmental neuroscience 32 (5–6):385–395. doi:10.1159/000321342 [PubMed: 21124000]
31. Uryu K, Chen XH, Martinez D, Browne KD, Johnson VE, Graham DI, Lee VM, Trojanowski JQ, Smith DH (2007) Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans. Experimental neurology 208 (2):185–192. doi:10.1016/j.expneurol.2007.06.018 [PubMed: 17826768]
32. Ikonomovic MD, Uryu K, Abrahamson EE, Ciallella JR, Trojanowski JQ, Lee VM, Clark RS, Marion DW, Wisniewski SR, DeKosky ST (2004) Alzheimer's pathology in human temporal cortex surgically excised after severe brain injury. Experimental neurology 190 (1):192–203. doi:10.1016/j.expneurol.2004.06.011 [PubMed: 15473992]
33. Newell KL, Boyer P, Gomez-Tortosa E, Hobbs W, Hedley-Whyte ET, Vonsattel JP, Hyman BT (1999) Alpha-synuclein immunoreactivity is present in axonal swellings in neuroaxonal dystrophy and acute traumatic brain injury. Journal of neuropathology and experimental neurology 58 (12):1263–1268 [PubMed: 10604751]
34. Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL (1991) A controlled cortical impact model of traumatic brain injury in the rat. Journal of neuroscience methods 39 (3):253–262 [PubMed: 1787745]
35. Choi I, Zhang Y, Seegobin SP, Pruvost M, Wang Q, Purtell K, Zhang B, Yue Z (2020) Microglia clear neuron-released alpha-synuclein via selective autophagy and prevent neurodegeneration. Nat Commun 11 (1):1386. doi:10.1038/s41467-020-15119-w [PubMed: 32170061]
36. Kiechle M, von Einem B, Hofs L, Voehringer P, Grozdanov V, Markx D, Parlato R, Wiesner D, Mayer B, Sakk O, Baumann B, Lukassen S, Liss B, Ekici AB, Ludolph AC, Walther P, Ferger B, McLean PJ, Falkenburger BH, Weishaupt JH, Danzer KM (2019) In Vivo Protein Complementation Demonstrates Presynaptic alpha-Synuclein Oligomerization and Age-Dependent Accumulation of 8–16-mer Oligomer Species. Cell reports 29 (9):2862–2874 e2869. doi:10.1016/j.celrep.2019.10.089 [PubMed: 31775051]
37. Bernis ME, Babila JT, Breid S, Wusten KA, Wullner U, Tamguney G (2015) Prion-like propagation of human brain-derived alpha-synuclein in transgenic mice expressing human wild-type alpha-synuclein. Acta neuropathologica communications 3:75. doi:10.1186/s40478-015-0254-7 [PubMed: 26612754]
38. Liu L, Sun T, Liu Z, Chen X, Zhao L, Qu G, Li Q (2014) Traumatic brain injury dysregulates microRNAs to modulate cell signaling in rat hippocampus. PloS one 9 (8):e103948. doi:10.1371/journal.pone.0103948 [PubMed: 25089700]
39. Kokjohn TA, Maarouf CL, Daugs ID, Hunter JM, Whiteside CM, Malek-Ahmadi M, Rodriguez E, Kalback W, Jacobson SA, Sabbagh MN, Beach TG, Roher AE (2013) Neurochemical profile of dementia pugilistica. Journal of neurotrauma 30 (11):981–997. doi:10.1089/neu.2012.2699 [PubMed: 23268705]
40. Impellizzeri D, Campolo M, Bruschetta G, Crupi R, Cordaro M, Paterniti I, Cuzzocrea S, Esposito E (2016) Traumatic Brain Injury Leads to Development of Parkinson's Disease Related Pathology in Mice. Frontiers in neuroscience 10:458. doi:10.3389/fnins.2016.00458 [PubMed: 27790086]

41. Acosta SA, Tajiri N, de la Pena I, Bastawrous M, Sanberg PR, Kaneko Y, Borlongan CV (2015) Alpha-synuclein as a pathological link between chronic traumatic brain injury and Parkinson's disease. Journal of cellular physiology 230 (5):1024–1032. doi:10.1002/jcp.24830 [PubMed: 25251017]
42. Abeliovich A, Schmitz Y, Farinas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JM, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25 (1):239–252 [PubMed: 10707987]
43. Anwar S, Peters O, Millership S, Ninkina N, Doig N, Connor-Robson N, Threlfell S, Kooner G, Deacon RM, Bannerman DM, Bolam JP, Chandra SS, Cragg SJ, Wade-Martins R, Buchman VL (2011) Functional alterations to the nigrostriatal system in mice lacking all three members of the synuclein family. The Journal of neuroscience : the official journal of the Society for Neuroscience 31 (20):7264–7274. doi:10.1523/JNEUROSCI.6194-10.2011 [PubMed: 21593311]
44. Greten-Harrison B, Polydoro M, Morimoto-Tomita M, Diao L, Williams AM, Nie EH, Makani S, Tian N, Castillo PE, Buchman VL, Chandra SS (2010) alphabetagamma-Synuclein triple knockout mice reveal age-dependent neuronal dysfunction. Proceedings of the National Academy of Sciences of the United States of America 107 (45):19573–19578. doi:10.1073/pnas.1005005107 [PubMed: 20974939]
45. Wang L, Das U, Scott DA, Tang Y, McLean PJ, Roy S (2014) alpha-synuclein multimers cluster synaptic vesicles and attenuate recycling. Current biology : CB 24 (19):2319–2326. doi:10.1016/j.cub.2014.08.027 [PubMed: 25264250]
46. Betzer C, Movius AJ, Shi M, Gai WP, Zhang J, Jensen PH (2015) Identification of synaptosomal proteins binding to monomeric and oligomeric alpha-synuclein. PloS one 10 (2):e0116473. doi:10.1371/journal.pone.0116473 [PubMed: 25659148]
47. Diao J, Burre J, Vivona S, Cipriano DJ, Sharma M, Kyoung M, Sudhof TC, Brunger AT (2013) Native alpha-synuclein induces clustering of synaptic-vesicle mimics via binding to phospholipids and synaptobrevin-2/VAMP2. eLife 2:e00592. doi:10.7554/eLife.00592 [PubMed: 23638301]
48. Cabin DE, Shimazu K, Murphy D, Cole NB, Gottschalk W, McIlwain KL, Orrison B, Chen A, Ellis CE, Paylor R, Lu B, Nussbaum RL (2002) Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking alpha-synuclein. The Journal of neuroscience : the official journal of the Society for Neuroscience 22 (20):8797–8807 [PubMed: 12388586]
49. Gureviciene I, Gurevicius K, Tanila H (2009) Aging and alpha-synuclein affect synaptic plasticity in the dentate gyrus. Journal of neural transmission 116 (1):13–22. doi:10.1007/s00702-008-0149-x [PubMed: 19002552]
50. Wu N, Joshi PR, Cepeda C, Masliah E, Levine MS (2010) Alpha-synuclein overexpression in mice alters synaptic communication in the corticostriatal pathway. Journal of neuroscience research 88 (8):1764–1776. doi:10.1002/jnr.22327 [PubMed: 20029978]
51. Steidl JV, Gomez-Isla T, Mariash A, Ashe KH, Boland LM (2003) Altered short-term hippocampal synaptic plasticity in mutant alpha-synuclein transgenic mice. Neuroreport 14 (2):219–223. doi:10.1097/01.wnr.0000054961.21656.2d [PubMed: 12598733]
52. Liu S, Ninan I, Antonova I, Battaglia F, Trinchese F, Narasanna A, Kolodilov N, Dauer W, Hawkins RD, Arancio O (2004) alpha-Synuclein produces a long-lasting increase in neurotransmitter release. The EMBO journal 23 (22):4506–4516. doi:10.1038/sj.emboj.7600451 [PubMed: 15510220]
53. Watson JB, Hatami A, David H, Masliah E, Roberts K, Evans CE, Levine MS (2009) Alterations in corticostriatal synaptic plasticity in mice overexpressing human alpha-synuclein. Neuroscience 159 (2):501–513. doi:10.1016/j.neuroscience.2009.01.021 [PubMed: 19361478]
54. Scheff SW, Price DA, Hicks RR, Baldwin SA, Robinson S, Brackney C (2005) Synaptogenesis in the hippocampal CA1 field following traumatic brain injury. Journal of neurotrauma 22 (7):719–732. doi:10.1089/neu.2005.22.719 [PubMed: 16004576]
55. Uryu K, Giasson BI, Longhi L, Martinez D, Murray I, Conte V, Nakamura M, Saatman K, Talbot K, Horiguchi T, McIntosh T, Lee VM, Trojanowski JQ (2003) Age-dependent synuclein pathology following traumatic brain injury in mice. Experimental neurology 184 (1):214–224 [PubMed: 14637093]

56. Carlson SW, Dixon CE (2018) Lithium improves dopamine neurotransmission and increases dopaminergic protein abundance in the striatum after traumatic brain injury. Journal of neurotrauma. doi:10.1089/neu.2017.5509
57. Marti MJ, Tolosa E, Campdelacreu J (2003) Clinical overview of the synucleinopathies. Movement disorders : official journal of the Movement Disorder Society 18 Suppl 6:S21–27. doi:10.1002/mds.10559 [PubMed: 14502652]
58. Wong YC, Krainc D (2017) alpha-synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies. Nature medicine 23 (2):1–13. doi:10.1038/nm.4269
59. Chiba-Falek O (2017) Structural variants in SNCA gene and the implication to synucleinopathies. Current opinion in genetics & development 44:110–116. doi:10.1016/j.gde.2017.01.014 [PubMed: 28319736]
60. Halliday GM, Holton JL, Revesz T, Dickson DW (2011) Neuropathology underlying clinical variability in patients with synucleinopathies. Acta neuropathologica 122 (2):187–204. doi:10.1007/s00401-011-0852-9 [PubMed: 21720849]
61. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276 (5321):2045–2047 [PubMed: 9197268]
62. Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atares B, Llorens V, Gomez Tortosa E, del Ser T, Munoz DG, de Yebenes JG (2004) The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Annals of neurology 55 (2):164–173. doi:10.1002/ana.10795 [PubMed: 14755719]
63. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388 (6645):839–840. doi:10.1038/42166 [PubMed: 9278044]
64. Lippa CF, Fujiwara H, Mann DM, Giasson B, Baba M, Schmidt ML, Nee LE, O'Connell B, Pollen DA, St George-Hyslop P, Ghetti B, Nochlin D, Bird TD, Cairns NJ, Lee VM, Iwatsubo T, Trojanowski JQ (1998) Lewy bodies contain altered alpha-synuclein in brains of many familial Alzheimer's disease patients with mutations in presenilin and amyloid precursor protein genes. The American journal of pathology 153 (5):1365–1370 [PubMed: 9811326]
65. Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, Iwatsubo T (1998) Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. The American journal of pathology 152 (4):879–884 [PubMed: 9546347]
66. Trojanowski JQ, Lee VM (2001) Parkinson's disease and related neurodegenerative synucleinopathies linked to progressive accumulations of synuclein aggregates in brain. Parkinsonism & related disorders 7 (3):247–251. doi:10.1016/s1353-8020(00)00065-1 [PubMed: 11331194]
67. Nuber S, Rajsombath M, Minakaki G, Winkler J, Muller CP, Ericsson M, Caldarone B, Dettmer U, Selkoe DJ (2018) Abrogating Native alpha-Synuclein Tetramers in Mice Causes a L-DOPA-Responsive Motor Syndrome Closely Resembling Parkinson's Disease. Neuron 100 (1):75–90 e75. doi:10.1016/j.neuron.2018.09.014 [PubMed: 30308173]
68. Nuber S, Harmuth F, Kohl Z, Adame A, Trejo M, Schonig K, Zimmermann F, Bauer C, Casadei N, Giel C, Calaminus C, Pichler BJ, Jensen PH, Muller CP, Amato D, Kornhuber J, Teismann P, Yamakado H, Takahashi R, Winkler J, Masliah E, Riess O (2013) A progressive dopaminergic phenotype associated with neurotoxic conversion of alpha-synuclein in BAC-transgenic rats. Brain 136 (Pt 2):412–432. doi:10.1093/brain/aws358 [PubMed: 23413261]
69. McKee AC, Stern RA, Nowinski CJ, Stein TD, Alvarez VE, Daneshvar DH, Lee HS, Wojtowicz SM, Hall G, Baugh CM, Riley DO, Kubilus CA, Cormier KA, Jacobs MA, Martin BR, Abraham CR, Ikezu T, Reichard RR, Wolozin BL, Budson AE, Goldstein LE, Kowall NW, Cantu RC (2013) The spectrum of disease in chronic traumatic encephalopathy. Brain 136 (Pt 1):43–64. doi:10.1093/brain/aws307 [PubMed: 23208308]
70. Byrd M, Dixon CE, Lucke-Wold B (2018) Examining the Correlation between Acute Behavioral Manifestations of Concussion and the Underlying Pathophysiology of Chronic Traumatic Encephalopathy: A Pilot Study. Journal of neurology and psychology 6 (1). doi:10.13188/2332-3469.1000037

71. Albensi BC, Sullivan PG, Thompson MB, Scheff SW, Mattson MP (2000) Cyclosporin ameliorates traumatic brain-injury-induced alterations of hippocampal synaptic plasticity. Experimental Neurology 162(2):385–89. doi: 10.1006/exnr.1999.7338 [PubMed: 10739643]

72. Norris CM, Scheff SW (2009) Recovery of afferent function and synaptic strength in hippocampal CA1 following traumatic brain injury. Journal of Neurotrauma 12:2269–78. doi: 10.1089/neu.2009.1029

73. Bondi CO, Cheng JP, Tennant HM, Monaco CM, Kline AE (2014) Old dog, new tricks: the attentional set-shifting test as a novel cognitive behavioral task after controlled cortical impact injury. Journal of Neurotrauma 31:926–37. doi: 10.1089/neu.2013.3295 [PubMed: 24397572]

74. Vonder Haar C, Anderson GD, Emore BE, Moore LH, Wright AM, Kantor ED, Farin FM, Bammler TK, MacDonald JW, Hoane MR (2014) Comparison of the effect of minocyline and simvastatin on functional recovery and gene expression in a rat traumatic brain injury model. Journal of Neurotrauma 31:961–75. doi: 10.1089/neu.2013.3119 [PubMed: 24308531]

75. Mountney A, Bramlett HM, Dixon CE, Mondello S, Dietrich DW, Wang KK, Caudle K, Empey PE, Poloyac SM, Hayes RL, Povlishock JT, Tortella FC, Kochanek PM, Shear DA (2016) Simnvastatin treatment in traumatic brain injury: Operation Brain Trauma Therapy. Journal of Neurotrauma 33(6):567–80. doi: 1089/neu2015.4130. [PubMed: 26541177]

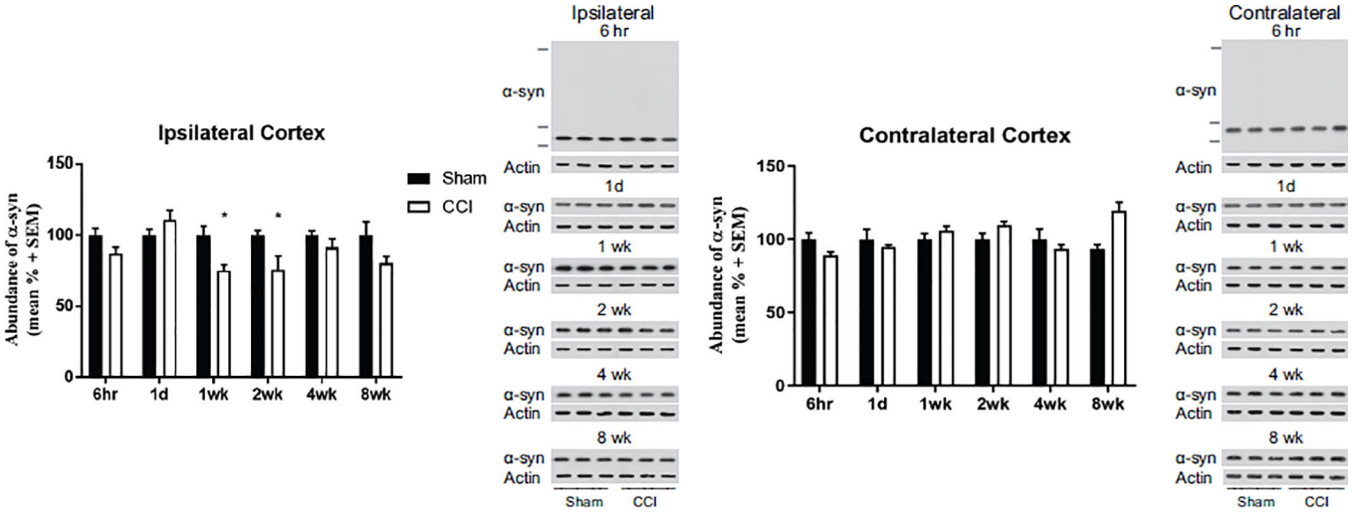

Figure 1. Controlled cortical impact (CCI) altered cortical α-synuclein abundance post-injury.

Representative western blot images of α-synuclein and actin in cortical whole cell lysates post-injury. The 6 hr immunoblot images show the representative single band pattern for monomeric α-synuclein (18 kDa, markers are 15, 20, 160 kDa). Subsequent images were cropped due to the number of immunoblots and space. Semiquantitative measurements of cortex homogenates from CCI-injured or sham operated rats sacrificed at 6 hour, 1 day, 1 week, 2 weeks, 4 weeks and 8 weeks post-injury were assessed by Western blot analysis. Assessment of α-synuclein (18 kDa, markers are 15 and 20 kDa) revealed significantly reduced abundance at 1 and 2 weeks post-injury in the ipsilateral cortex (*$p<0.05$). The abundance of α-synuclein in the contralateral cortex was not changed post-injury. α-synuclein was normalized to actin (42 kDa, marker is 37 kDa; n = 6 per group per time point).

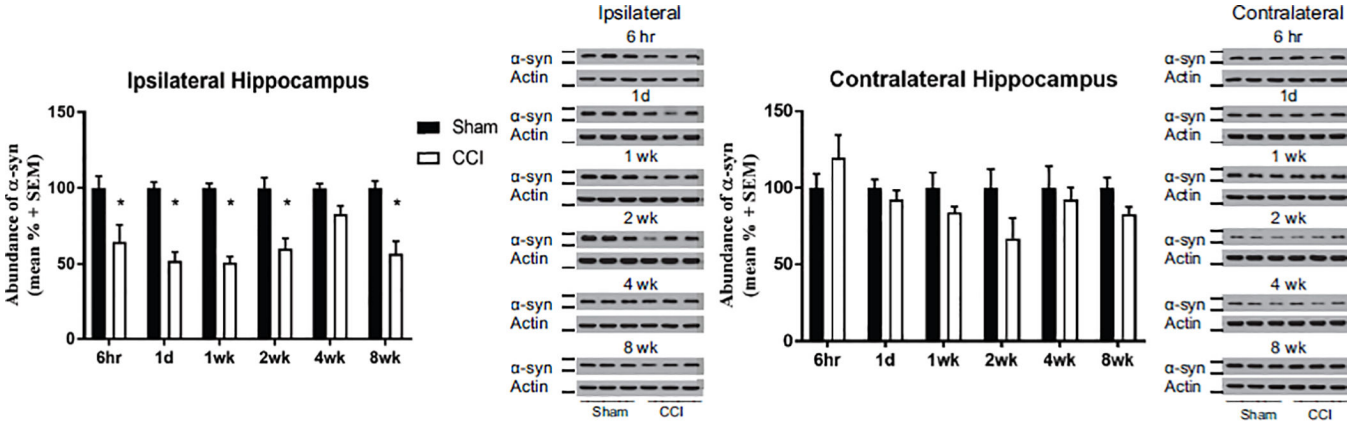

Figure 2. Controlled cortical impact (CCI) reduced ipsilateral hippocampal α-synuclein abundance at multiple time points over the 8 weeks post-injury.

Representative western blot images of α-synuclein and actin in hippocampal whole cell lysates post-injury. Semiquantitative measurements of hippocampal homogenates from CCI-injured or sham operated rats sacrificed at 6 hour, 1 day, 1 week, 2 weeks, 4 weeks and 8 weeks post-injury were assessed by Western blot analysis. Assessment of α-synuclein (18 kDa, markers are 15 and 20 kDa) revealed significantly lower protein in the ipsilateral hippocampus at 6 hours, 1 day, 1, 2, and 8 weeks, but not 4 weeks after CCI injury (*$p<0.05$), compared to sham injury. The abundance of α-synuclein in the contralateral hippocampus was not changed post-injury. α-synuclein was normalized to actin (42 kDa, marker is 37 kDa; n = 6 per group per time point).

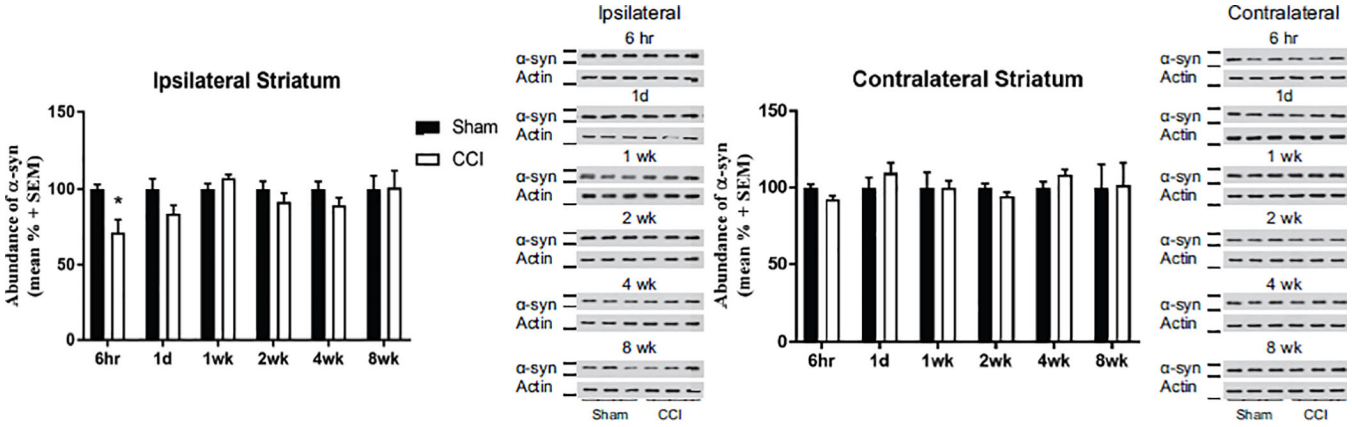

Figure 3. Controlled cortical impact (CCI) reduced ipsilateral striatal α-synuclein abundance at 6 hours post-injury.

Representative western blot images of α-synuclein and actin in striatal whole cell lysates post-injury. Semiquantitative measurements of striatal homogenates from CCI-injured or sham operated rats sacrificed at 6 hour, 1 day, 1 week, 2 weeks, 4 weeks and 8 weeks post-injury were assessed by Western blot analysis. Assessment of α-synuclein (18 kDa, markers are 15 and 20 kDa) revealed significantly lower protein in the ipsilateral striatum at 6 hours after CCI injury (* $p<0.05$), compared to sham injury. The abundance of α-synuclein in the contralateral hippocampus was not changed post-injury. α-synuclein was normalized to actin (42 kDa, marker is 37 kDa; n = 6 per group per time point).

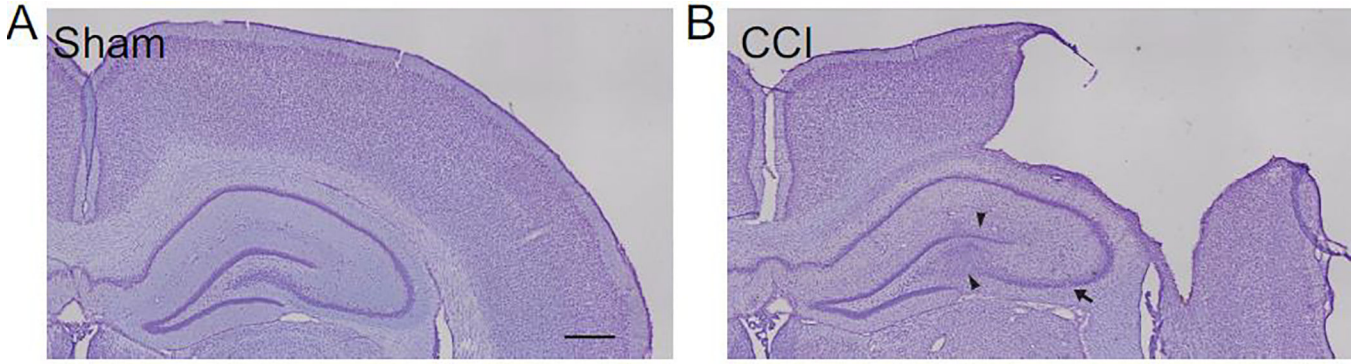

Figure 4. Controlled cortical impact produces cortical and hippocampal cellular loss at 1 week post-injury.

The anatomical position of the CCI is illustrated by representative cresyl violet stained sections of the ipsilateral cortex and hippocampus at 1 week after (A) sham control (−3.48mm bregma) or (B) CCI injury (−3.60mm bregma). CCI resulted in pronounced tissue loss at the epicenter of injury at 1 week post-injury. In the hippocampus, cell loss is observed in the CA3 (arrow), dentate gyrus and hilus (arrowheads). Scale bar is equal to 500μm.

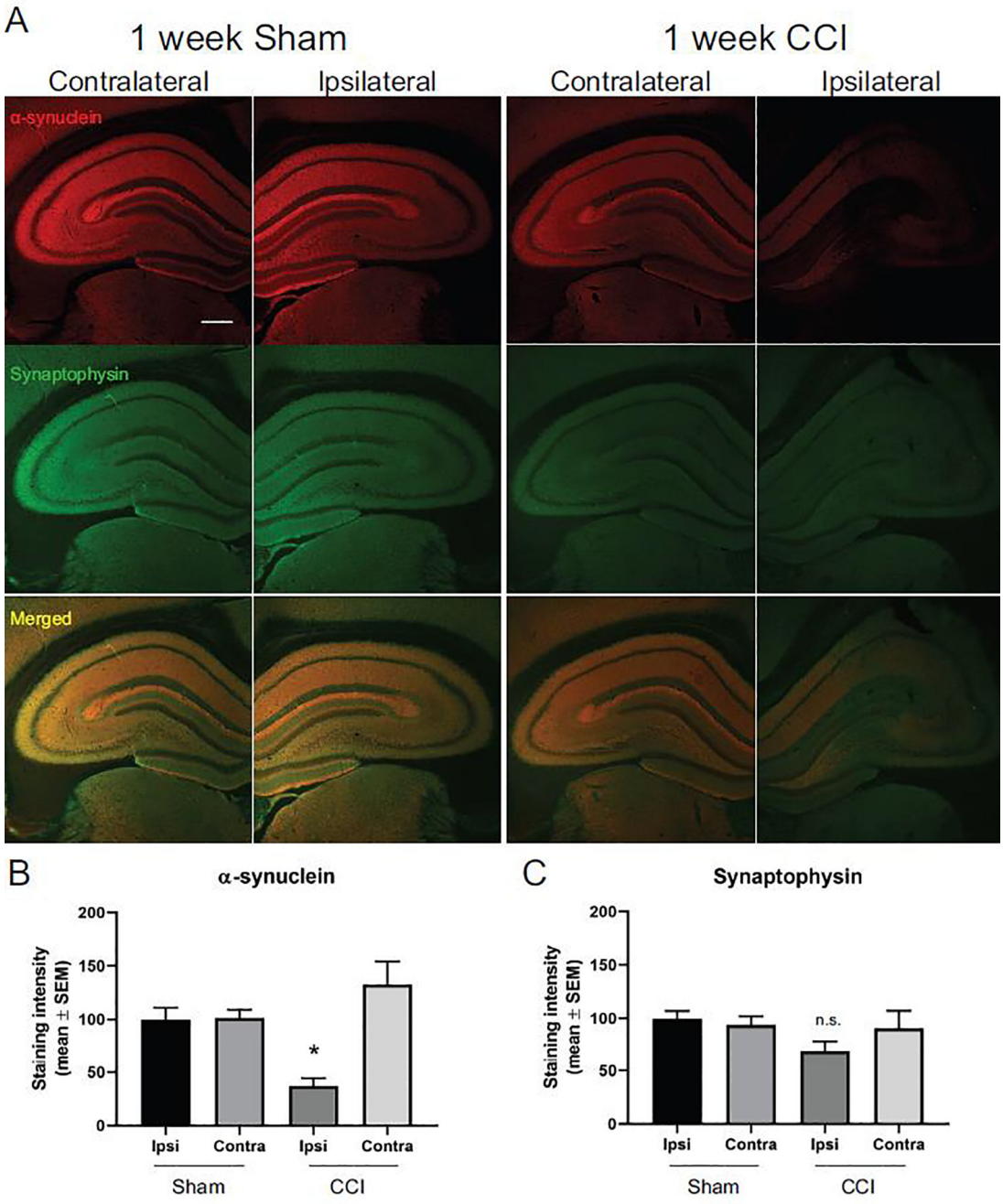

Figure 5. Controlled cortical impact (CCI) reduced α-synuclein immunoreactivity in ipsilateral hippocampus at 1 week post-injury.

Representative images of hippocampal α-synuclein and synaptophysin immunoreactivity in sham and CCI-injured brain sections at 1 week post-injury (A). CCI resulted in reduced α-synuclein immunoreactivity in the ipsilateral hippocampus. Quantitative assessment of α-synuclein immunoreactivity revealed a significant reduction in pixel intensity in the ipsilateral hippocampus at 1 week following CCI (* $p<0.01$), as compared to sham injury (B). However, intensity measurements of synaptophysin in the same sections revealed no

significant differences (one-way ANOVA; p=0.26; C). Scale bar is equal to 500μm (n=6 per group at each time point).

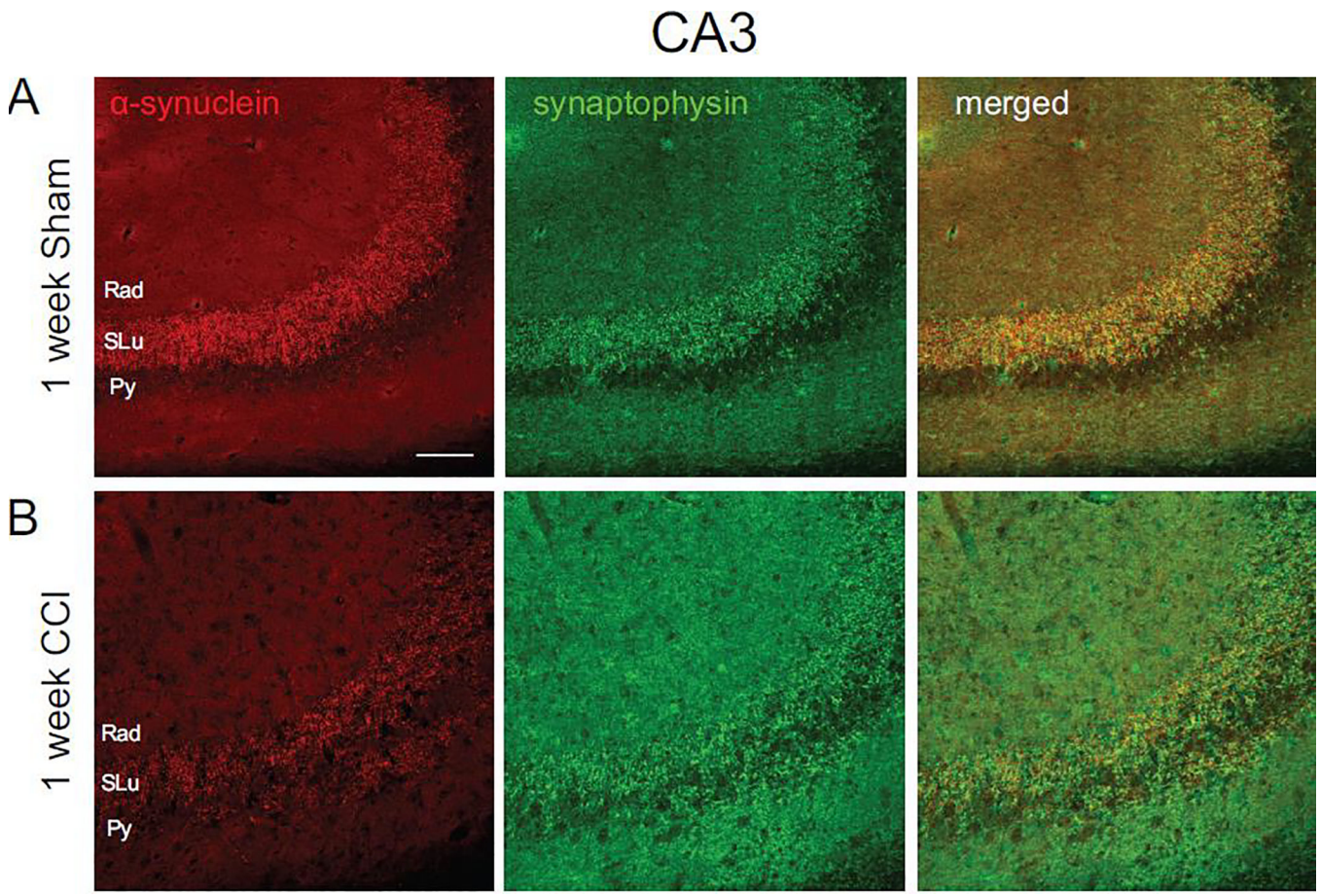

Figure 6. Controlled cortical impact (CCI) reduced hippocampal α-synuclein and synaptophysin immunoreactivity in CA3 at 1 week post-injury.

Representative images of immunofluorescent staining for α-synuclein (red) and presynaptic terminal marker synaptophysin (green) shows decreased immunoreactivity in the stratum lucidum (SLu) of CA3 (A,B) at 1 week post-injury. Stratum radiatum (Rad) and pyramidal cell layer (Py). Scale bar represents 100μm (n=6 per group at each time point).

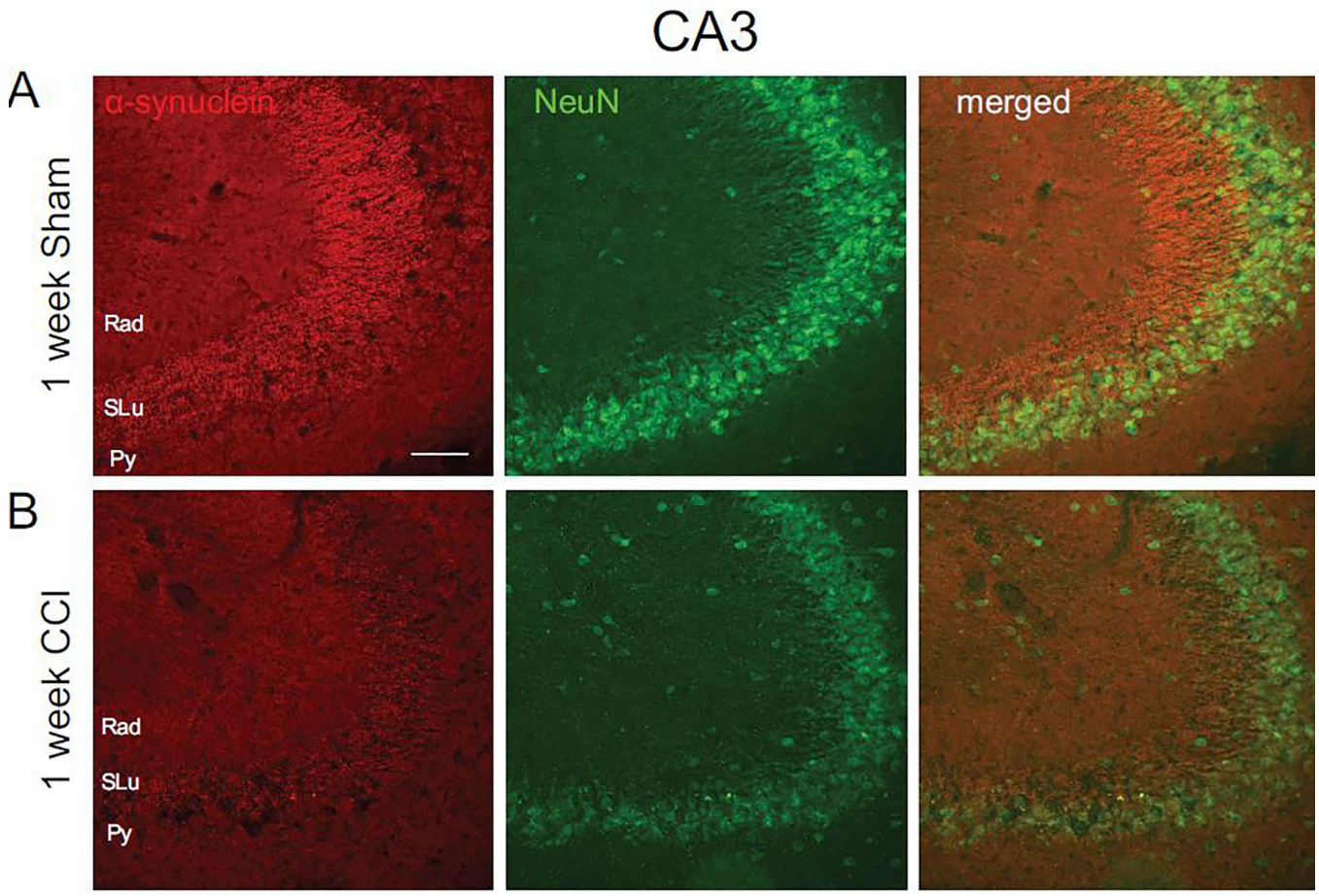

Figure 7. Controlled cortical impact (CCI) reduced hippocampal α-synuclein and NeuN immunoreactivity in CA3 at 1 week post-injury.

Representative images of immunofluorescent staining for α-synuclein (red) and the neuronal nuclei marker NeuN (green) show decreased immunoreactivity for both markers in CA3 (A,B) at 1 week post-injury. Stratum radiatum (Rad), pyramidal cell layer (Py), stratum lucidum (SLu), Scale bar represents 100μm (n=6 per group at each time point).

Table 1:

Hippocampal α-synuclein immunoreactivity following controlled cortical impact

Time point	Sham (ipsilateral)	Sham (contralateral)	CCI (ipsilateral)	CCI (contralateral)
6hr post-injury	100 ± 14%	113 ± 13%	73 ± 12%	98 ± 11%
24hr post-injury	100 ± 4%	115 ± 7%	75 ± 21%	132 ± 13%
1 week post-injury	100 ± 11%	102 ± 19%	37 ± 7% *	133 ± 21%
2 week post-injury	100 ± 3%	100 ± 7%	33 ± 3% #	88 ± 14%
4 week post-injury	100 ± 9%	100 ± 8%	55 ± 9% *	101 ± 13%
8 week post-injury	100 ± 5%	84 ± 7%	56 ± 11% *	76 ± 10%

Analysis of α-synuclein immunoreactivity by mean pixel intensity. Mean pixel values are depicted as percentage values of sham ipsilateral hemisphere measurements. All quantified sections were within the −3.24mm and −3.60mm bregma position range. Statistical comparison of one-way ANOVA was completed for each time point.

When appropriate a post hoc Sidak multiple corrections t-test was completed, with significance depicted (* $p < 0.05$ or # $p < 0.001$ as compared to sham ipsilateral). N=6 per group.