Traumatic axonal injury is a major contributor to morbidity after severe traumatic brain injury (TBI). Reduction and avoidance of increases in intracranial pressure (ICP) continues to be the mainstays of treatment. It remains unclear whether elevations in ICP influence axonal injury.

Six week old male mice (C57BL/6J) were subjected to either controlled cortical impact (CCI) (N = 48) or sham surgery (SHAM, N = 12). Immediately after CCI, injured animals were randomized to a loose fitting plastic cap (OPEN) or replacement of the previously removed bone flap (CLOSED). Animals were sacrificed at 1 day, 7 days and 4 weeks post injury. Brain parenchymal ICP measurements were taken via a contralateral burr hole. Severity of white matter axonal injury was quantified utilizing stereological methods of beta amyloid precursor protein (B-APP) stained sections at 1 day and 7 days post injury.

Elevated ICP was observed in CLOSED animals 15 minutes and 1 day after injury compared to OPEN and SHAM (15 min 21.4 ± 4.2 vs. 12.3 ± 2.9 and 8.8 ± 1.8 mm Hg, $P < 0.0001$; 1 day 17.8 ± 3.7 vs. 10.6 ± 2.0 and 8.9 ± 1.9 mm Hg, $P < 0.0001$). Stereologic quantification of B-APP staining in the corpus callosum and ipsilateral external capsule revealed increased axonal swellings and bulbs in CLOSED compared to OPEN animals at 1 day (136 ± 24 vs. 94 ± 29 10^3 axons/mm^3, $P < 0.01$) and 7 days (99 ± 29 vs. 58 ± 15 10^3 axons/mm^3, $P < 0.001$) post injury. At 4 weeks post injury, CLOSED animals had increased white matter atrophy compared to OPEN and SHAM, resulting in smaller corpus callosum and external capsule volume (1.2 ± 0.1 vs. 1.5 ± 0.2 and 2.0 ± 0.1 mm^3, $P < 0.0001$).

Following controlled cortical impact, even moderate elevations in intracranial pressure were associated with increased axonal injury and white matter atrophy. Therapeutic interventions that ameliorate intracranial hypertension may influence white matter injury severity.

Key words
axonal injury, intracranial pressure, mouse, seconday injury

C3-12

IMPAIRED SYNAPTIC VESICLE DOCKING IS A NOVEL CONTRIBUTOR TO REDUCED NEUROTRANSMISSION AFTER TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) impairs neuronal function and can culminate in lasting cognitive impairment. While impaired acetylcholine release has been well established after experimental TBI, little is known about the mechanisms underlying this consequence. We hypothesized that alterations in synaptic vesicle distribution and reduced vesicular docking at the pre-synaptic membrane contribute to impaired neurotransmission. To examine the ultrastructural distribution of synaptic vesicles, Sprague-Dawley rats received 2.7 mm controlled cortical impact (CCI) or sham injury (n = 6/group) and the brains were processed for transmission electron microscopy at 1 week post-injury. In each animal, 20 randomly selected synaptic nerve terminals from the molecular layer of the hippocampus were imaged at 100 k magnification. Synaptic vesicle distribution was assessed by measuring the distance of each vesicle from the active zone for all terminals. CCI resulted in a significant reduction in vesicle frequency within 200 nm of the active zone ($p < 0.01$ compared to sham, repeated measures one-way ANOVA). Recent reports highlight that reduced vesicular density within 100nm of the active zone impairs vesicular docking and blunts neurotransmitter release. In a normal synapse, vesicular docking and neurotransmitter release requires formation of the SNARE complex. To examine the effect of TBI on the SNARE complex, rats received CCI or sham injury and were sacrificed at 6 hr, 1 d, 1, 2, or 4 weeks post-injury (n = 6/injury/time). Immunoblotting of unboiled hippocampal homogenates showed that SNARE complex formation, identified by SNAP-25 and syntaxin immunoreactivity, was reduced by at least 48% at 1 week ($p < 0.05$) and 2 weeks ($p < 0.01$) after CCI. Neurotransmitter release deficits have been well characterized at 1 and 2 weeks post-injury, suggesting that changes in synaptic vesicle docking contributes to impaired neurotransmission. In this study, we provide the first evidence that TBI alters synaptic vesicle distribution using quantitative ultrastructural analysis of electron micrographs. Our findings suggest that reductions in the standing pool of readily releasable vesicles and impaired SNARE complex formation are two novel mechanisms that contribute to the impaired neurotransmission after TBI.

Key words
electron microscopy, neurotransmission, synapse, vesicle

C3-13

SECONDARY MEMBRANE DAMAGE AND THE POTENTIAL FOR MEMBRANE-TARGETED NEUROPROTECTION

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Traumatic Brain Injuries (TBI) result in primary and secondary damage. The extended timescale of secondary injuries provides a larger window of opportunity for therapeutic interventions, but unfortunately there are currently no clinically successful pharmaceutical treatments for TBI. This indicates a need for a better understanding of the mechanisms and pathways of cellular degeneration and dysfunction after injury in order to better develop effective therapeutic interventions for patients with TBI. Plasma membrane damage, calcium influx, mitochondrial damage, and increased oxidative stress have all been identified as key players in the TBI pathway. Membrane damage has been hypothesized to be an initiating factor in the secondary damage pathway and previous studies have shown that sealing the damage using Poloxamer 188(P188) is neuroprotective after mechanical shear stress injury. However, the therapeutic potential of P188 and targeted membrane protection is limited if membrane damage is only occurring at the beginning of the secondary damage pathway. The results here make the case that secondary membrane damage is occurring and that it can be targeted using P188. Aspects of the injury pathway downstream of initial membrane damage were targeted and induced using Calcium Ionophore A23187 to increase intracellular calcium without general membrane damage, CCCP to damage mitochondria, and the hydroperoxide donor tert-butyl hydroperoxide to increase oxidative stress. These treatments were used to create isolated perturbations of pieces of the TBI pathway in cultured chick forebrain neurons. Axonal injury was quantified by normalizing the number of focal swellings (beads) by the length of the axon. P188 used in combination with each of these resulted in a statistically significant reduction in beading. Membrane permeability was quantified by measuring the loss of intracellular Calcein Red/Orange. Together, these results provide evidence for the presence of secondary membrane damage and the possibility of targeted membrane sealing as a therapeutic tool for treating TBI.

Key words
calcium, membrane damage, membrane protection, mitochondria, oxidative stress, poloxamer 188