



Role of microtubule-associated protein 6 in axonal repair and degeneration after traumatic axonal injury *in-vitro*

Hailong Song, Frank J. Rauscher IV, Jean-Pierre Dollé, Douglas H. Smith

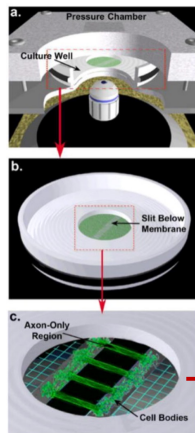
Department of Neurosurgery, Center for Brain Injury and Repair, University of Pennsylvania, Philadelphia, PA, 19104, USA



Introduction

As a key pathological substrate of concussion, traumatic axonal injury (TAI) can result in disruption of the axonal cytoskeleton. Specifically, dynamic stretch injury of axons induces immediate breaking and damage to axonal microtubules (MTs), which impairs protein transport and ultimately leads to the formation of varicose swellings. Although the entire axon tract receives the same mechanical deformation, only a subset of axons within the tract go on to degenerate. This suggests that injury triggers a selection process that drives some axons towards repair/survival and others towards degeneration. Here, we explored potential mechanistic pathways of axonal survival and degeneration by examining specific MT-associated protein (MAP) expression and interactions.

Methodology: *in-vitro* stretch injury of axons



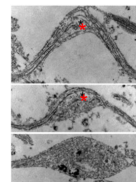
- Rat cortical neurons are plated on a deformable elastomeric membrane
- Cells mature over 14 days and extend axons along an axon-only lane region (0.2mm width x 2mm length x 0.2mm height) resembling axon tracts within the brain.
- Cells were fixed at various timepoints, and standard immunocytochemistry (ICC) procedures performed.

- Pulsed air pressure induces rapid deformation/stretching of axon-only tracks, mimicking the stretch injury of axons during a concussion.
- Dynamic stretch injury causes MT breakage (below: asterisk), resulting in altered MAP distribution along the axon.
- We examined altered levels and selective regulation of MAP6 in response to MT damage.

Concussion



TAI

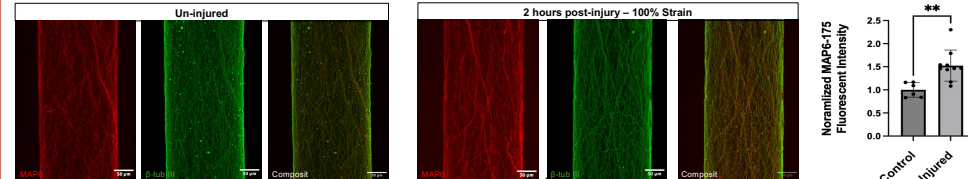


Acknowledgments

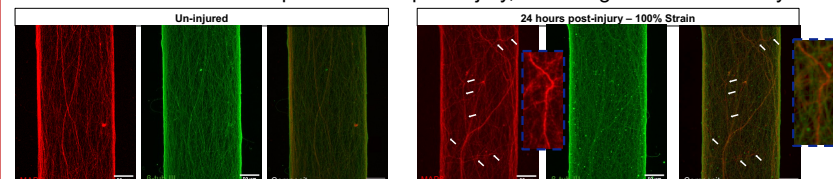
Research supported by: NIH grants: U54NS115322, U01NS137500, R01NS038104, R01NS094003, R21NS35384; and Alzheimer's Association grant AARFD-23-1144656.

Results

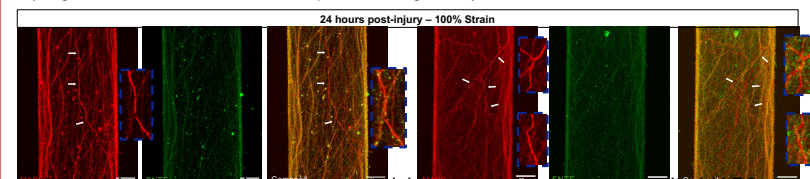
- Global axon MAP6 levels were greatest at 2 hours post-injury.



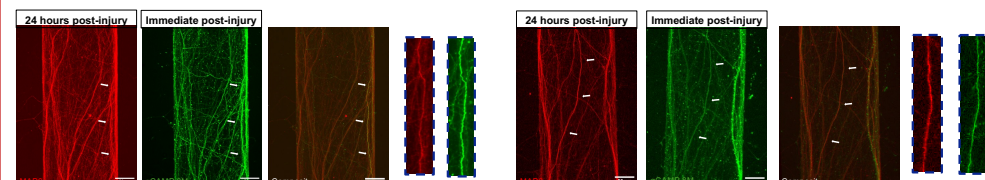
- Select axons are MAP6+ up to 24 hours post-injury, indicating increased stability.



- MAP6+ axons at 24 hours post-injury do not display degenerative morphologies or colocalize with SNF (degenerative axon marker - Spectrin fragment).



- High levels of injury induced axonal calcium influx that colocalizes with MAP6+ axons at 24 hours.



Key Takeaways

- Enhanced axonal MAP6 levels at 2 hours post-injury.
- Selective axons show sustained MAP6 levels up to 24 h post-injury, suggesting a prolonged MT stabilization response to injury.
- MAP6+ axons at 24 hours show no colocalization with degenerative morphologies (periodic swellings) or markers: APP (transport interruption) or SNF (axonal spectrin fragments)

