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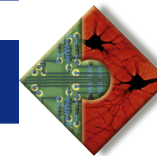
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# Shear Stress Injury Induces Morphological and Structural Changes in Cultured Chick Forebrain Neurons

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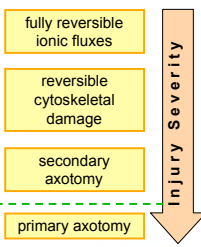
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## Background

Traumatic brain injury (TBI) is an important pathology associated with closed head trauma and effects around 2 million people each year.

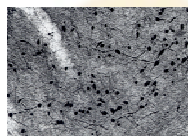
Diffuse axonal injury (DAI) is common form of TBI and involves widespread brain dysfunction.

Primary axotomy is the immediate disruption of axons and requires severe injury. Secondary axotomy is a progressive event that develops over minutes to days.



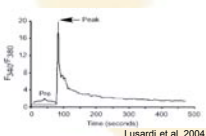
### Axonal beading

- is the "hallmark" morphology of Traumatic Axonal Injury (TAI)
- is associated with impaired axonal transport and consequent accumulation of organelles and proteins that are being transported.



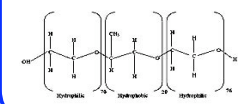
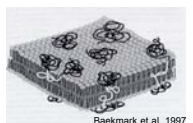
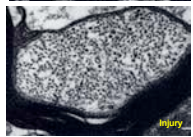
### Mechanisms of Secondary Injury

- Axolemmal permeability increases following injury and depends on magnitude and onset rate of trauma.
- Intracellular calcium ion concentration  $[Ca^{2+}]_i$  shows an acute rise following injury.
- Calpains (Ca-activated non-specific proteases) get activated following injury. Calpains are known to degrade various cytoskeletal proteins including tubulin.
- Loss of microtubules were reported 15 minutes following stretch injury.



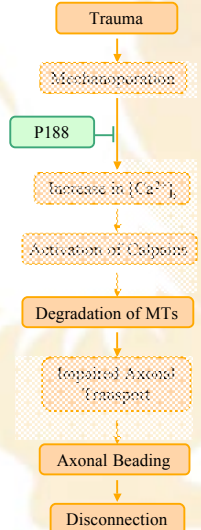
### Neuroprotection by P188

- Poloxamer 188 (P188) is a triblock co-polymer (MW: ~8400), that is widely used in the medical field as a non-toxic surfactant
- P188 promotes resealing of injured membranes and to increase cell viability to control levels (Serbest et al 2005).



## Hypotheses

- FSSI induces changes in MT structure that and thereby increases axonal beading.
- Post-injury application of P188 can attenuate or decrease axonal beading.



## Significance

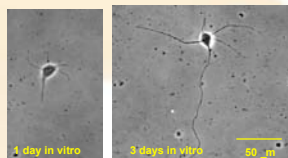
Our study will apply fluid shear stress injury to primary neuron culture and trace individual neurons using patterned coverslips.

Post-injury effects of P188 on primary neurons will be assessed using an in vitro model system.

## Methods

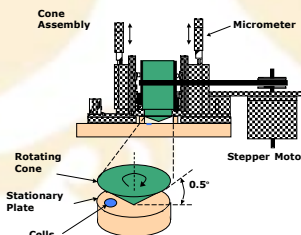
### Cell Culture (Heidemann et al, 2003)

- Embryonic (ED8) chick forebrain neurons dissected, freshly isolated and plated on polylysine coated or chemically altered glass coverslips.
- Cell cultures are incubated for 6 days before experimentation.



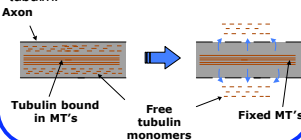
### Controlled Shear Stress Device (Blackman et al, 2000)

- Applies uniform shear stress over the entire area.
- Precise control on the onset rate and magnitude of the shear stress.
- Enables imaging before, during, and after the injury using an inverted microscope.



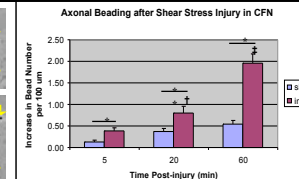
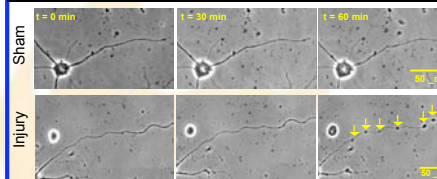
### Combined Fixing and Extraction (Gallo and Letourneau, 1999)

- Simultaneous fixation of MTs while permeating axolemma to remove free tubulin.



## Results

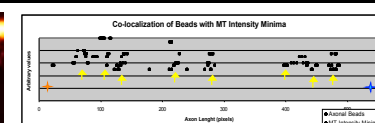
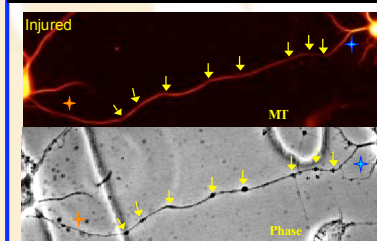
### Axonal Beading Increases with FSSI (45 dyn-cm<sup>-2</sup>, 30 ms onset)



Axonal beading is measured by normalizing the number of beads, that emerged post-injury and over the axon length.

n = 172 neurons (25 CSs) \* p<0.001, \*\* p<0.05 compared to sham controls; † p<0.05, ‡ p<0.001 compared to previous time point.

### Axonal Beads Co-localize with MT Discontinuities



MT mass is lost at the same exact locations where beads are formed.

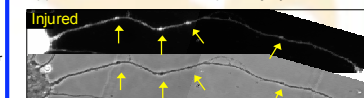
### Post-injury P188 Reduces Axonal Beading

P188 was added to the shearing medium 5-10 minutes following trauma (final concentration 100 μM).

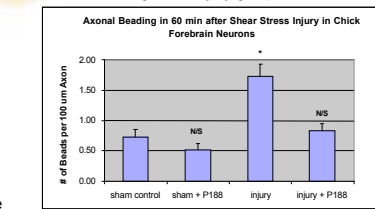
P188 had no significant effect on controls but decreased beading in the injury group to control levels.

### Beads Contain Accumulated Organelles

DiOC<sub>6</sub>(3) (membranous organelle staining dye) applied to cultures fixed 1h post-injury.



Dye concentrated at beads indicating organelle accumulation due to impaired axonal transport.



n = 145 neurons (23 CSs) \* p<0.001 compared to sham controls

## Conclusions

In vitro FSSI sufficiently induces TBI-like axonal beading on cultured neurons. Our finding that beads co-localizing with MT loss provides direct evidence for the idea that impaired transport is responsible for the accumulation of transported material at bead locations (also verified by organelle staining). Our future studies will concentrate on mechanoporation, Ca<sup>2+</sup> influx and Calpain activation following injury.

Our results verify the neuroprotective effect of P188 in terms of reduced axonal beading. It should be clarified if this effect is via resealing mechanically-induced axolemmal pores.

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