There is nothing “mild” about mild TBI

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NINDS, NICHD, VA
At least 1.4 million TBIs occur in the United States each year.

- 50,000 Deaths
- 235,000 Hospitalizations
- 1,111,000 Emergency Department Visits
- ??? Receiving Other Medical Care or No Care

***>80% of returning vets have sustained a TBI***
The major causes of TBI

- Firearms: 39%
- Vehicle accidents: 34%
- Falls: 10%
- Other: 17%
TBI affects multiple behavioral systems

**Sensory**
- blindness
- deafness
- loss of smell
- tolerance to light/sound/heat

**Emotions**
- Irritability
- Mood swings
- Depression
- Paranoia

**Cognition and executive function**
- Attention
- Amnesia
- Language-communication
- Spatial mapping, disorientation
- Problem solving

**Miscellaneous**
- Headaches
- Seizures
Traumatic brain injury: Contusions
Grades of Diffuse Axonal Injury

I – Axonal injury in parasagittal white matter in cerebrum

II – Grade I + focal lesions in corpus callosum

III – Grade II + focal lesions in cerebral peduncle
White matter damage presents as hemorrhagic focal lesions leading to atrophy.
White matter damage presents as axonal swellings leading to Wallerian degeneration.
The predominant pathology of pediatric brain trauma is **WHITE MATTER** injury

Ashwal et al
Human TBI

Pathophysiology
- Biomechanics
- Histopathology
- Behavior

Animal models
- Biomechanics
- Histopathology
- Neurochemistry
- Behavior

Mechanisms

In vitro models
- Biomechanics
- Cytopathology
- Neurochemistry
- Function

Therapy

Treatment
Biomechanics of TBI

Rotation (diffuse)

Impact (focal)

Rotation + impact (diffuse and focal)
ANIMAL MODELS OF TBI

DIFFUSE Pathology
(axonal injury)

Midline fluid-percussion
Closed head impact
(cortical impact, no craniotomy)

FOCAL Pathology
(contusions)

Lateral fluid-percussion
Lateral cortical impact (craniotomy)
Head Trauma

Vascular (Pathophysiology)

- Altered Cerebral Blood Flow
- BBB Breakdown
- Altered Metabolism (insufficient oxygen & glucose; inadequate energy supply)
  - Influx of Na⁺, Cl⁻ & water
  - Cytotoxic
  - EDEMA

Neuro-Inflammation

- iGLUR Activation (NMDAR, AMPAR)
- Ca²⁺ Influx
  - Vasogenic
  - ROS, Cytokines

Cellular (Pathology)

- Glutamate Accumulation
- Structural Changes (Dendrite, Axon)
  - Kinase Activation (JNK)
  - Protease Activation (Calpains, Caspases)
  - Cell Death
Mechanical injury

Damage to gray matter

Necrosis (calpains-SBDP)

Excitotoxicity (NMDA, AMPA)

Apoptosis (AC3, Bax/Bcl-2, MAPKs)

Damage to white matter
We have been focusing on the tip of the iceberg!

- 50,000 Deaths
- 235,000 Hospitalizations
- 1,700,000 Emergency Department Visits
Epidemiology of Mild TBI/Concussion

- 1.7 million emergency room visits for TBI annually
- Approximately 80% (1.4 million) are treated and released without hospital admission
- Corrections for underreporting estimate an annual incidence as high as 3.8 million

(Faul M, Xu L, Wald MM, Coronado VG, 2010)
Brain damage after mild TBI is only visible by MR imaging
The structural basis for diffuse TBI is Traumatic Axonal Injury.

Mechanical injury

- Damage to gray matter
  - IAT – impaired axonal transport
  - NFC – neurofilament compaction
  - Apoptosis of oligodendrocytes

- Damage to white matter
  - Secondary axotomy, Wallerian degeneration, microglial reactivity
What is Traumatic Axonal Injury?

- Microtubule derangement
- Neurofilament compaction
- Disruption of transport
- Accumulation of proteins
- Secondary axotomy
  - Wallerian degeneration
A model of mild TBI
Mild TBI causes TRANSIENT cognitive deficits

Spatial Learning

![Graph A: Spatial Learning](image)

Working Memory

![Graph C: Working Memory](image)

Latency to Platform (sec)

Days Post Injury

Days Post Injury

Graphs A and C show the latency to platform for Sham and Injured groups over different days post injury, illustrating transient cognitive deficits.
Mild TBI does not result in a contusion. However, there is mild neuronal loss.
Mild TBI Causes Structural and Function Damage to Axons

**βAPP**
- **Sham**: Image A
- **Injured 24h**: Image B
- **Injured 3d**: Image C

**SYN**
- **Sham**: Image D
- **Injured 24h**: Image E
- **Injured 3d**: Image F

**SMI-32**
- **Sham**: Image G
- **Injured 24h**: Image H
- **Injured 3d**: Image I

**J**
- **24h**: Image K
- **3d**: Image K
- **7d**: Image K

**K**
- **Bar Graph**
  - **App IR (%)**
  - **Sham**
  - **Injured 24h**
  - **Injured 3d**
  - **Injured 7d**

**Legend**: *p < 0.05, **p < 0.01, # p < 0.05 compared to sham*
Functional alterations in white matter following TBI

Baker et al

Reeves et al
Mild TBI Causes Axonal Conduction Deficits

A

Sham

Injured

N1

N2

B

N1

Sham 24h

Injured 24h

Sham 14d

Injured 14d

Amplitude (mV)

0.0

0.5

1.0

1.5

2.0

10

20

30

40

50

60

70

80

90

100

D

N1

Sham ○

Injured ■

Time to 50% recovery (ms)

0

2

4

6

8

10

24h

14d

10

20

30

40

50

60

70

80

90

100

C

N2

Amplitude (mV)

0.0

0.5

1.0

1.5

2.0

Stimulus (% Maximum)

10

20

30

40

50

60

70

80

90

100

E

N2

Time to 50% recovery (ms)

0

5

10

15

20

24h

14d

*
Mild TBI Impairs in Retrograde Axonal Transport
Axonal Transport and JNK Activation

Horiuchi et al. 2007

\[ \beta\text{APP} \]

Injury

MAP3Ks

MAP2Ks

JNKs

\[ \text{Microtubule} \]

\[ \text{Myelinated axons} \]

\[ \text{Soma of oligodendrocyte} \]

\[ \text{Mitochondrion in axoplasm} \]

\[ \text{Node of Ranvier} \]

\[ \text{APP} \]

\[ N \]

\[ C \]
JNK is activated in axons
JNK activation occurs where intra-axonal APP accumulations are observed

<table>
<thead>
<tr>
<th>Time</th>
<th>Nissl</th>
<th>APP</th>
<th>pJNK</th>
</tr>
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<tbody>
<tr>
<td>6 hrs</td>
<td><img src="image1" alt="Nissl Image" /></td>
<td><img src="image2" alt="APP Image" /></td>
<td><img src="image3" alt="pJNK Image" /></td>
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<tr>
<td>24 hrs</td>
<td><img src="image4" alt="Nissl Image" /></td>
<td><img src="image5" alt="APP Image" /></td>
<td><img src="image6" alt="pJNK Image" /></td>
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<tr>
<td>72 hrs</td>
<td><img src="image7" alt="Nissl Image" /></td>
<td><img src="image8" alt="APP Image" /></td>
<td><img src="image9" alt="pJNK Image" /></td>
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</tbody>
</table>
pJNK colocalizes with accumulated APP in injured axons
The JNK3 isoform is implicated in neurodegeneration.
Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene

Derek D. Yang*†‡, Chia-Yi Kuan§§, Alan J. Whitmarsh¶¶, Mercedes Rincón∗§, Timothy S. Zheng*, Roger J. Davis†¶, Pasko Rakic§ & Richard A. Flavell††

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§ Immunobiology Program, Department of Medicine, University of Vermont, Burlington, Vermont 05405, USA
‡ These authors contributed equally to this work
JNK3 deletion reduces JNK activation in white matter below the impact site.
JNK3 deletion reduces APP phosphorylation in white matter below the impact site.
JNK3 deletion does not attenuate impaired axonal transport
A

White Matter

<table>
<thead>
<tr>
<th>Protein</th>
<th>Wild Type</th>
<th>JNK3 Knockout</th>
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</thead>
<tbody>
<tr>
<td>JNK1</td>
<td>54kDa</td>
<td>54kDa</td>
</tr>
<tr>
<td></td>
<td>46kDa</td>
<td>46kDa</td>
</tr>
<tr>
<td>JNK2</td>
<td>54kDa</td>
<td>54kDa</td>
</tr>
<tr>
<td></td>
<td>46kDa</td>
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</tr>
<tr>
<td>JNK3</td>
<td>54kDa</td>
<td>54kDa</td>
</tr>
<tr>
<td></td>
<td>46kDa</td>
<td>46kDa</td>
</tr>
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</table>

B

Cortex

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<th>Wild Type</th>
<th>JNK3 Knockout</th>
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The pan JNK inhibitor SP600125 decreases JNK activation.
The pan JNK inhibitor SP600125 decreases impaired axonal transport.
TBI results in separate populations of injured axons – neurofilament dephosphorylation
TBI results in separate populations of injured axons
Neurofilament Dephosphorylation

• Neurofilament dephosphorylation = Marker of TAI

• May lead to compaction

• TBI → Dephosphorylation by calcineurin → axonal swelling
FK506 reduced neurofilament dephosphorylation
FK506 reduced axonal degeneration
FK506 exacerbated CAP deficits of myelinated axons

Amplitude (mV) vs Current Steps

K, N1, L, N2 graphs showing the effect of FK506 and Vehicle on CAP deficits over 3 and 7 days.
Mild TBI results in
- intra-axonal accumulation of APP
- dephosphorylation of axonal NF
- impairment of axonal transport
- reduction of CAP

The JNK pathway is activated in injured axons
- Deletion of JNK3 reduces APP phosphorylation
- Deletion of JNK3 does not reverse transport impairment
- The pan-JNK inhibitor reduces APP accumulation
Concussion

• Symptoms can include:
  – somatic (headache)
  – cognitive (in a “fog”)
  – emotional (increased lability)
  – sleep disturbances

• Signs can include:
  – physical (loss of consciousness)
  – motor (postural instability)
  – behavioral and affective changes (irritability, depression)
  – cognitive impairments (working memory deficits)

• A clinical diagnosis
Concussion results in TRANSIENT working memory deficits
Concussion Does Not Result in Neurodegeneration, Apoptosis, or Traumatic Axonal Injury

<table>
<thead>
<tr>
<th>Test</th>
<th>Sham-Injured</th>
<th>1 Day Post-Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nissl-myelin (1x)</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Fluoro-Jade B (4x)</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Fractin (10x)</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>APP (10x)</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>
Concussion Induces Transient Hypoexcitability

- Sham-Injured
- 1 Day Post-Injury
- 3 Days Post-Injury

Dopamine Pathways
- Frontal cortex
- Nucleus accumbens
- VTA
- Functions: Reward (motivation), Pleasure, euphoria, Motor function (fine tuning), Compulsion, Perseveration

Serotonin Pathways
- Functions: Mood, Memory processing, Sleep, Cognition
- Hippocampus
- Nucleus raphe

Recording Electrode
Stimulating Electrode

Cg1

Concussion Induces Transient Hypoexcitability

1 mV
5 ms

Peak Amplitude
Latency to Peak
Slope (mV/ms)
Concussion results in an acute increase in extracellular Dopamine
Dopamine Neurotransmission

(http://www.ibibiobase.com/projects/db-drd4/drd4_synthesis.htm)
Internalization of DRD1 following concussion may explain the hypoexcitability of PFC neurons.
Pretreatment with the DRD1 antagonist SCH233390 reverses internalization.
Concussion decreases DRD1 activity

Sham-Injured

1 Day Post-Injury

Baseline 1 µM 10 µM 100 µM

Sham-Injured (n=6)

Injured (n=6)

* * *

#
The DRD1 Partial Agonist SKF 38393 Attenuates Concussion-Induced Working Memory Deficits

Latency to Platform (seconds)

Days Post-Injury

- Sham + Vehicle (n=5)
- Sham + SKF 38393 (n=5)
- Injured + Vehicle (n=7)
- Injured + SKF 38393 (n=7)
At the mildest end of the spectrum (concussion)
Cortical neurons become hypoexcitable
An acute increase in DA release is accompanied by
the internalization of the D1 receptor
Activation of the D1 receptor reverses working memory deficits
Concussion (Mild TBI)

- Behavior deficits
- White matter injury
- Contusion
- Microglia reactivity
- Astrocyte reactivity

Severe TBI

- Headaches/Nausea/Dizziness
- Learning and Memory
- Executive function
- Mood/Emotion
- Motor
- Traumatic axonal injury
- Oligo cell death
- Delayed neuronal death
- Synaptic dysfunction
- Acute neuronal death
- Vascular damage
- Neurodegeneration
- Wallerian degeneration
- Repair
- Neurodegeneration
- Glutamate sink
- $K^+$ sink
- Scar formation
Concussion = mild TBI = TBI

- Cell death (neurons, oligos)
- Axonal degeneration
- Functional deficits
  - Cognition
  - Motor function
  - Neurotransmission
  - Seizures
- Cell dysfunction
  - Glial reactivity (astrocytes, microglia)
Jack and Jill went up the hill to fetch a pail of water
Jack fell down and **broke his crown**
And Jill came tumbling after.

Up got Jack, and home did trot
As fast as he could caper
He went to bed and **bound his head**
With **vinegar** and **brown paper**.

- Children’s nursery rhyme, 1795
Concussion Recovery - Neurosafe (25.4 oz, 720 g)

NeuroSafe | Neuroprotective drink which helps protect Athletes from head injuries and may Reduce Brain Injury and help you Recover Quicker from Concussions.

Add to Shopping Cart (Secure Server)
Currency US Dollars Each $98.00

NeuroSafe Benefits:
• Neuroprotective Drink
• Reduce Risk From Brain Injury
• Help Brain Recover Quicker After Traumatic Brain Injury

NeuroSafe Directions:
Mix One (1) level scoop (included) of NeuroSafe once daily with 8 - 12 oz. of cold water. Mix well and feel the SHIELD!

NeuroSafe Ingredients:
Tocotrienols - Neuroprotectant, blocks glutamate-induced cell death.
Creatine Monohydrate - Offers protection from synaptic dysfunction & cortical tissue loss.
Magnesium - Effective pretreatment prevents vital nutrient depletion.
Zinc - Essential to normal brain function and neuronal repair.
Alpha-Lipoic Acid - Antioxidant used to prevent free radical damage.
Uptake Technology - Specific carb matrix ensures uptake of nutrients from the stomach to the brain.

Other Ingredients: Creatine Monohydrate, Maltodextrin, Dextrose, Fructose, Silicon Dioxide, Tocotrienols, Natural Flavors and Colors, Citric Acid, Natural Mixed Tocopherols, Alpha-Lipoic Acid, Sucralose, Caramine, Ace K Bioperine.

Allergen Statement: Produced in a facacility that also processes wheat, dairy, soy and eggs.

*These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.
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The MORAL imperative – what you can do outside the laboratory

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- Blogs
- Community meetings
- School visits

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- Letters to Congress (local and national)
- Lab tours

Public Outreach

- School visits
- Lab tours
- Support groups