Biomarkers and treatments for mild traumatic brain injury: from bench to fieldside

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The Long-Term Effects of Sports Concussion on Retired Australian Football Players: A Study Using Transcranial Magnetic Stimulation

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**Table 2. Neuropsychological and Fine Motor Control Assessment (n = 20; Mean ± SD)**

<table>
<thead>
<tr>
<th></th>
<th>Elite</th>
<th>Amateur</th>
<th>Combined AF groups</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time</td>
<td>256.42 ± 63.99</td>
<td>262.69 ± 61.82</td>
<td>259.55 ± 55.46</td>
<td>217.44 ± 36.58*</td>
</tr>
<tr>
<td>Stimulus to onset</td>
<td>337.27 ± 50.01</td>
<td>308.84 ± 42.70</td>
<td>323.02 ± 43.86</td>
<td>289.26 ± 36.88*</td>
</tr>
<tr>
<td>Movement</td>
<td>382.79 ± 69.37</td>
<td>370.95 ± 60.13</td>
<td>378.20 ± 64.08</td>
<td>336.76 ± 59.90*</td>
</tr>
</tbody>
</table>
Preliminary findings from NRL

<table>
<thead>
<tr>
<th></th>
<th>Retired Athletes</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41</td>
<td>39.4</td>
</tr>
<tr>
<td>Education (years)</td>
<td>12.1</td>
<td>12.6</td>
</tr>
</tbody>
</table>

**Neuropsychology**
- Attention and concentration
- Processing speed
- Visuospatial ability
- Learning and memory
- Executive function
- Fine motor and balance
- Language

**MRI**
- $T_1$ structural scan
- Susceptibility-weighted (SWI)
- Diffusion tensor imaging (DTI)
- MRS
- Resting state fMRI

Gardner et al., ongoing
Preliminary findings from NRL

Complex Visual Memory

RCFT copy

**RCFT Immediate Recall**

**RCFT Delayed Recall**

**RCFT Recognition**

Gardner et al., ongoing
Preliminary findings from NRL

Word Retrieval

Verbal Reasoning

Fine Motor

Gardner et al., ongoing
Preliminary findings from NRL

Controls

Athletes

Gardner et al., ongoing
Chronic traumatic encephalopathy (CTE)

- Neurodegenerative condition induced by mild brain trauma
  - Severe cognitive, emotional, and motor symptoms
  - Cortical atrophy, neuronal loss, axonal injury, enlarged ventricles, BBB disruption, proteinopathies

adapted from McKee et al., 2009, 2010, 2013
The issue of mild TBI in sport

The New York Times

Pro Football

N.F.L. Agrees to Settle Concussion Suit for $765 Million

Published: August 29, 2013

The National Football League has agreed to pay $765 million to settle a lawsuit brought by more than 4,500 players and their families, largely closing the legal front in the league’s battle against accusations that it concealed what it knew about the dangers of repeated hits to the head.
The issue of concussion in sport

• Cumulative and chronic effects of concussions?
  – Why?

• Can cumulative and chronic effects be prevented?
  – Biomarkers to aid diagnosis and return decisions
  – Therapeutic interventions

• Difficult to study in patients
  – Mild nature of injury
  – Chronic nature of disease process

Animal models may provide important insight
Mild fluid percussion injury model

Adapted from Xiong et al., 2013
## Single concussion model

<table>
<thead>
<tr>
<th>Concussion</th>
<th>Mild FPI in Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amnesia</td>
<td>✔</td>
</tr>
<tr>
<td>Cognitive deficits</td>
<td>✔</td>
</tr>
<tr>
<td>Emotional disturbances</td>
<td>✔</td>
</tr>
<tr>
<td>Impulsivity</td>
<td>✔</td>
</tr>
<tr>
<td>Axonal injury</td>
<td>✔</td>
</tr>
<tr>
<td>Neuroinflammation</td>
<td>✔</td>
</tr>
<tr>
<td>Metabolic dysfunction</td>
<td>✔</td>
</tr>
<tr>
<td>No structural damage</td>
<td>✔</td>
</tr>
<tr>
<td>Negligible neuronal loss</td>
<td>✔</td>
</tr>
<tr>
<td>Recovery</td>
<td>✔</td>
</tr>
</tbody>
</table>

Shultz et al., 2011, 2012; Hylin et al., 2013; DeRoss et al., 2002; Gurkoff et al., 2006
### Repeated concussion and CTE model

<table>
<thead>
<tr>
<th>Repeated concussion and CTE</th>
<th>Repeated mild FPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative and chronic cognitive deficits</td>
<td>✔</td>
</tr>
<tr>
<td>Depression</td>
<td>✔</td>
</tr>
<tr>
<td>Anxiety</td>
<td>✔</td>
</tr>
<tr>
<td>Motor abnormalities</td>
<td>✔</td>
</tr>
<tr>
<td>Neuronal loss</td>
<td>✔</td>
</tr>
<tr>
<td>Neurodegeneration</td>
<td>✔</td>
</tr>
<tr>
<td>Axonal injury</td>
<td>✔</td>
</tr>
<tr>
<td>Oxidative Stress</td>
<td>✔</td>
</tr>
<tr>
<td>Neuroinflammation</td>
<td>✔</td>
</tr>
<tr>
<td>Hyperphosphorylated tau</td>
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Shultz et al., 2012, 2013; DeRoss et al., 2002; Xiong et al., 2013
Mechanisms and treatments

• Hyperphosphorylated tau is pathological hallmark of CTE

McKee et al., 2009, 2010, 2013
Hyperphosphorylated tau

- Tau is a microtubule associated phospho-protein
  - Microtubule stabilization, axonal transport, and neuronal health
- Phosphorylation is regulated by kinases and phosphatases

Kinases and phosphatases as pharmacological targets?

Ballatore et al., 2007; Morris et al., 2011
PP2A (PR55)

- Major tau phosphatase (i.e. dephosphorylates tau)
  - Hypofunction implicated in Alzheimer’s
  - Decreased after experimental TBI

Sodium selenate

- PP2A (PR55) agonist
  - Beneficial in numerous tauopathy models
  - Clinical trials for Alzheimer’s
Study aim and design

**Aim:** Study the role of hyperphosphorylated tau in repeated mTBI, and the therapeutic effects of sodium selenate

**Hypothesis:** Sodium selenate (SS) will increase PP2A (PR55), reduce phosphorylated tau, and improve outcomes

**Experimental groups:**
1. SHAM + VEH
2. SHAM + SS (1 mg/kg/day)
3. 3mFPI + VEH
4. 3mFPI + SS (1 mg/kg/day)
   - Male long-evans rats (n=12-14/group)

<table>
<thead>
<tr>
<th>Injury 1</th>
<th>Injury 2</th>
<th>Injury 3</th>
</tr>
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<tbody>
<tr>
<td>1d</td>
<td>5d</td>
<td>10d</td>
</tr>
<tr>
<td>12w</td>
<td></td>
<td></td>
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Continuous treatment via subcutaneous osmotic pumps

1.) Behavioural testing
2.) MRI
3.) Post-mortem

Tan et al., in preparation
Selenate increases PP2A (PR55)

Tan et al., in preparation
Selenate reduces phospho-tau

Tan et al., in preparation
Selenate reduces atrophy

Ipsilateral Cortex

Ipsilateral Corpus Callosum

Tan et al., in preparation
Selenate reduces axonal injury

SHAM+VEH  SHAM+SS  3mFPI+VEH  3mFPI+SS

Corpus Callosum FA

Corpus Callosum Tracks

Tan et al., in preparation
Selenate reduces behavioral impairments

Tan et al., in preparation
Study summary

• 3mFPI + VEH consistent with CTE

• Selenate treatment:
  – Increases PP2A (PR55)
  – Decreases phosphorylated tau
  – Decreases cortical and axonal injury
  – Decreases cognitive and motor deficits

• Selenate represents a translatable treatment

• PP2A and tau mediated?
  – Other protective properties of sodium selenate?

• Other mechanisms?
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Shultz et al., 2012, 2013; DeRoss et al., 2002; Xiong et al., 2013
Progesterone

• Neurodevelopmental hormone

• Protective in brain injury
  – Anti-inflammatory
  – Reduces ROS and free radical damage
  – Preservation of the Blood Brain Barrier
  – Anti-apoptotic

• Ongoing Phase III clinical trials in moderate-severe TBI
  – ProTECT
  – SyNAPSe

Roof et al, 1993; Vagnerova et al, 2008; Attella et al, 1987; Maghool et al, 2013; Schumacher et al, 2003; Cutler et al, 2007; Xiao et al., 2008; Wright et al., 2006; Chen et al, 1999; Biggio et al, 2001; Cervantes et al, 2002; Giulian and Robertson, 1990
Aim: Study the therapeutic effects of progesterone in repeated mTBI context

Hypothesis: Progesterone will reduce inflammation, apoptosis, and oxidative stress, and improve long-term outcomes after repeated mTBI

Experimental groups:
1. SHAM + VEH
2. SHAM + PROG (8 mg/kg)
3. 3mFPI + VEH
4. 3mFPI + PROG (8 mg/kg)

- Male long-evans rats (n=10-12/group)

Injury 1
Injury 2
Injury 3

1d 5d 10d 15d

1.) Behavioural testing
2.) MRI
3.) Post-mortem

12w

Daily treatment

Webster et al., ongoing
PROG reduces lipid peroxidation

Webster et al., ongoing
PROG reduces cognitive impairment

Webster et al., ongoing
PROG reduces motor deficits

Webster et al., ongoing
Study summary

• 3mFPI + VEH consistent with CTE

• PROG treatment:
  – Decreases lipid peroxidation
  – Decreases cognitive and motor deficits

• Much work to be done

• PROG represents a translatable treatment
The need for concussion biomarkers

- Management relies on neuropsych testing and symptoms
  - Brain abnormalities persist after resolution of symptoms
- Cumulative effects due to 2nd insult during vulnerable period?
- Important to diagnose concussion and determine recovery
- More sensitive biomarkers?
Magnetic resonance imaging (MRI)

- MRI is a common and readily available clinical tool
  - Advanced methods may be sensitive to changes in concussion and CTE

Wright et al., in preparation
Study aims and design

Aims
1. Can MRI detect and assess recovery from mTBI?
2. Relationship between MRI and behavioural biomarkers?
3. Effects of repeated mTBI on these biomarkers?

Groups
1. Sham-injured
2. Single mFPI
3. Two mFPI
   • male Long-Evans rats (n = 8/group)

Wright et al., in preparation
Biomarkers

**Behaviour**
- Water maze
  - Cognition
- Beam task
  - Sensorimotor
- Open field
  - Activity
- Elevated-plus
  - Anxiety
- Forced-swim
  - Depression

**MRI**
- $T_2$-weighted MRI
  - Structural damage
- Perfusion (ASL)
  - Blood flow
- Diffusion-weighted (DWI)
  - Axonal injury/connectivity
- Susceptibility-weighted MRI
  - Hemorrhaging
- MRS
  - Metabolites

Wright et al., in preparation
Cognition

Water Maze Search Time

Total search time (s)

Day Post-injury

Sham
Single mTBI
Repeated mTBI

Wright et al., in preparation
Beam Slips & Falls

- Sham
- Single mTBI
- Repeated mTBI

# Slips and Falls

Day Post-injury

Wright et al., in preparation
Activity

Open Field Distance Traveled

- Sham
- Single mTBI
- Repeated mTBI
T$_2$-weighted MRI

Wright et al., in preparation
Fractional anisotropy (DWI)

Wright et al., in preparation
Tractography

Wright et al., in preparation
Study summary

• Advanced MRI more sensitive than behaviour
  – Consistent with human studies

• Symptom-based biomarkers in concussion management?
  – 2\textsuperscript{nd} injury after behavioral recovery = exacerbated outcomes

• Other biomarkers (e.g. blood, saliva)
# Blood biomarkers

<table>
<thead>
<tr>
<th>Event</th>
<th>Mechanism</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal and Glial Cell Death</td>
<td>Activation of factors triggering necrosis and/or apoptosis</td>
<td>Neuronal: NSE, CK-BB, Glial: S100B, GFAP, MBP</td>
</tr>
<tr>
<td>Axonal Injury</td>
<td>Mechanical injury; Neuronal degeneration</td>
<td>Tau and P-tau, NF-H</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Cytokine release and cellular stress</td>
<td>IL-1β, IL-6, IL-8, TNF-α, IFN-γ</td>
</tr>
<tr>
<td>Metabolic Changes</td>
<td>Hypoxia; altered energy demand, ion homeostasis and neurotransmission; increased repair processes</td>
<td>Ceruloplasmin, HIF-1α</td>
</tr>
</tbody>
</table>
Study summary

• Advanced MRI more sensitive than behaviour
  – Consistent with human studies

• Symptom-based biomarkers in concussion management?
  – 2\textsuperscript{nd} injury after behavioral recovery = exacerbated outcomes

• Other biomarkers (e.g. blood, saliva)

• Applicable to concussed humans?
  – Beginning studies next NRL preseason
NRL Concussion Biomarker Study

• Beginning next offseason
  – Entire team list of NRL club (approx. 40 players)

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**Concussed**

- Pre-season
- Non-concussive match
- 1-3 days after concussion
- Symptom resolution
- 1wk, 1m, 1yr post-resolution

**Non-concussed**

- Neuropsychology, MRI, Blood
Study summary

• Advanced MRI more sensitive than behaviour
  – Consistent with human studies

• Symptom-based biomarkers in concussion management?
  – 2nd injury after behavioral recovery = exacerbated outcomes

• Other biomarkers (e.g. blood, saliva)

• Applicable to concussed humans?
  – Beginning studies next NRL preseason

• Prevents chronic effects?
  – Animal model studies
Conclusions

• mTBI is a serious international health concern
  – Difficult to study in patients

• Complimentary bench to fieldside to bench approach
  – Rodent models allow for rigorous and invasive studies in a time/cost sensitive manner
  – Evidence-based translation to clinical setting
    • Biomarkers
    • Mechanisms
    • Treatments
Acknowledgements

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Thank you!

Comments and Questions?