

THE AUSTRALASIAN  
**NEUROTRAUMA**  
NETWORK

# 12<sup>th</sup> Australasian Neurotrauma Symposium

2<sup>nd</sup> – 3<sup>rd</sup> December 2023



The 12th Australasian Neurotrauma Symposium  
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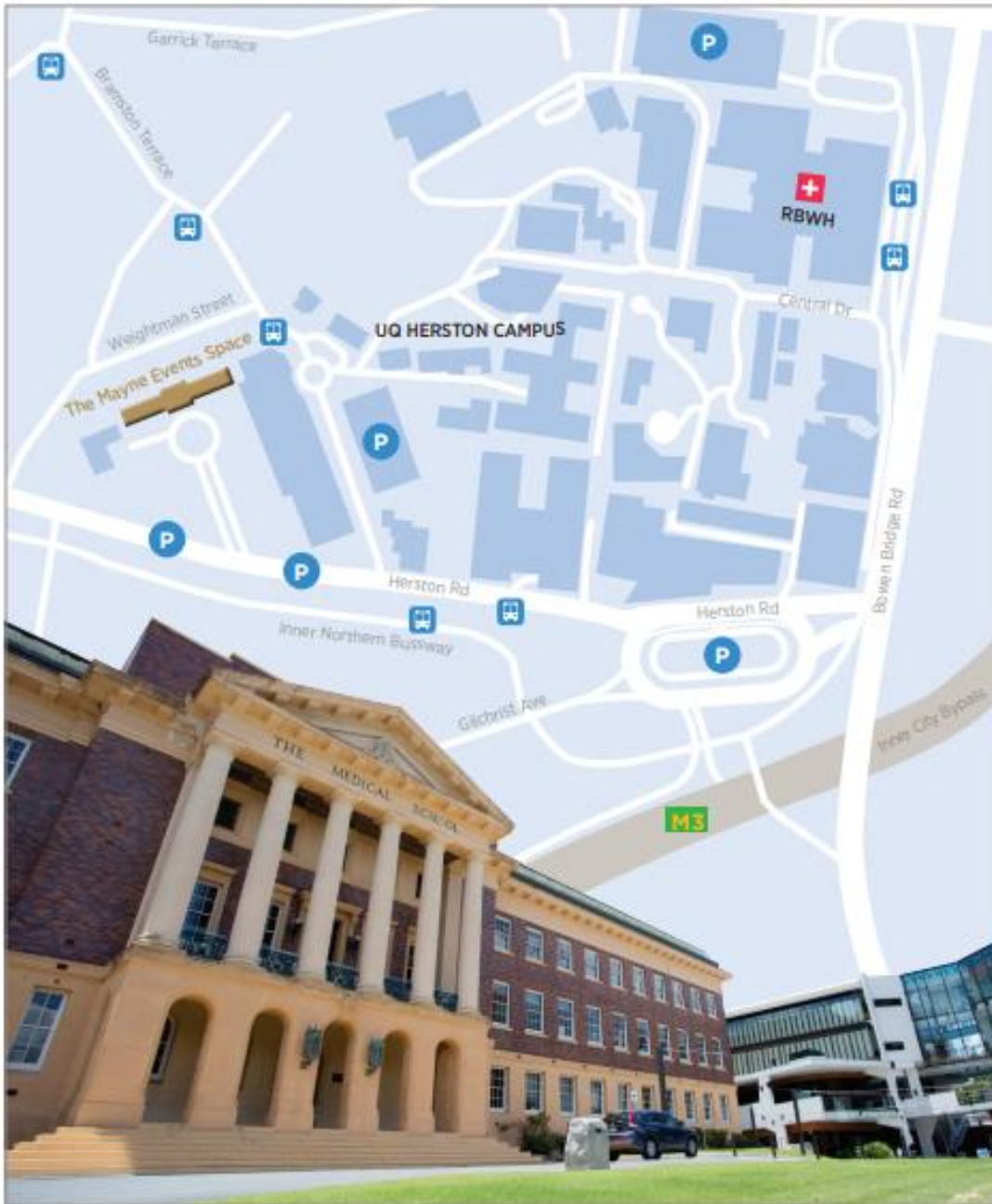
The Australasian Neurotrauma Symposium is an Official Satellite Meeting of ANS 2023

A banner for the Australasian Neuroscience Society (ANS) 2023 41st Annual Scientific Meeting. It features a stylized brain icon on the left, the text "ANS 2023 AUSTRALASIAN NEUROSCIENCE SOCIETY 41ST ANNUAL SCIENTIFIC MEETING" in the center, and the ANS logo on the right. Below the main text, it says "4-7 December 2023 | W, Brisbane, Queensland" and "EXCITING THE NETWORK".

ANS 2023 AUSTRALASIAN NEUROSCIENCE SOCIETY  
41<sup>ST</sup> ANNUAL SCIENTIFIC MEETING  
4-7 December 2023 | W, Brisbane, Queensland  
**EXCITING THE NETWORK**

Australasian  
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The Symposium will be held in the ES Meyer Lecture Theatre, located on level 4 of the Mayne Medical School building (288 Herston Road, Herston).



*ANTS 2023 Organising Committee:*

A/Prof. Marc J. Ruitenberg, The University of Queensland (Chair)

A/Prof Bridgette Semple (Monash University)

Dr Jenna Ziebell (University of Tasmania)

Dr Anna Leonard (University of Adelaide)

## PROGRAM

### Saturday December 2<sup>nd</sup>, 2023 - DAY 1

8:30 Registration & Refreshments

08:50 – 09:00 Welcome & Housekeeping

09:00 – 10:30 Session I: Neuroinflammation & Neuroprotection

Time	Speaker	Title	Page
09:00	Faith Brennan (Queen's University, Canada)	Microglia coordinate cellular interactions during spinal cord repair in mice – <i>keynote</i>	
09:30	Simon O'Carroll (University of Auckland)	The clinically used kappa opioid receptor agonist nalfurafine improves inflammation in a rodent model of spinal cord injury	<u>8</u>
09:45	Seung Jae Kim (University of Queensland)	Central IL-6 trans-signalling prevents tissue damage and motor dysfunction following ischemic stroke in mice	<u>9</u>
10:00	Chidozie C. Anyaegbu (Curtin University)	Poly-arginine-18d (R18d) peptide is neuroprotective following moderate traumatic brain injury in rats	<u>10</u>
10:15	Glenn Yamakawa (Monash University)	Time restricted feeding alters behavioural and physiological outcome to repetitive mild traumatic brain injury	<u>12</u>

10:30 – 11:00 Morning Tea

11:00 – 12:15 Session II: Neurostimulation Approaches to TBI & SCI

Time	Speaker	Title	Page
11:00	Dinesh Palipana (Griffith University)	From patient to doctor to researcher: how a spinal cord injury lead to research for recovery – <i>keynote</i>	
11:30	Bruce Harland (University of Auckland)	Daily electroceutical treatment promotes functional recovery in a rat contusion model of SCI	<u>14</u>
11:45	Ryan Dorrian (University of Adelaide)	Peripheral nerve stimulation via a bio-adhesive graft-antenna for the treatment of spinal cord injuries: An experimental animal study	<u>15</u>
12:00	Athena Stein (University of Queensland & Child Health Research)	Functional network biomarkers of attention improvement after tDCs in children with acquired brain injury: a sham-controlled clinical trial	<u>17</u>

12:15 – 13:30 Lunch (Poster Viewing & Networking Opportunities)

13:30 – 14:00 TALK-TBI: What do individuals know about traumatic brain injury?  
Jenna Ziebell (University of Tasmania)

14:00 – 15:30 **Session III: Secondary Sequelae of Neurotrauma & Neurodegeneration I**

Time	Speaker	Title	Page
14:00	Bridgette Semple (Monash University)	Clinical and preclinical insights into the mechanisms of post-traumatic epilepsy – <i>keynote</i>	
14:30	Yasmine Doust (University of Tasmania)	Neurofilament light chain deletion is associated with increased neuropathology following diffuse traumatic brain injury	<u>22</u>
14:45	Eleanor Bowley-Schubert (University of Adelaide)	Examining the spread of Alzheimer’s-like pathology following a single mild TBI	<u>23</u>
15:00	Chelsie Osterman (University of Auckland)	Utilising multiplex immunohistochemistry to characterise neuroinflammatory changes in Chronic Traumatic Encephalopathy	<u>24</u>
15:15	Srisankavi Sivasankar (University of Adelaide)	The effect of spinal cord injury severity on chronic cognitive dysfunction in a cervical hemi-contusion rodent model	<u>25</u>

15:30 – 16:00 Student Award Presentations & Poster Blitz

16:00 – 17:30 Afternoon Tea & Poster Viewing

18:00 Evening Social (Victoria Park Golf Club, 309 Herston Road, Herston)

## Sunday December 3<sup>rd</sup>, 2023 – DAY 2

09:00 – 9:30 Arrival & Refreshments

09:30 – 11:00 Session I: Acquired Brain Injury: Biomarkers & Prognosis

Time	Speaker	Title	Page
09:30	Tracey Farr (University of Nottingham)	Neuroimaging biomarkers and novel targets for vascular dementia – <i>keynote</i>	
10:00	Jacinta Thorne (Curtin University)	Symptoms associated with exercise intolerance and resting heart rate following mild traumatic brain injury in adults: results from the prospective observational CREST cohort study	<u>27</u>
10:15	James Hickey (Monash University)	Markers of Autonomic Dysfunction in the Sub-Acute Period Following Sport-Related Concussion	<u>29</u>
10:30	Lauren Evans (Monash University)	Associations Between In-Match Head Acceleration Events Measured by Instrumented Mouthguards and Post-Match Serum GFAP and NfL Levels in Amateur Male Australian Football Players	<u>30</u>
10:45	Linfeng Liu (Queensland Brain Institute)	The Added Value of using DTI data to Predict TBI Patients' Outcome using Multi-Modal Data	<u>32</u>

11:00 – 11:30 Morning Tea

11:30 – 12:30 Session II: TBI Sequelae & Neurodegeneration II

Time	Speaker	Title	Page
11:30	Lyndsey Collins-Praino (University of Adelaide)	Forecasting Impairment and Neurodegenerative Disease Risk following Traumatic Brain Injury (FIND-TBI): A focus on the link between TBI and Parkinson's disease	<u>34</u>
11:45	Sam Stuart (University of Queensland)	IL-6 Trans-Signalling Protects Dopaminergic Neurons in Mouse Model of Traumatic Brain Injury	<u>35</u>
12:00	Shannon Stuckey (University of Adelaide)	The Role of Neuroinflammation in Delayed Neurodegeneration following Photothrombotic Stroke in Rats	<u>36</u>
12:15	Rosie Costigan-Dwyer (University of Adelaide)	Exploring the long-term neuroinflammatory and blood-brain-barrier alterations associated with secondary neurodegeneration in an ovine stroke model	<u>37</u>

12:40 – 13:00 Prizes, Concluding Remarks & Farewell

DAY 1 – SESSION I

# Neuroinflammation & Neuroprotection

## The Clinically Used Kappa Opioid Receptor Agonist Nalfurafine Improves Inflammation In A Rodent Model Of Spinal Cord Injury

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**Background:** The selective KOR receptor agonist U50,488 has shown protection in models of spinal cord injury. However, U50,488 causes a number of adverse side-effects, limiting its potential use in the clinic. Newer KOR agonists have potential to address this issue. The KOR agonist nalfurafine has greatly enhanced potency, is well-tolerated and has fewer adverse effects compared to U50,488. Nalfurafine is biased towards the G-protein pathway activation compared to U50,488 with a lower potency for p38 activation, which is a major contributor to SCI and involved in SCI inflammation. Therefore, we trailed different doses of nalfurafine for its ability to improve inflammation following spinal cord injury.

**Methods:** Male and female Sprague-Dawley rats were given a moderate (175 kDyne) contusion spinal cord injury at T10 using the Infinite Horizons impactor. Animals were randomly divided into 3 groups (n = 8/group) and received daily i.p injections of vehicle, 0.03 or 0.1 mg/kg nalfurafine daily for 4 weeks. Animals underwent open field testing to measure hind limb function and co-ordination using the Basso-Beattie-Bresnahan (BBB) rating scale and tissue was collected at the end of the study for immunohistochemical analysis.

**Findings:** Treatment with 0.03 mg/kg showed a significant decrease in total and M1 microglia compared to vehicle (one-way ANOVA, Bonferroni's post-hoc test,  $p < 0.05$ ). No significant difference in M2 microglia was seen between the groups. No significant difference was seen with the 10 mg/kg dose. No difference in astrocytes was seen between the groups. Although not powered to determine a difference in the BBB score, 0.03 mg/kg treated animals showed improved hind-limb function at 4 weeks (control,  $10.4 \pm 0.64$  vs nalfurafine,  $12.2 \pm 0.28$ ).

**Conclusion:** This preliminary study suggests that treatment with nalfurafine may be a potential treatment for spinal cord injury.

**Funding:** CatWalk Spinal Cord Injury Trust

## Central IL-6 *Trans*-Signalling Prevents Tissue Damage And Motor Dysfunction Following Ischemic Stroke In Mice

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Queensland Brain Institute, The University of Queensland

Globally, stroke affects 1 in 4 people during their lifetime, with survivors experiencing poor functional and neurological outcomes due to ischemic brain damage. Clinical and pre-clinical studies show that ischemic stroke increases the plasma levels and neural bioactivity of the cytokine interleukin-6 (IL-6), with high IL-6 levels correlated with increasing severity of neural damage, as measured by infarction volume and reduced neurological outcomes. Stimulation of IL-6 *trans*-signalling pathway using a designer fusion protein, hyperIL-6, is profoundly neuroprotective after brain injury, sparing neurons from cell death and markedly improving functional outcomes. Therefore, we hypothesised that upregulation of IL-6 has neuroprotective benefits to ischemic damage in neural tissue. Here, we tested whether hyperIL-6 prevents neural tissue damage and promotes functional recovery after ischemic stroke. C57BL/6J mice underwent either sham-surgery or 1hr of middle cerebral artery occlusion (MCAO; to mimic ischemic stroke), followed briefly thereafter with injection of saline or hyperIL-6 into the lateral ventricles. At 24hrs post-MCAO, mice underwent Bederson neurological scoring and behavioural testing (tapered beam task), and infarct volumes were measured. Stimulating IL-6 *trans*-signalling using hyperIL-6 in an MCAO model of ischemic stroke significantly improved neurological scores and restored fine motor function. Compared with saline, hyperIL-6 treatment reduced infarct volume after MCAO by approximately 50%. IL-6 *trans* pathway plays a significant neuroprotective role after MCAO by preventing the spread of ischemic tissue damage. In turn, this improves neurological and behavioural outcomes.

## Poly-Arginine-18D (R18D) Peptide Is Neuroprotective Following Moderate Traumatic Brain Injury In Rats

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**Background:** Moderate traumatic brain injury (mod-TBI) occurs from many incidents including falls and vehicle crashes. Mod TBI causes secondary degeneration of initially spared tissue around, or distal from, the primary damage, leading to diffuse axonal damage, neuroinflammation and neurological dysfunction. There are no treatments targeting secondary degeneration in neurotrauma. Poly-arginine-18 D enantiomer (R18D) is a small cationic peptide with preclinical efficacy in multiple stroke models. We hypothesised that R18D will be neuroprotective in mod-TBI, since TBI and stroke share neurodegenerative features.

**Methods:** Male Sprague Dawley rats received mod-TBI using an impact-acceleration model of diffuse axonal injury. R18D (3mg/kg; n=10) or saline vehicle (n=10) was administered intravenously 30min post mod-TBI. Sham injured rats (n=10) received saline vehicle. At 1d, 5d and 10d post mod TBI, the open-field (anxiety), beam-walk and rotarod (coordination) tests were conducted. At 11d, brains were immunohistochemically assessed for axonal pathology ( $\beta$  amyloid precursor protein [APP], neurofilament heavy [NF-H]) and neuroinflammation (Iba1+ microglia). Behaviour was analysed using repeated measures two way ANOVA with Tukey's post-hoc. One-way ANOVA with Tukey's post-hoc was used for immunohistochemistry data. Investigators were blind to group assignment until data analysis was complete.

**Findings:** R18D prevented mod TBI induced weight loss at acute (5d, p=0.049) and subacute (10d, p=0.004) timepoints. R18D reduced travel velocity of injured animals in the open-field at 10d (p=0.046). Vehicle treated mod-TBI rats showed increased thigmotaxis over time (p=0.0425). In contrast, no changes in thigmotaxis were observed in R18D treated mod-TBI rats, suggesting that R18D may ameliorate anxiety-like behaviour. R18D reduced APP (p=0.010), NF-H (p=0.001) and Iba1 density of immunoreactivity (p=0.008) in the pyramidal tract post mod TBI. R18D also decreased Iba1 density of immunoreactivity in the corpus callosum (p=0.048).

**Interpretation:** R18D mitigates mod TBI induced weight loss, anxiety-related movement dysfunction, axonal damage and neuroinflammation, particularly in the pyramidal tracts,

indicating potential regulation of movement patterns, eating behaviour and inflammatory associated neurodegeneration.

**Funding:** Argenica Therapeutics Limited, the owner of R18D.

## Time Restricted Feeding Alters Behavioural And Physiological Outcome To Repetitive Mild Traumatic Brain Injury.

Aidil Zaini<sup>1</sup>, Pooranee K Morgan<sup>2</sup>, Elaina Vlassopoulos<sup>3</sup>, Marissa Sgro<sup>3</sup>, Crystal Li<sup>3</sup>, Sabrina Salberg<sup>3</sup>, Jennaya Christensen<sup>3</sup>, Andrew J Murphy<sup>2</sup>, Peter J Meikle<sup>2</sup>, Benjamin J Marsland<sup>1</sup>, Richelle Mychasiuk<sup>3</sup> & Glenn R Yamakawa<sup>3</sup>

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**Background:** Time restricted feeding has numerous health benefits including reduced body weight, disease risk, inflammation, and increases to gut microbiota diversity. Conversely, feeding at the wrong time, alters biological synchrony between the brain and gut with evidence suggesting that desynchrony can exacerbate symptoms following repetitive mild traumatic brain injury (RmTBI). The aim of this study was to determine if day or night restricted feeding could alter outcomes following RmTBI.

**Methods:** Male and female adolescent Sprague Dawley rats (n=72) received three mild traumatic brain injuries, or sham injuries. Immediately following the first injury, rats were randomly assigned to have food restricted to the active period (night) or inactive period (day). The animals were administered a behavioural test battery to assess for post-concussive symptomology. Once completed, the hypothalamus was collected for RNAseq, while the small intestine, serum, and prefrontal cortex were collected for lipidomics.

**Findings:** Night fed animals showed higher body weight, body mass index, and food consumption but lower brain weights, more footslips on the beamwalk test, and altered thermal nociception. Day fed males showed altered hypothalamic transcriptomes that were not apparent in the females. These changes involved downregulation of sequences involved in immune function and upregulation of epigenetic, bone, cellular function sequences. Injured animals no longer showed these transcriptome changes. Lipidomics analysis is ongoing.

**Interpretation:** The day fed animals were forced to entrain to the new eating schedules but given that they also received injuries and behavioural testing during the day this may have been beneficial. RNAseq showed males and females had different responses to feeding and injury and that injury may be interfering with compensatory transcriptome changes in response to challenges. This study highlights the importance of sex and food timing in response to brain injury.

**Funding:** This work was supported by the GIN Discovery program seed funding awarded to AZ and GRY

# Neurostimulation Approaches to TBI & SCI

## Daily Electroceutical Treatment Promotes Functional Recovery in a Rat Contusion Model of SCI

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**Background:** Spinal cord injury (SCI) is a devastating condition that can result in permanent neurological impairment and profoundly affect quality of life. Regenerative treatments for SCI based on electrical stimulation developed sufficiently to be tested in a clinical trial  $\approx$  2 decades ago. However, technologies at the time were incapable of achieving safe and stable electric fields localised within the spinal cord. Our approach is to use an ultrathin polyimide bioelectronic implant inserted beneath the dura mater with super capacitive electrodes capable of delivering a much stronger but safe electric field across the injury site.

**Methods:** Three groups of 2-3 month old female Sprague-Dawley rats ( $n = 8-10$ ) underwent a laminectomy of the T10-T12 spinal processes and bioelectronic implants were inserted into the subdural space. Two groups then received a 175 kdyn contusion at T11 using an Infinite Horizons Impactor, while the third group received no injury. Starting the next day, one SCI group received a daily 1-hour bi-phasic 1.66 Hz electrical stimulation treatment from electrodes positioned either side of the injury. Treatment was administered 5 days per week for 12 weeks during which all groups were assessed weekly using the BBB-scale in an open field, an error ladder task, and an electronic Von Frey task.

**Findings:** Rats receiving the electroceutical treatment showed significant improvement in BBB-scores indicating recovery of hind limb function from week 6 onwards compared with non-treated controls (Two-way repeated ANOVA, Bonferroni post-hoc's,  $p$ 's  $< 0.05$ ). Electroceutical treatment also restored touch sensitivity to the Von Frey filament to a similar level to non-injured rats.

**Interpretation:** Preliminary data suggests that subdural electrical stimulation is a promising treatment for SCI. Blinded re-assessment of BBB and error ladder videos is currently underway alongside histology.

**Funding:** CatWalk Trust, Health Research Council NZ, Neurological Foundation

## Peripheral Nerve Stimulation Via A Bio-Adhesive Graft-Antenna for the Treatment of Spinal Cord Injuries: An Experimental Animal Study

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**Background:** Peripheral nerve stimulation (NS) represents a promising intervention for spinal cord injury (SCI), having demonstrated functional improvements and neuropathic pain relief. However, current NS devices are large, invasive, and incorporate complex circuitry. These factors risk device failure, restrict clinical applications and create access barriers. Hence, we have developed the graft-antenna, a biodegradable device that facilitates wireless NS. While the graft-antenna has not been utilised post-SCI, the device has successfully promoted regeneration following nerve transections. Similarly, peripheral NS may improve outcomes post-SCI by promoting tissue regeneration and modulate neuroinflammation following SCI. As such, we hypothesised that NS via the graft-antenna would alleviate neuropathic pain and improve motor and bladder function post-SCI by modulating neuroinflammation and promoting tissue regeneration.

**Methods:** Male Sprague Dawley rats (11-weeks-old, n=7/group, Ethics: M-2021-038) were randomised into groups (naïve, sham, SCI-only, SCI + unilateral stimulation [US], or SCI + bilateral stimulation [BS]), and endpoints (3-days/8-weeks post-SCI). Graft-antenna's (US/BS) or an inactive adhesive (SCI-only) were implanted on the sciatic nerve one week before T10 SCI induction (200kdyne, Infinite Horizon). NS was administered immediately post-injury and weekly thereafter (1Hz, 1hr/antenna). Researchers blinded to treatment groups assessed neuropathic pain (von Frey, place-escape-avoidance-paradigm [PEAP]), motor function (BBB open-field, Horizontal Ladder), and bladder function (Void spot assay, retained urine weight) at various timepoints post-SCI.

**Findings:** Preliminary results (n=completed to date/total number) suggest the graft-antenna may alleviate neuropathic pain, with SCI-only animals having a lower foot withdrawal force (-21.66% change from baseline) than NS animals (US: -5.97%, BS: -8.68%) and exhibiting greater pain-avoidance behaviour. However, no improvement in motor or bladder function in NS groups has been detected. Ongoing analysis is evaluating potential cellular and molecular mechanisms.

**Interpretation:** If preliminary findings are confirmed, the graft-antenna would provide a more accessible treatment option to improve quality of life for individuals suffering neuropathic pain post-SCI.

**Funding:** Neurosurgical Research Foundation, Morton Cure Paralysis Fund.

## Functional Network Biomarkers of Attention Improvement After tDCs in Children with Acquired Brain Injury: A Sham-Controlled Clinical Trial.

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**Background:** About 1 in 4 children who sustain an acquired brain injury (ABI) have attention difficulties impacting education, employment, and community functioning. These problems relate to dysfunction in brain networks such as the executive and salience networks. Transcranial direct current stimulation (tDCS) in adult ABI has shown potential to improve attention but response to treatment varies. We investigated whether pre-tDCS functional brain connectivity predicted tDCS-related attention improvement in children with ABI.

**Methods:** In a randomised, single-blind, counterbalanced, sham-controlled clinical trial, n=15 children with ABI (age: mean 12.7 (SD 3.33) years) and n=15 healthy controls (HCs) of similar age and sex received three single tDCS sessions during attention training (Go/No-Go and continuous performance): 20 minutes, 1 mA, 5x5cm, bilateral (1) dorsolateral prefrontal cortex, (2) inferior frontal gyrus, and (3) sham. Flanker reaction time (RT) was measured immediately pre- and post-tDCS. Brain connectivity was estimated from high-density (128-channel) EEG and derived using network-based statistics. Using linear mixed modelling, the effect of connectivity on post-tDCS RT (controlling for pre-tDCS RT) was explored.

**Results:** There was no difference in post-tDCS flanker RT across tDCS sessions in ABI or HC participants. In the ABI group only, fronto-parietal delta or theta connectivity were significantly associated with post-tDCS RT (delta:  $F=11.73$ ,  $p=0.004$ ; theta:  $F=9.47$ ,  $p=0.009$ ) where increased connectivity was associated with slower post-tDCS RT.

**Conclusions:** Similar to Castellanos et al. 2010, greater fronto-parietal slow wave connectivity at baseline may be associated with poor recovery from TBI and may predict poorer performance following combined tDCS and cognitive training, but not tDCS alone.

# Consumer Engagement How & where to?

## TALK-TBI: What Do Individuals Know About Traumatic Brain Injury?

Jenna M. Ziebell<sup>1</sup>, Hannah L. Fair<sup>1</sup>, Yasmine V. Doust<sup>1</sup>, Claire Eccleston<sup>1</sup>, James C. Vickers<sup>1</sup>, Peta S. Cook<sup>2</sup>, Melinda Fitzgerald<sup>3</sup>, Anna E. King<sup>1</sup>, Kathleen Doherty<sup>1</sup>, Christine Padgett<sup>4</sup>

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TALK-TBI aims to improve the understanding of TBI throughout Australia. To do this, we first need to understand what individuals currently know about TBI and the experiences of individuals living with TBI. Together, these pieces of data will enable us to incorporate evidence-based information which fills identified knowledge gaps into the Understanding TBI massive open online course (MOOC). To determine individual's knowledge of TBI we have integrated a modified common misconceptions of TBI survey into the understanding TBI MOOC. This survey is a previously validated instrument that includes new items related to concussion. To date, 2,322 Australians have completed the misconceptions survey. The median age is 48 years (range 16-89), with a very even spread from 20-69 years of age. Most participants (1,501; 64.6%) reported working in allied health (e.g. OT, nurse, care, paramedic, physiotherapy). Of the 2,322 participants, 516 (22.2%) have a family member who has sustained a TBI, and 1,954 (84.1%) identify as female. When worldwide data are analysed the cohort distribution is similar. In addition to misconceptions of TBI, we are recording lived experiences of individuals who have sustained a TBI or the perspective of those caring for these individuals. These experiences are captured via a variety of mechanisms including journals and interviews. Data collection is ongoing. The data from surveys and lived-experience will identify knowledge gaps that can be addressed by improvements to our educational programs, e.g. TBI MOOC. Once the enhanced suite of educational programs is released, we will evaluate their impact on knowledge gain.

# Secondary Sequelae of Neurotrauma & Neurodegeneration ~ I

## Neurofilament Light Chain Deletion Is Associated With Increased Neuropathology Following Diffuse Traumatic Brain Injury.

Yasmine V. Doust (PhD), Ross C. Langley (BMedResHons), Emily A. Garratt (BMedRes), Anna E. King (PhD), Jenna M. Ziebell (PhD)

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Breakdown of the axonal cytoskeleton is a key component in traumatic brain injury (TBI)-induced neuropathology. We hypothesised that an altered neuronal cytoskeleton (neurofilament knock-out; NFL-KO) would attenuate neuropathology after diffuse TBI, evidenced through amyloid precursor protein (APP) accumulation. Diffuse TBI was modelled in male and female NFL-KO and wildtype (C57/Bl6; WT) mice by midline fluid percussion injury. Immunohistochemical analysis was conducted on 132 brains from naïve and injured mice at 3 hours, 1- and 3-days post-injury and matched for age, biological sex, and genotype (n = 5-6/group). The number of APP-positive (APP+) axonal profiles were quantified using the *trainable WEKA segmentation* plugin in Fiji (ImageJ) and analysed using a mixed effects negative binomial regression model in the R statistical computing environment. TBI-induced neuropathology (APP-positive axons) was evident in the corpus callosum ( $p < 0.0001$ ), primary sensory barrel field ( $p < 0.0001$ ), dentate gyrus (DG) of the hippocampus ( $p = 0.0003$ ), and ventral posteromedial nucleus (VPM) of the thalamus ( $p = 0.0002$ ), regardless of time post-injury, genotype, or biological sex. Surprisingly, APP+ axonal injury was more extensive in all brain regions of injured NFL-KO mice compared with WT (corpus callosum:  $p = 0.0133$ ; primary sensory barrel field:  $p = 0.0470$ ; DG:  $p = 0.0345$ ; VPM:  $p = 0.0130$ ), regardless of time post-injury or biological sex. Biological sex differences were also evident across time post-injury where APP+ neuropathology was more robust in male mice compared with females that occurred in a region-specific manner (corpus callosum:  $p = 0.0468$ ; primary sensory barrel field:  $p = 0.0064$ ; VPM:  $p = 0.0302$ ). These results show that in the initial stages following TBI, the structure of the axonal cytoskeleton is important in the sequelae of diffuse TBI pathophysiology in both biological sexes. This research was funded by the JO and JR Wicking Trust.

## Examining The Spread Of Alzheimer's-Like Pathology Following A Single Mild TBI

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Traumatic brain injury (TBI) is one of the strongest environmental risk factors for the later development of neurodegenerative diseases, including Alzheimer's disease (AD), that are characterised by the accumulation of hyperphosphorylated tau (p-tau). This p-tau spreads from neuron to neuron following a stereotypical pattern originating in the locus coeruleus, a small brainstem nuclei, with widespread noradrenergic projections to the whole brain including the pre-frontal cortex (PFC) and hippocampus (CA1 and DG). Evidence indicates LC neurodegeneration to be the earliest site of AD pathology and may be particularly vulnerable to the development of p-tau following TBI. This study aimed to examine the spread of p-tau aggregates in a mild diffuse model of TBI in non-transgenic mice and confirm the relationship between previously established cognitive deficits and AD-pathology. It was hypothesised that p-tau pathology and degeneration would initiate within the LC and spread to synaptically-connected regions, PFC and hippocampus. 10-week-old mixed sex C57BL/6J mice (n=14-16 per group) were randomly allocated to either sham or TBI surgery. TBI was delivered via the Closed Head Impact Model of Engineered Rotational Acceleration model (CHIMERA), and animals were taken to 24h, 1m and 6m. Immunohistochemical analysis was undertaken to assess neurodegeneration and inflammation within the LC, PFC and hippocampus. Following mTBI, previous work established injury-dependent cognitive deficits within this cohort at 6m in prefrontal cortex executive function ( $p=0.0360$ ), hippocampal dependent spatial memory ( $p=0.0495$ ) and anxiety-like behaviour ( $p=0.0092$ ). Development of p-tau within the LC was found to have an age effect, not injury from 24h PI ( $p=0.0103$ ), suggesting pathology does not initiate here, however, no neuronal loss was observed within the LC. CA1 observed a decrease in p-tau with age ( $p=0.0002$ ), potentially indicating an anatomical location from which p-tau may spread from. Inflammation may play a role with a comparable age-dependent increase in average process length per microglial cell within PFC ( $p=0.0060$ ), CA1 ( $p=0.0112$ ) and DG ( $p=0.0028$ ), suggesting inflammation decreased with age following injury. These results suggest normal ageing causes development of p-tau pathology and injury did not exacerbate this effect, whilst age seems to reduce inflammation. Further work required to elucidate the exact mechanism driving these injury-dependent cognitive deficits.

## Utilising Multiplex Immunohistochemistry To Characterise Neuroinflammatory Changes In Chronic Traumatic Encephalopathy

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**Background:** Chronic Traumatic Encephalopathy (CTE) is a neurodegenerative disease caused by repetitive head injuries and defined by the presence of hyperphosphorylated tau (p-tau) within the depths of the cortical sulci.

Neuroinflammation has known associations with both neurodegeneration and traumatic brain injury, however, there has been limited characterisation of gliosis in CTE compared to other tauopathies such as Alzheimer's disease (AD).

Previous studies reported astrogliosis at the grey-white matter interface (GWMI) in CTE post-mortem tissue, but whether this is specific to CTE is unknown. We sought to further characterise GWMI astrogliosis using a range of markers for glial reactivity and compare the distribution of these markers between CTE, AD and neurologically normal brains.

**Methods:** We examined the superior frontal gyrus from 9 CTE, 9 AD, and 9 neurologically normal post-mortem human brains. Multiplex immunohistochemistry was utilized to label 36 proteins of interest relating to glial and vascular changes and pathological proteins. This was achieved via an iterative process of 5-plex immunohistochemical labelling and imaging on the same tissue section. For analysis, all images acquired from a single section were combined and the mean fluorescent intensity of each marker was measured in the GWMI.

**Results:** Markers associated with glial reactivity, such as GFAP, CHI3L1, CD44, CD68, and L-ferritin were increased at the GWMI in CTE and AD compared to normal cases, with more focal reactivity around the vasculature in CTE and amyloid-beta plaques in AD. There were also qualitative differences in the distribution pattern of astrocyte proteins AQP4 and GLT1 throughout the grey matter between CTE and AD.

**Conclusion:** Markers of reactive gliosis within the GWMI are increased relative to the grey matter in CTE and AD cases compared to neurologically normal cases. These results implicate the GWMI as a region susceptible to inflammatory changes in a tauopathy caused by head injury.

## The Effect Of Spinal Cord Injury Severity On Chronic Cognitive Dysfunction In A Cervical Hemi-Contusion Rodent Model.

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**Background:** Spinal cord injury (SCI) individuals are 13 times more likely to develop chronic cognitive dysfunction (CCD) with age. Previous studies have acknowledged CCD post-SCI but overlooked SCI's heterogeneity. Immediate outcomes are severity-dependent, with severe SCIs resulting in poor prognosis. However, the impact of severity on CCD remains unclear. This study aims to explore SCI severity's effect on long-term cognition.

**Methods:** 12-week-old male Sprague-Dawley rats underwent a C5 hemi-contusion (Infinite Horizon device, ethics number: M-2020-083) and were randomised into severity groups (sham, mild and severe SCI) and timepoints (3- and 6-months post-SCI, n=10-14 group/timepoint). At pre-determined timepoints, behavioural tasks assessed specific cognitive domains (Novel object recognition, Barnes maze, and Five-choice reaction time), whilst motor function was assessed using the Irvine, Beatties, and Bresnahan (IBB) scale and beam test to evaluate fine and gross motor functions, respectively. All data were analysed via two-way ANOVA, except for Barnes maze old escape-box (one-way).

**Findings:** No significant differences in any of the cognitive assessments were observed between groups at either timepoints. However, the IBB scores indicate significantly poor fine motor function in the severe group compared to the mild group at 3-months ( $5.86 \pm 0.49$  vs  $8.88 \pm 0.08$ ) and 6-months ( $5.89 \pm 0.66$  vs  $8.61 \pm 0.23$ ). The beam test revealed significant differences in ipsilateral forelimb slips between sham and severe groups at 1- ( $12.57 \pm 5.30$  vs  $60.13 \pm 10.36$ ), 4- ( $10.54 \pm 3.42$  vs  $38.93 \pm 6.93$ ;  $p=0.03$ ), and 12-weeks ( $12.10 \pm 3.94$  vs  $39.26 \pm 7.13$ ;  $p=0.02$ ) for the 3-month group, and at 1-week ( $10.14 \pm 3.94$  vs  $51.86 \pm 9.83$ ) for the 6-month group.

**Interpretation:** The results from this study confirm graded injury severity on motor outcomes, comparable to others within the literature. The lack of CCD observed at 3- and 6-months post-SCI, suggests that CCD may not be detected until after 6-months post-SCI. However, future histological assessment of cognitive regions of the brain will provide further insight into early (3-6 month) changes.

**Funding:** NeuroSurgical Research Foundation and Brain Foundation

# Acquired Brain Injury: Biomarkers & Prognosis

## Symptoms Associated With Exercise Intolerance And Resting Heart Rate Following Mild Traumatic Brain Injury In Adults: Results From The Prospective Observational CREST Cohort Study

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**Background:** People may experience a myriad of symptoms after mild traumatic brain injury (mTBI), but the relationship between symptoms and objective assessments is poorly characterised. This study sought to investigate the association between symptoms, resting heart rate (HR) and exercise tolerance in individuals following mTBI, with a secondary aim to examine the relationship between symptom-based clinical profiles and recovery.

**Methods:** Prospective observational study of adults aged 18-65 years who had sustained mTBI within the previous 7 days. Symptoms were assessed using the Post-Concussion Symptom Scale, HR was measured at rest and exercise tolerance assessed using the Buffalo Concussion Bike Test. Symptom burden and symptom-based clinical profiles were examined with respect to exercise tolerance and resting HR.

**Findings:** Data from 32 participants were assessed (mean age  $36.5 \pm 12.6$  years, 41% female,  $5.7 \pm 1.1$  days since injury). Symptom burden (number of symptoms and symptom severity) was significantly associated with exercise intolerance ( $p=0.002$  and  $p=0.025$ , respectively). Physiological and vestibular-ocular clinical profile composite groups were associated with exercise tolerance ( $p=0.001$  and  $p=0.014$  respectively), with individuals who were exercise intolerant having higher mean number of symptoms in each profile compared to those who were exercise tolerant. Mood-related and autonomic clinical profiles were associated with higher resting HR (greater than 80bpm) ( $p=0.048$  and  $p=0.028$  respectively), suggesting altered autonomic response for participants with symptoms relating to this profile. After adjusting for age and mechanism of injury (sport- or non-sport related), having a higher mood-related clinical profile was associated with persisting symptoms at three months post-injury (adjusted odds ratio = 2.08; 95% CI=1.11-3.90;  $p=0.013$ ).

**Interpretation:** Symptom-based clinical profiles, in conjunction with objective measures such as resting HR and exercise tolerance, are important components of clinical care for those having sustained mTBI. These results provide preliminary support for the concept that specific symptoms are indicative of autonomic dysfunction following mTBI.

## Markers Of Autonomic Dysfunction In The Sub-Acute Period Following Sport-Related Concussion

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**Context:** Return-to-play (RTP) decisions after sport-related concussion (SRC) heavily hinge on symptoms. However, neurobiological recovery may extend beyond symptom resolution, prompting a need for objective tools to delve into recovery facets. While there is some evidence that SRC may alter autonomic function, including markers such as heart-rate-variability (HRV) and pupillometry, a knowledge gap at the typical RTP clearance period of 11-12 days exists. Our aim was to compare HRV and pupillometry measures between athletes with and without SRC specifically at the 11-day mark. Additionally, we explored the associations of these measures with symptoms and biomarkers of glial (GFAP) and axonal (NfL) injury.

**Methods:** A prospective, cross-sectional study, of thirty-seven male and female amateur Australian football players (20 SRC, 17 non-SRC) was conducted. Blood collection and symptom questionnaires were completed at 24-hours and 11-days. At 11-days, a five-lead ECG setup measured HRV. Additionally, a virtual reality tool (CONVIRT) with eye tracking technology measured pupillometry. Both measures were recorded over 30-minutes, during which participants completed cognitive and meditation tasks in CONVIRT.

**Findings:** Multiple HRV metrics, including low-frequency power and R-R interval standard deviation, were significantly reduced in SRC compared to control athletes. Pupillometry measures did not differ between SRC and controls, and no measures correlated with symptom burden. A positive correlation between serum NfL and HRV was identified in SRC athletes.

**Interpretation:** The decrease in HRV at 11-days implies its potential as an indicator of autonomic dysfunction after SRC and may offer promise for informing RTP decisions. The unexpected positive association between serum NfL and HRV raises intriguing questions, and may suggest a differential HRV response in the presence of more significant axonal injury. Subsequent studies in a larger sample are essential to delve deeper into this and establish how HRV metrics evolve over the weeks following SRC.

**Funding:** This study was funded by a grant (2020/GNT2002689) awarded to SM by the NHMRC, and internal funding through Monash University.

## Associations Between In-Match Head Acceleration Events Measured By Instrumented Mouthguards And Post-Match Serum GFAP And NfL Levels In Amateur Male Australian Football Players

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**BACKGROUND:** There is increasing concern for potential neurobiological consequences of repeated exposure to head acceleration events (HAEs) in collision sports. No studies have investigated how head impact kinematics from non-concussive impacts align with sensitive blood biomarkers of axonal and glial pathology.

**AIM:** To quantify non-concussive head impact exposure and the association with emerging blood biomarkers in male amateur Australian football players.

**METHODS:** Forty-one amateur male Australian football players from the Victorian Amateur Football Association were recruited and underwent in-season (24h post-match) and post-season blood collections. Twenty-six players wore custom-fitted HitIQ Nexus A9 Instrumented Mouthguards, which measure peak linear (PLA) and rotational (PRA) acceleration per impact. Game footage was used to verify impacts and code the match play situations in which HAEs occurred. Blood biomarker quantification was completed using a Simoa HD-X Analyser.

**FINDINGS:** In-season levels of serum GFAP ( $p=0.046$ ), NfL ( $p=0.001$ ), and p-tau181 ( $p<0.0001$ ) were significantly elevated compared to post-season ( $n=24$ ). Maximum PLA ( $p=0.029$ ) and PRA ( $p=0.046$ ) in a single game was associated with post-match GFAP levels. Similarly, cumulative PLA ( $p=0.010$ ) and PRA ( $p=0.009$ ) in a single game was associated with post-match GFAP levels. No such post-match correlations were observed for NfL and p-tau181; however, cumulative PLA ( $p=0.011$ ) and PRA ( $p=0.027$ ) across two consecutive games correlated with changes in NfL levels across this period. Tackles accounted for 41% ( $n=202$ ) of video-verified true positive HAEs ( $n=484$ ).

**INTERPRETATION:** This data suggests that non-concussive impacts sustained during one or two matches may lead to subtle increases in blood markers linked to brain cell injury. Ongoing validation studies with a larger, diverse sample, including females, are in progress.

**FUNDING:** Australian NHMRC Grant (2020/GNT2002689) awarded to SM, and internal funding through Monash University.

## The Added Value Of Using DTI Data To Predict TBI Patients' Outcome Using Multi-Modal Data

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Accurate prognosis of Traumatic Brain Injury (TBI) outcomes is crucial for healthcare prioritization, rehabilitation planning, and managing patient and family expectations. This study investigates the predictive power of clinical biomarkers and neurological assessments within two weeks post-TBI in forecasting Glasgow Outcome Scale Extended (GOSE) scores at 3, 6, and 12 months. Additionally, it explores whether incorporating diffusion Magnetic Resonance Imaging (MRI) acquired at the two-week mark enhances prognostic accuracy.

We utilized data from the Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) observational cohort, comprising 393 TBI patients with diffusion MRI. The diffusion MRI data were processed into Diffusion Tensor Imaging (DTI) and Neurite Orientation Dispersion metrics (NODM) and spatially normalized. Predictor variables included blood test results, demographic information, and the 2-week neurological assessments. The outcome variable was categorized as incomplete recovery (GOSE < 8) or complete recovery (GOSE > 8). Principal Component Analysis was performed on the predictor variables to simplify and ensure statistical independence.

Results demonstrated that non-imaging predictor variables alone achieved reasonable performance for predicting outcomes at 3 months (AUC: 0.8322, overall accuracy: 76.15%), 6 months (AUC: 0.8551, overall accuracy: 75.73%), and 12 months (AUC: 0.8798, overall accuracy: 80.33%). The inclusion of imaging features significantly improved accuracy: 3 months (AUC: 0.8814, overall accuracy: 82.85%), 6 months (AUC: 0.8568, overall accuracy: 76.15%), and 12 months (AUC: 0.8892, overall accuracy: 81.59%). The validity of the multiple logistic regression model was confirmed through the Hosmer-Lemeshow goodness-of-fit hypothesis testing. The best model achieved 78.38% and 84.85% accuracy in predicting complete and incomplete recovery at 3 months.

Key contributors to the most effective prognosis model included white matter NODM, Insomnia Severity Index (ISI) scores, and FA in the grey matter. We introduced a feature extracting model from diffusion MRI for regression modelling and demonstrated that adding acute diffusion MRI data to a prognosis model significantly enhances its predictive performance.

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# Secondary Sequelae of Neurotrauma & Neurodegeneration ~ 2

## Forecasting Impairment and Neurodegenerative Disease Risk Following Traumatic Brain Injury (FIND-TBI): A Focus on the Link between TBI and Parkinson's Disease

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Although once considered an acute event, it is now recognised that traumatic brain injury (TBI) leads to long-lasting disability in a subset of individuals, including persistent impairments in memory, decision making and motor function. In addition, TBI is associated with significantly elevated risk of developing neurodegenerative disease, including dementia, motor neuron disease and, perhaps most strikingly, Parkinson's disease (PD). Multiple studies have found a link between TBI and later development of PD, with a study in US military veterans reporting that mild TBI increases risk of PD by 56%, while moderate/severe TBI increases risk by 83 percent. Recently, a meta-analysis also supported an elevated risk of PD in those with prior history of TBI (RR = 1.48; 95% CI = 1.22-1.74). Further, studies in animal models have shown that experimental TBI is associated with two neuropathological hallmarks of PD, progressive nigrostriatal dopamine loss and increased alpha-synuclein deposition in the substantia nigra.

Despite this, the brain mechanisms that underlie such persistent functional impairments and heightened neurodegenerative disease risk remain largely unknown. To address this, FIND-TBI, which is funded by the MRFF Mission for TBI, will use a combination of innovative neuroimaging techniques, including quantitative magnetisation transfer imaging of the locus coeruleus, Nigrosome-1 visualisation and PET imaging of neuroinflammation, to assess brain pathology as a function of both initial severity and time since injury in individuals who have experienced a TBI compared to those with established idiopathic PD. This will be coupled with functional assessment using custom-designed cognitive and motor testing, as well as a comprehensive panel of neuroinflammatory and cell stress markers, to assess patterns of change. Machine learning and computational neurology techniques will be used to generate risk algorithms. Ultimately, this could improve our ability to predict an individual's long-term prognosis following TBI, allowing for earlier, more targeted therapeutic interventions.

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## IL-6 Trans-Signalling Protects Dopaminergic Neurons in Mouse Model of Traumatic Brain Injury

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**Background:** Traumatic brain injury (TBI) causes loss of dopaminergic neurons in the substantia nigra pars compacta (SN pc). Such pathogenic changes in the SN pc typically occur secondary to the initial insult. We previously demonstrated that repopulating microglia mitigate secondary pathology in an interleukin 6 (IL-6) trans-signalling dependent manner. Here we aimed to investigate whether such approaches can also mitigate dopaminergic neuron loss after TBI. We hypothesized that these approaches would attenuate dopaminergic neuronal degeneration after TBI.

**Methods:** We utilised a unilateral controlled cortical injury model of TBI in mice, delivering an impact to the motor cortex. Adult female mice were used. To repopulate microglia, CX<sub>3</sub>CR1 x iDTR mice were used. In C57BL6/J mice, to stimulate IL-6 trans-signalling, we used the designer cytokine, Hyper IL-6 (200ng, intracerebroventricular injected at 1-day post-TBI). Tyrosine hydroxylase (TH) expressing dopaminergic neurons were labelled using immunostaining and their densities within the SN pc were quantified using stereological methods. All statistical significance was  $p < 0.05$ .

**Findings:** After TBI, we observed a significant unilateral loss (~50%) of TH-positive dopaminergic neurons compared with sham-operated controls. Repopulating microglia significantly attenuated dopaminergic neuron loss post-injury compared with vehicle TBI controls. Hyper IL-6 treatment conferred similar benefits, increasing remaining dopaminergic neuron numbers after TBI.

**Interpretation:** These findings suggest that repopulating microglia can have protective effects on dopaminergic neurons, mitigating their loss that otherwise occurs after TBI. Such neuroprotective effects can also be recapitulated by stimulating IL-6 trans-signalling, without the need to turnover microglia. With protection of dopaminergic neurons in the SN pc, understanding how stimulating IL-6 trans signalling effects gross dopaminergic signalling throughout the brain represents an interesting avenue for further investigation.

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## The Role Of Neuroinflammation In Delayed Neurodegeneration Following Photothrombotic Stroke In Rats

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**Background:** Many patients suffer long-term functional deficits following stroke. This is linked with delayed neuronal death in areas distal from the infarct site, termed secondary neurodegeneration (SND). Increasing evidence implicates neuroinflammation as a potential driver of SND, although, the current long-term implications of this are not understood.

**Aim:** Investigate the spatiotemporal profile of neuroinflammation and neurodegeneration, and associated functional changes, long-term following stroke.

**Method:** Male Sprague-Dawley rats (n=44/gp; 12 weeks) underwent photothrombotic stroke or sham surgery. Brain tissue and serum were collected at 12- or 15-months post-stroke (n=30 stroke; n=14 sham/gp). Motor outcomes (step test and open field), anxiety (open field), and cognitive decline (Barnes maze and paired-associates learning task) were assessed at 12- and 15-months post-stroke. Neuroinflammation was analysed using a cytokine/chemokine multiplex (Millipore). Data was analysed using three-way ANOVAs, with Tukey's post hoc test.

**Findings:** Motor deficits were observed to be greater at 15-months. Specifically, a decrease from baseline (p=0.0016) motor function was seen in the 15-month post-stroke group (-1.9±0.4) when compared to 12-month group (0.07±0.4). Unexpectedly, animals showed higher levels of anxiety (p=0.001) and cognitive decline (p=0.03) at 12-months (48.9 ± 7.0; 48.8±8.8) compared with 15-months post-stroke (6.6±10.7; 26.6±4.5). Preliminary investigation showed increased levels of pro-inflammatory cytokines (IL-6, TNF- $\alpha$ , IL-12, IFN- $\gamma$  and MCP-1; p<0.05) within the serum of both the 12- and 15-month post-stroke groups.

**Interpretation:** Greater motor decline at 15-months suggests that pathological progression is still occurring >15 months post-stroke. This is supported by the preliminary serum results which demonstrate a persistent neuroinflammatory reaction, highlighting a need for inclusion of long-term timepoints in experimental stroke research. Given recovery on cognitive and neuropsychiatric measures at 15-months post-stroke, investigation into brain mechanisms that may account for this is the focus of our ongoing research.

**Funding and Ethics:** This study was funded by the Neurosurgical Research Foundation and Perpetual and was approved by The University of Adelaide Animal Ethics Committee (M-2020-072).

## Exploring The Long-Term Neuroinflammatory and Blood-Brain-Barrier Alterations Associated with Secondary Neurodegeneration in an Ovine Stroke Model.

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**Background:** Cognitive impairment is seen in >70% of stroke survivors and may worsen over time, partially attributed to secondary neurodegeneration (SND). SND involves degeneration of regions synaptically connected yet distal to the stroke site e.g., thalamus. Although the exact mechanisms are unknown, neuroinflammation and blood-brain barrier (BBB) alterations have been observed out to 3 months post-stroke in SND regions in rodent models, but the long-term temporal and spatial distribution of such pathological features are unknown. This study sought to characterise the temporal profile of neuroinflammatory and BBB changes in post-stroke SND using a clinically relevant ovine model.

**Methods:** 20 Merino sheep (2-3 years) underwent 2h middle cerebral artery occlusion. Serum and CSF collection were performed pre-stroke and at either 1- or 6-months post-stroke (n=10/timepoint; 5F;5M), then brains collected at endpoint. Samples were analysed for levels of neuroinflammatory markers (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IFN- $\gamma$ , MIP-1a, VEGF-a, IL-10, and IL-4) using multiplex protein analysis. T1 (anatomy) and DCE (perfusion/BBB leakage) MRIs were also taken at baseline and endpoint; with analysis of BBB alterations being conducted on Quantiphyse.

**Findings:** Serum IL-10 and MIP-1a levels were significantly elevated at 6-months post stroke compared with both baseline (p=0.0002, p=0.0004 respectively) and 1-month (p=0.0006, p=0.0185 respectively). Contralateral IL-10 and IFN- $\gamma$  levels were significantly reduced within the thalamus at 6-months post-stroke compared with 1-months (p=0.0283 and p=0.0386, respectively). There were no significant changes observed regarding the other neuroinflammatory markers. Along with this, DCE-MRI processing and analysis protocols have been established and analysis is currently underway.

**Interpretation:** Altered levels of pro- and anti-inflammatory cytokines were observed within the serum and thalamus at 1- and 6-months post-stroke. This provides evidence of a long-term inflammatory response occurring within distal regions post-stroke. These findings provide important information towards understanding the spatio-temporal profile of SND progression following stroke and identifying potential treatment targets.

**Funding:** Neurosurgical Research Foundation

## Poster Abstracts

Speaker	Title	Page
L. Akrapongpisak	Acute Factors as Predictors and Modifiers Of Post-Concussion Symptoms From TRACK TBI: An Observational Cohort Study	40
T. N. Ashenden	Repopulating Microglia Increase CD74 Expressing Cells In A Mouse Model Of Traumatic Brain Injury	42
I.M. Bilecki	Alterations to Blood-Brain Barrier Structure and Function in an Aged Preclinical Model of Stroke.	43
G. E. Bright	The Supraspinal Impact of Traumatic Spinal Cord Injury on Cerebral Microarchitecture and Psychogenic Sexual Function: An Exploratory Clinical DTI And fMRI Study.	44
M. Dierich	Protecting Motor Function Through the Stimulation of IL-6 Signalling Following Traumatic Brain Injury.	45
K. Dunn	Designer cytokine IC7Fc attenuates neuronal loss in a mouse model of traumatic brain injury.	46
S. Edwards	Characterising synergistic effects of traumatic brain injury and pesticide exposure on the development of neurodegeneration in a novel rat model of disease	48
M. G Papini	Evidence for Altered White Matter Integrity after Mild Traumatic Brain Injury: A Scoping Review on the Use of Diffusion MRI and Blood-Based Biomarkers to Investigate Acute Pathology and Relationship to Persistent Post-Concussion Symptoms.	49
L. P. Giesler	Exploring the diagnostic and prognostic potential of plasma brain-derived neurotrophic factor in mild traumatic brain injury	50
D. Hamlin	Characterisation of Neurofibrillary Tangle Maturity in Alzheimer's disease using Tau Immunophenotype Signatures	52
S. Harris	Developing and characterising a mouse model of abusive head trauma	53
R. Hood	A temporal analysis of cerebrovascular dysregulation at sites of hippocampal secondary neurodegeneration following cortical photothrombosis in mice	54
F. I. Isik	Investigating the Immunomodulatory Role of GDF15-GFRAL in Traumatic Spinal Cord Injury Using Murine Models	56
S. Jenkner	A novel pre-conditioning technique using white blood cells improves stem cell survival in ex vivo inflammatory neurotrauma microenvironments	58
S. L. Joubert	MRI volumetric analysis: secondary neurodegeneration is evident at 1- and 6- months following stroke	60
J. Keeves	AUS-mTBI: designing and implementing novel health informatics approaches to improve outcomes for people with mild TBI across Australia	61
J. Krieg	Evaluation of diffuse axonal injury in a paediatric gyrencephalic ferret model of TBI.	65
H. W. Lao	Exploring the Use of iPSC-derived Propriospinal Interneuron Transplants to Restore Function in a Mouse Model Of Spinal Cord Injury	66

H. Le	Behavioural and histological outcomes following 50% compression of the spinal cord in adult and infant rats.	67
P. Marciano	Quantitative changes of femur trabecular and cortical bone in a concomitant TBI and SCI rodent model.	68
W. O'Brien	Divergent trajectories of serum GFAP and NFL are associated with loss of consciousness after sport-related concussion.	69
C. Osterman	Investigating the pathological signature of Chronic Traumatic Encephalopathy using multiplex immunohistochemistry	71
B. Raos	An investigation of electrophysiological activity in the spinal cords of rats to develop biomarkers of injury	72
S. Salberg	A rodent model of intimate partner violence: Perinatal hypoxic and traumatic brain injury combined with early life stress increases offspring susceptibility to pain sensitivity	73
K. Skein	A new animal model to evaluate concomitant traumatic brain injury and its role in developing neuropathic pain following spinal cord injury	74
A. Stein	Abnormal Default Mode Network Functional Connectivity Is Associated With Poorer Attention In Children With Acquired Brain Injury: A HD-EEG Study.	76
A. Stein	Home based tDCS in children with acquired brain injury: preliminary results from the hrtDCS-attention trial.	77
A. Stein	Using NODDI to characterise longitudinal changes in free water in children with chronic mild traumatic brain injury.	79
A. Stein	Cortical electric field predicts tDCS-related attention improvement in children with acquired brain injury: a sham-controlled clinical trial.	81
A. Stein	Investigating the association between brain network connectivity and attention following acquired brain injury: a systematic review of structural and functional measures.	82
E. F. Willis	ROCK2 Regulates Microglia Proliferation And Neuronal Survival In A Mouse Model Of Traumatic Brain Injury.	84
Y. Xu	Turnover Dynamics of CNS Border-Associated Macrophages in Adult Mice	86

## Acute Factors as Predictors And Modifiers Of Post-Concussion Symptoms From TRACK TBI: An Observational Cohort Study

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Little is known regarding prognostic factors in post concussive syndrome among patients with traumatic brain injury (TBI). We aimed to identify acute biomarkers and predictors associated with post-concussive symptoms in those with mild TBI (mTBI). Adult mTBI patients (> 18 years) with a Glasgow Coma Scale score of 13 or more, and a negative CT head with twelve-month Rivermead Post Concussion Questionnaire (RPQ) outcomes were extracted from the Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) observational cohort study. Ethical approval was provided by the San Francisco General Hospital Panel Institutional Review Board (IRB #: 12-09465; Reference #: 313687). Participants provided written informed consent to participate. 687 participants were grouped into complete recovery (RPQ score of 0), mild (1-32 RPQ), and moderate/severe (33-72 RPQ) post-concussive symptoms based on their RPQ score at twelve months. Principal component regression analyses were performed to predict groupings. RPQ score at 2 weeks (parameter estimate ( $B$ ) = 0.0145, 95% CI: [0.01247,0.01654],  $p < 0.0001$ ), chloride levels ( $B$  = 0.01176 [0.001999, 0.02153],  $p = 0.0184$ ), carbon dioxide levels ( $B$  = 0.01566 [0.005984,0.02533],  $p = 0.0016$ ), leukocyte count ( $B$  = 0.01336 [0.008176,0.01854],  $p < 0.0001$ ), age ( $B$  = 0.002753 [0.0004098,0.00509],  $p = 0.0215$ ), previous psychiatric history ( $B$  = 0.07639 [0.001323,0.1515], $p = 0.0461$ ), loss of consciousness ( $B$  = 0.1006 [0.005226,0.1959],  $p = 0.0388$ ), and black race ( $B$  = -0.03237 [-0.04827,-0.01647],  $p < 0.0001$ ) were associated with a moderate/severe RPQ score compared to complete recovery at twelve months. Hosmer Lemeshow goodness-of-fit testing confirmed model correctness ( $p = 0.6640$ , selected model is correct if  $p > 0.05$ ). These acute factors can help to prognosticate the persistence of post-concussive symptoms in mTBI patients one year post-injury. Furthermore, these predictors can guide clinical management and treatment of mTBI. This study was limited by the restricted availability of TRACK TBI data and may be subject to bidirectional ambiguity due to the observational nature of the study. No funding was required.

## Repopulating Microglia Increase CD74 Expressing Cells In A Mouse Model Of Traumatic Brain Injury

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**Background:** Traumatic brain injury (TBI) is a major cause of disability and death. TBI morbidity is attributed to secondary pathology occurring over time and includes hippocampal atrophy and long-term cognitive deficits. We previously demonstrated that repopulating microglia induces a neuroprotective phenotype, mitigating secondary hippocampal pathology after TBI. Early during microglial repopulation, these microglia upregulate CD74, a protein which plays important roles in cell survival and proliferation. Here we aimed to investigate whether either injury and/or repopulating microglia alters CD74-expressing cells in the hippocampus.

### Methods

*Study design:* Mice were randomly assigned to their groups. To repopulate microglia, mice received PLX5622 incorporated chow diet at 1200ppm for 3 weeks prior to TBI, returning to a standard chow after surgery. Vehicle mice received standard chow diet. Mice underwent a craniotomy above the somatosensory cortex, with a unilateral controlled cortical impact inducing TBI. Cell counts were completely blinded.

*Subjects:* Three-month-old C57BL/6J mice, conducted with approval from the UQ ethics committee.

*Interventions:* Three days post-injury, mice were euthanised and brains prepared for histology. Free-floating 40µm serial sections were collected using a sliding microtome and immune-stained for CD74 and other cell-type expression markers (e.g., IBA1 for microglia).

**Findings:** In sham-operated controls, no CD74-expressing cells found in the hippocampus, while TBI resulted in a significant increase. Repopulating microglia significantly increased CD74 expressing cells compared with vehicle mice after TBI (P=0.041). Repopulating microglia also significantly increased microglia expressing CD74 compared with TBI / vehicle mice (P=0.029).

**Interpretations:** Numbers of cells expressing CD74 is increased after TBI and elevated further by repopulating microglia. As repopulating microglia are associated with a neuroprotective effect, increased CD74 expression could be a potential target for therapeutic intervention.

**Funding:** Senior Medical Research Fellowship from the Sylvia and Charles Viertel Foundation (2020001416) (JV); National Health and Medical Research Council (NHMRC) project grant (1124503) (JV).

## Alterations To Blood-Brain Barrier Structure And Function In An Aged Preclinical Model Of Stroke.

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**Background:** Disruption to cerebral blood flow during ischaemic stroke not only causes focal and acute cellular death but also disruption the blood-brain barrier (BBB). Additionally, functional BBB breakdown is also observed with physiological ageing, via disruption of paracellular tight junction complexes and dysregulated transcellular transport. Despite 70% of ischaemic stroke cases occur in individuals over 65 years, the effect of age on stroke pathological outcome is vastly underrepresented in pre-clinical research. Thus, this study aimed to characterise the degree of BBB dysfunction in the aged rat post-stroke compared to a typical young preclinical cohort. It was hypothesised that BBB breakdown would be exacerbated in the aged brain post-stroke.

**Methods:** Male Sprague-Dawley rats aged 18-months underwent photothrombotic stroke or sham surgery (n=9-10/group)(AEC#M-2019-103). 12-week-old male Sprague-Dawley injury matched archival tissue (n=5-6/group)(AEC#M-2015-112) was used for comparison. Brain tissue was collected 1- or 7-days post-stroke to assess protein extravasation (IgG, albumin), BBB integrity (claudin-5, caveolin-1, occludin), extracellular matrix proteins (Collagen type IV, MMP-9), astrocytes (GFAP) and blood vessels (Lycopersicon esculentum lectin). Cell counts, colocalization and fluorescence intensity parameters were quantified using QuPath. Data was analysed using two-way ANOVA with Tukey's post-hoc test.

**Findings:** Increased IgG and albumin extravasation was observed 1-day post-injury in the aged stroke brain (interaction effect: IgG [ $F_{(1, 18)}=6.942$ ,  $p=0.0168$ ]; albumin [ $F_{(1, 19)}=9.027$ ,  $p=0.0073$ ]). Increased peri-infarct caveolin-1 immunoreactivity was observed in both the aged [ $F_{(1,37)}=26.45$ ,  $p<0.0001$ ] and stroke [ $F_{(1,37)}=11.15$ ,  $p=0.0019$ ] brain. Increased claudin-5 immunoreactivity was observed in the aged brain 7-days post-stroke [ $F_{(1,18)}=17.69$ ,  $p=0.0005$ ].

**Interpretation:** The early abnormal transcytosis observed 1-day, followed by structural alterations to paracellular BBB regulation by 7-days in the aged brain suggests that permeability recovery mechanisms may be occurring acutely post-stroke. Functionally, this might indicate repair of the BBB and warrant further investigation towards identifying novel treatment targets.

**Funding:** Neurosurgical Research Foundation.

## The Supraspinal Impact of Traumatic Spinal Cord Injury on Cerebral Microarchitecture And Psychogenic Sexual Function: An Exploratory Clinical DTI And fMRI Study

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**Background:** Sexual dysfunction is the most severe and undertreated secondary health issue among Australians with spinal cord injury (SCI). Despite this, our understanding of cerebral processes governing sexual function and the potential impact of SCI is relatively nascent. Our study aimed to develop a neuroimaging protocol for the spatiotemporal interrogation of cerebral microarchitecture and function related to visually-evoked psychogenic sexual arousal and supraspinal reorganisation following chronic traumatic SCI.

**Methods:** Following written informed consent, participant recruitment for this prospective investigator-initiated pilot study was conducted through Project SCIN (2021/HRE00106; SSA00193; MyIP: 15168). Two patients (each male, mean age 36 yrs) with diagnosed chronic traumatic SCI (complete; ASIA A, levels C5 and T6) and nine healthy volunteers (six male, mean age 35.5 yrs) underwent cerebral diffusion-weighted and functional magnetic resonance imaging. A novel audiovisual stimulation (AVS) paradigm, consisting of two 5-minute intervals separated by 1.5-minute baseline (blank screen) periods, evoked psychogenic sexual arousal. Diffusion and functional data were preprocessed and analysed using DSI Studio and FSL, and CONN Toolbox, respectively. Functional image analyses yielded grouped connectivity maps of AVS-evoked changes in regional cerebral blood flow (2x two-sample *t*-test,  $p < 0.05$ ) from previously identified regions of interest (weighted general linear model,  $p < 0.05$ ). The microarchitecture and integrity of white matter tracts between functionally significant cluster regions were spatially mapped and equated (unpaired *t*-test,  $p < 0.05$ ).

**Findings:** Compared to healthy volunteers, patients with SCI had a global decrease in fractional anisotropy, a measure of white matter integrity. Similarly, connectivity analysis displayed significant differences in the number of regions engaged during visual sexual stimuli in patients compared to healthy volunteers; however, the number of connections was not significant compared to healthy male-only participants.

**Interpretation:** Our findings give an impression of cerebral processing and perception of visual sexual stimuli following SCI. Further work is needed to power this study for analysis.

**Funding:** Lifetime support Authority

## Protecting Motor Function Through The Stimulation Of IL-6 Signalling Following Traumatic Brain Injury

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Traumatic brain injury (TBI) frequently leads to neurological dysfunction, due to brain damage arising from both the primary impact of the injury, and the secondary spread of tissue damage. Our lab has previously shown that the secondary injury can be significantly reduced by local stimulation of interleukin-6 (IL-6) *trans* signalling using the designer fusion protein, HyperIL-6. In a closed cortical impact (CCI) model of TBI, local injection of HyperIL-6 spared neurons in the secondary injury site from cell death and markedly improved functional outcomes (Willis et al., 2020). However, it was unclear whether (1) HyperIL-6 treatment was neuroprotective at the primary injury site; and (2) whether diffuse stimulation of IL-6 *trans* signalling would be neuroprotective following TBI. Here, we used CCI to injure the motor cortex of adult C57Bl6 mice, and stimulated IL-6 *trans* signalling one day after injury by injecting HyperIL-6 either locally (into the cerebral ventricles), or intravenously (IV). Motor function was tested behaviourally for up to 30 days post injury. With either local or IV delivery of HyperIL-6, we saw long-lasting protective effects, with mice performing significantly better than saline-treated controls in fine motor tasks, up to 30 days post injury. These findings indicate that both central and peripheral stimulation of IL-6 *trans* signalling is neuroprotective following TBI.

# Designer Cytokine IC7Fc Attenuates Neuronal Loss In A Mouse Model Of Traumatic Brain Injury

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**Background:** Traumatic brain injury (TBI) is a world leading cause of death and disability. TBI induces functional impairment, and this is attenuated by inducing Interleukin-6 (IL-6) trans-signalling. Recently, a designer cytokine was developed, consisting of IL-6 fused with CNTF, capable of stimulating IL-6 trans-signalling. Able to be administered peripherally, IC7Fc does not induce an immunogenic response typically associated with IL-6. We aimed to identify whether IC7Fc administration can attenuate neuronal loss following TBI, where it was hypothesized that IC7Fc would reduce motor neuron loss after motor cortex injury.

## Methods

*Study Design:* Emx1-Cre<sup>ERT2</sup> x tdTomato<sup>fllox</sup> mice fate label pyramidal upper motor neurons. Mice were orally gavaged with tamoxifen (0.125mg/kg) for five days, two weeks prior to surgeries to induce tdTomato expression. Mice were randomly allocated to sham, TBI / vehicle or TBI / IC7Fc groups. A craniotomy was performed above the motor cortex and TBI was induced with using a controlled cortical impactor. Cell counts were completed blinded.

*Subjects:* Three-month old transgenic mice were used (11M, 15F), conducted with approval from the UQ animal ethics committee.

*Interventions:* Mice were administered either IC7Fc (1mg/kg) or an Fc vehicle via tail vein injection one to four days post-surgery. Thirty days post injury, mice were sacrificed, and brains processed for histology. Brains were serial sectioned using a sliding microtome and Emx1<sup>pos</sup> neurons within layer five of the primary motor cortex quantified using stereological methods.

**Analysis** Statistical differences were analysed with a one-way ANOVAs with Bonferroni post comparison using GraphPad Prism.

**Findings:** TBI / vehicle mice had significant loss of Emx1<sup>pos</sup> neurons in the ipsilateral motor cortex (33.5% loss, P=0.006) compared with sham controls. IC7Fc treatment significantly reduced Emx1<sup>pos</sup> motor neuron loss (37.4% increase, P=0.0012).

**Interpretation:** Peripheral IC7Fc treatment induces neuroprotective effects post TBI, indicating a potential for therapeutic targets in future.

**Funding:** Funding was received from the Senior Medical Research Fellowship from the Sylvia and Charles Viertel Foundation held by J.V. and imaging was performed at the Queensland Brain Institute's Advanced Microscopy Facility using a Diskovery spinning disk confocal microscope, Imaris software, and the stereology microscope, supported by the Australian Government through the ARC LIEF grant LE100100074.

## Characterising Synergistic Effects of Traumatic Brain Injury and Pesticide Exposure on The Development Of Neurodegeneration in A Novel Rat Model Of Disease

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Traumatic Brain Injury (TBI) and exposure to pesticides have been independently associated with increased risk of developing Parkinson's Disease (PD). However, it is becoming increasingly clear that development of PD is complex and likely involves multiple factors. There are currently few preclinical models of neurodegeneration that reflect this, and thus elucidating mechanisms of disease pathogenesis presents considerable challenges. The aim of this study was to develop and characterise a novel "two-hit" model of PD combining TBI and low-level rotenone exposure, to induce underlying inflammation and dopaminergic neuronal loss in the Substantia Nigra prior to injury. 8-10 week-old male Sprague-Dawley rats were randomly allocated to receive vehicle or rotenone treatment, and sham or TBI surgery (n=16 per group). Animals were subcutaneously injected with 2% DMSO or rotenone (1.5mg/kg) every 48 hours for 12 days, followed by a moderate-severe TBI using the Marmarou weight-drop model, or sham surgery, 24 hours after the final injection. Animals were tested on various motor domains at 3-months post-TBI to assess the development of Parkinsonian symptoms. Gross motor function, involuntary movement, forelimb dexterity and grip strength were not significantly affected by injury or rotenone, or the two in combination. TBI significantly affected motor coordination, as assessed by latency (p=0.020) and faults (p=0.0006) on the beam walk, and sensorimotor function, as assessed by step adjustments (p=0.0055), and time to contact (0.027) and removal (p=0.026) in the tape removal test, however there was no interaction with rotenone exposure. While a synergistic effect of TBI and rotenone exposure was not observed 3-months post-injury, deficits may emerge at a later time-point post-injury. Thus, further characterisation of our two-hit model is currently underway, including histological analysis of tissue collected 1-month post-injury, cognitive testing at 3-months post-injury and broad functional testing at 6-months post-injury to assess chronic manifestations of disease.

This project was funded by the Neurosurgical Research Foundation

Approved by the University of Adelaide Animal Ethics Committee under ethics number M-2022-004

## Evidence for Altered White Matter Integrity After Mild Traumatic Brain Injury: A Scoping Review on the Use Of Diffusion MRI and Blood-Based Biomarkers to Investigate Acute Pathology and Relationship to Persistent Post-Concussion Symptoms.

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**Background:** Although most people recover from mild traumatic brain injury (mTBI), up to 50% experience symptoms for >3 months, termed persistent post-concussive symptoms (PPCS). Subtle white matter (WM) microstructural damage is thought to underlie PPCS. Mounting evidence suggests that diffusion magnetic resonance imaging (dMRI) and blood-based biomarkers could be used as objective surrogate markers of WM integrity. A systematic scoping review was conducted to collate evidence for the use of dMRI and blood-based biomarkers of WM integrity in mTBI and PPCS, and to assess documented relationships between these dMRI and blood biomarkers.

**Methods:** The review was conducted according to PRISMA-Scr guidelines. A comprehensive literature search was performed across five databases. Original research studies were included if they assessed blood and/or dMRI WM biomarkers in humans aged 16-65 with uncomplicated mTBI and/or PPCS. Data extracted included participant characteristics, blood and dMRI analysis techniques and associations between biomarkers and outcome measures.

**Findings:** Of 102 eligible studies, 69 analysed dMRI, 29 assessed blood-based biomarkers and 4 used both. Most studies, (70%), assessed acute mTBI, 16% investigated PPCS and 15% incorporated both modalities. Blood biomarker studies focused predominantly on diagnosis/screening and pathophysiological alteration, while dMRI studies primarily investigated only the latter. Almost half the participants were aged between 16 and 34 years and recruited from professional or student sporting clubs. Blood biomarker studies commonly assessed markers of the axonal cytoskeleton (i.e. tau); dMRI studies assessed measures of WM integrity: fractional anisotropy and mean diffusivity. Significant alterations in biomarkers were often associated with heightened symptom severity and number.

**Interpretation:** dMRI and blood-based biomarkers may be useful proxies of WM integrity, although few studies have assessed these complementary measures in parallel. Further studies are warranted to assess the benefit of a combined dMRI and blood biomarker approach for mTBI screening and prediction of PPCS.

**Funding:** None

## Exploring The Diagnostic And Prognostic Potential Of Plasma Brain-Derived Neurotrophic Factor In Mild Traumatic Brain Injury

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**INTRODUCTION:** Comprehension of the pathophysiological cascade following mild traumatic brain injury (mTBI) has been steadily improving. Although heterogeneous, mechanisms like inflammation and apoptosis may be key determinants of outcome after TBI. As such, it has been hypothesised that brain-derived neurotrophic factor (BDNF) may serve as a reliable biomarker of mTBI due to its key role in these processes. We aim to investigate the diagnostic and prognostic utility of BDNF as a biomarker of mTBI.

**METHODS:** 190 adults (135 mTBI, 55 healthy controls) aged 18-50 were recruited from either The Alfred Emergency & Trauma Centre or the Victorian Amateur Football Association. Participants underwent symptom evaluation using the Rivermead Post-Concussion Questionnaire, and CogState cognitive testing at <48hrs, 1-week, and 1-month post-injury. Blood was collected at <48hrs and 1-week for plasma biomarker quantification of BDNF using a Simoa HD-X Analyser.

**FINDINGS:** mTBI participants had significantly elevated plasma BDNF levels at <48hrs compared to healthy controls ( $p=0.005$ ), but no differences were found at 1-week. However, plasma BDNF had a poor ability to distinguish mTBIs from controls at <48hrs (AUROC=0.63; 95%CI=0.54-0.72). Plasma BDNF levels at <48hrs ( $p=0.026$ ) and 1-week ( $p=0.045$ ) were elevated in participants with high symptom severity compared to those with low severity or no symptoms at 1-week post-injury, although classification accuracy was poor at both time-points (48h: AUROC=0.63; 95%CI=0.52-0.73; 1w: AUROC=0.62; 95%CI=0.51-0.73).

**CONCLUSIONS:** Plasma BDNF levels were significantly elevated in mTBI compared to controls at <48hrs; however, diagnostic utility was relatively poor compared to other biomarkers such as GFAP we have previously shown to have utility in this cohort. Importantly, elevated BDNF levels in patients with more severe symptoms at 7 days suggest potential as a predictor of mTBI recovery and warrant further investigation.

**FUNDING:** Australian National Health and Medical Research Council grant (2020/GNT2002689) awarded to SM and internal funding through Monash University.

## Characterisation of Neurofibrillary Tangle Maturity in Alzheimer's Disease Using Tau Immunophenotype Signatures

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**Background:** Neurofibrillary tangles (NFTs) comprised of tau protein are a hallmark pathology of both Alzheimer's Disease (AD) and Chronic Traumatic Encephalopathy (CTE), a neurodegenerative disease associated with repetitive head trauma. NFT maturity is traditionally classified by labelling with a phosphorylated tau antibody and qualitatively assessing NFT morphology.

To facilitate more efficient and reproducible studies of NFT maturity progression throughout disease, we sought to identify a multiplexed antibody labelling signature which could be used to classify NFTs across three distinct maturity levels; Pre-tangle, mature and ghost tangles, which are often discussed in AD literature. We also tested if this could be applied to CTE tangles.

**Methods:** Using fluorescent immunohistochemistry, we labelled postmortem human brain tissue from normal and AD cases with Braak stage I-VI pathology with four antibodies targeting different tau epitopes; each associated with different levels of NFT maturity. We analyzed individual NFTs and measured the mean fluorescent intensity of each antibody. Spectral clustering was then used to identify distinct NFT immunophenotypes. We later repeated this with superior frontal gyrus sections from CTE cases provided by collaborating brain banks and compared the NFT immunophenotypes between the subpial and grey matter regions of the sulcus.

**Findings:** In AD we identified five unique populations of NFTs with immunophenotypes that indicate immature, mature, ghost, and potential intermediary maturities. These immunophenotypes were also observed in the grey matter NFTs from CTE cases. Immature NFT immunophenotypes were more prevalent in later affected regions in AD and in the CTE lesions. In non-AD Braak stage I-II cases, there was a unique phosphorylated tau immunophenotype which was also prevalent in the subpial region of CTE cases.

**Interpretation:** Our data validates the use of immunophenotypes for quantifying NFT maturity and provides an accurate and more efficient framework for studying the progression of NFT pathology.

## Developing and Characterising a Mouse Model of Abusive Head Trauma

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**Background:** Abusive head trauma (AHT) previously known as “shaken baby syndrome” is a traumatic brain injury that is primarily induced by a trusted caregiver in early postnatal life. AHTs occur when a child is grasped and shaken with a force great enough to overwhelm the underdeveloped neck muscles and can occur either with or without a blunt impact. Unfortunately, AHTs occur an average of 10 times to a single infant and are often challenging to diagnose due to lack of transparency from caregivers. AHT can result in lifelong developmental, social, emotional, cognitive, and visual impairments. To date, there have been limited efforts to create an animal model that accurately replicates the brain injuries and developmental pathologies seen in human children.

**Methods:** On postnatal days 8-12 unanaesthetised mice were restrained and placed on a shaking device for either 15 or 60 seconds. Mice received either 1, 3, or 5 shaking injuries or were allocated to a sham injury group where they were only restrained. Following injuries mice were euthanised and brain and eye tissue was collected for gene expression analysis and immunohistochemistry.

**Findings:** 15 second AHTs resulted in minor gene expression changes however there were no observed immunohistological changes. 60s AHTs resulted in brain swelling 48 hours post-injury and gene expression alterations in the hippocampus and prefrontal cortex. Regarding the retina, pathology visualised using immunohistochemistry is evident after injury.

**Interpretations:** A mouse model of AHT can guide insight to human AHT injuries. Retinal pathology may arise before brain pathology and provide a biomarker to aid in AHT diagnosis.

**Funding:** This work has been supported by funding to Richelle Mychasiuk by the National Health and Medical Research Council (NHMRC) of Australia.

## A Temporal Analysis of Cerebrovascular Dysregulation at Sites of Hippocampal Secondary Neurodegeneration Following Cortical Photothrombosis in Mice

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**Background:** We have previously demonstrated that a cortical stroke causes persistent impairment of hippocampal-dependent cognitive tasks concomitant with secondary neurodegenerative processes such as amyloid- $\beta$  accumulation in the hippocampus, a region remote from the primary infarct. Interestingly, there is emerging evidence suggesting that deposition of amyloid- $\beta$  around cerebral vessels may lead to cerebrovascular structural changes, neurovascular dysfunction, and disruption of blood-brain barrier integrity. However, there is limited knowledge about the temporal changes of hippocampal cerebrovasculature after cortical stroke. Here, we aimed to characterise the spatiotemporal cerebrovascular changes after cortical stroke.

**Method:** Photothrombotic stroke was induced in 10 week old male C57BL/6 mice (n=65 stroke; n=20 sham) targeting the motor and somatosensory cortices (University of Newcastle ethics approval A-2013-340). Cerebrovascular morphology as well as the colocalization of amyloid- $\beta$  with vasculature and blood-brain-barrier integrity were assessed via immunofluorescence in the peri-infarct area, as well as the CA1 and dentate gyrus of the hippocampus at 7, 28 and 84 days post-stroke. Data were analysed using two-way ANOVA.

**Findings:** Transient cerebrovascular remodelling was observed in the ipsilateral peri-infarct area. In the hippocampus, remodelling was sustained out to 84 days post-stroke. We observed a decrease in vessel diameter at 84 days in the peri-infarct area and CA1 sub-

region that was exacerbated in vessels with amyloid- $\beta$  deposition ( $p \leq 0.0006$ ). Lastly, we observed sustained vascular leakage, indicative of a compromised blood-brain-barrier.

**Interpretation:** Collectively, our results suggest that cortical stroke induces remote hippocampal cerebrovascular dysregulation, particularly reduction of vessel diameter, as well as BBB leakage, which may partly contribute to the progression of post-stroke SND and cognitive impairment. Hippocampal vasculature may represent an important therapeutic target to mitigate the progression of post-stroke cognitive impairment.

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## Investigating The Immunomodulatory Role Of GDF15-GFRAL in Traumatic Spinal Cord Injury Using Murine Models

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Traumatic spinal cord injury (SCI) is the leading preventable cause of disability in young people. Although largely irreversible [1], patient outcomes may be improved by minimising secondary neuronal loss. Targeting neuroinflammation [2] is thought to be beneficial, however previous immunomodulatory therapies have generally failed. Growth differentiation factor 15 (GDF15), a GDNF family cytokine that regulates appetite and metabolism via its recently identified receptor GDNF family receptor  $\alpha$ -like (GFRAL), is induced by cellular stress and injury [3]. In murine contusive SCI, we found that transgenic GDF15 overexpression was associated with improved functional recovery related to upregulated *CCL2* and altered recruitment of immune cells [4]. However, the underlying mechanism for this effect is unknown. We hypothesize that GDF15 binds to GFRAL to activate signalling cascades that are neurotrophic and orchestrate a more beneficial inflammatory cell recruitment in SCI. To test this hypothesis, we will (1) characterise the expression and localisation of GDF15-GFRAL in SCI, and (2) define its downstream signalling pathways. We will use adult (8-9 week), female mice randomly allocated to T10 dorsal laminectomy with contusive SCI (70 Kdyn) or laminectomy only conditions, and sample tissue at 1-, 3-, 7-, 14- and 28-days post injury (n = 8 per group) in mixed mouse lines. First, GFRAL expression in astrocytes (GFAP+), microglia (Iba1+), motor neurons (Isl1+) and oligodendrocytes (Olig2+) will be defined in GFRAL-reporter SCI mice. GFRAL expressing cells will be further examined for their role in reducing SCI, focussing on neuronal preservation. Secondly, we will extend these findings to confirm that pharmacological administration of GDF15 at the time of SCI is

as efficacious as endogenous expression in GDF15 transgenic mice. These studies will build upon our understanding of the role of GDF15 in SCI and serve as essential preclinical data to justify human therapeutic trials.

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## A Novel Pre-Conditioning Technique Using White Blood Cells Improves Stem Cell Survival in *Ex Vivo* Inflammatory Neurotrauma Microenvironments

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Stem cell therapy is considered the best treatment candidate for tissue regeneration following neurotrauma. However, low stem cell survival rates of <3% in harsh neurotrauma microenvironments post-transplantation greatly hinder clinical translation. Therefore, new strategies to enhance stem cell viability and efficacy are greatly required. Previously, we and others have demonstrated that *ex vivo* pre-conditioning of stem cells with inflammatory cytokines, peripheral blood mononuclear cells (PBMCs) or other factors can significantly improve stem cell viability. However, whether such improvements can be maintained within the harsh secondary injury microenvironment following neurotrauma has yet to be determined. Therefore, our aims were firstly to determine the inflammatory factors that contribute to poor stem cell survival, followed by characterisation of the therapeutic efficacy of various pre-conditioning techniques in simulated neurotrauma microenvironments *ex vivo*.

Human dental pulp stem cells (DPSCs) were pre-conditioned with PBMCs from healthy male control donors (n=3) and/or recombinant cytokines (TNF $\alpha$ , IFN $\gamma$  and/or IL17A), followed by exposure to inflammatory microenvironments of innate immunity (LPS), excitotoxicity (glutamic acid), hypoxia (cobalt chloride) and oxidative stress (H<sub>2</sub>O<sub>2</sub>). After 48 hours of pre-conditioning and exposure, DPSC cytotoxicity and viability was measured by lactate dehydrogenase (LDH) assays and flow cytometry, respectively.

DPSCs showed remarkable resilience to acellular stressors. However, the presence of various inflammatory cytokines reduced cell viability (24-57%) and increased cytotoxicity (45-66%), particularly under hypoxic and oxidative stress. In contrast, PBMC pre-conditioning without cytokines greatly improved DPSC viability (61-97%) and proliferation and reduced cytotoxicity (16-25%) under the same microenvironmental stressors.

Our data indicates that commonly utilised methods for stem cell pre-conditioning in the context of neurotrauma treatment may increase stem cell death when exposed to subsequent cellular secondary injury microenvironments. PBMC pre-conditioning may be an effective method to improve stem cell survival and therapeutic efficacy under these conditions.

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## MRI Volumetric Analysis: Secondary Neurodegeneration Is Evident At 1- And 6- Months Following Stroke

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**INTRODUCTION:** Post-stroke secondary neurodegeneration (SND) is associated with dementia and cognitive decline, linked to neuronal loss and degeneration within brain regions (e.g., thalamus) distal to, yet synaptically connected to the original stroke site. However, the spatiotemporal profile of this degeneration has yet to be elucidated, which is essential in understanding disease progression towards identifying the therapeutic window to halt/prevent SND. *The aim of this study was to determine the spatiotemporal profile of brain atrophy post-stroke in a clinically-relevant ovine model.*

**METHODS:** Twenty merino sheep (50-70kg; 2-3 years) underwent 2h transient middle cerebral artery occlusion. Pre-stroke and at 1- or 6- months post-stroke (n=5M:5F/timepoint) T1 and T2 MRI was acquired. Manual segmentation and volumetric analysis were performed on the thalamus, both hemispheres, and total brain using ITK-SNAP.

**FINDINGS:** Total thalamic volume was significantly reduced at both 1- and 6- months post-stroke (both  $p < 0.0001$ ). Similarly, a significant reduction of ipsilateral and contralateral thalamic volume was present at both timepoints (all  $p < 0.0001$ ). A reduction in both ipsilateral (1-month:  $p = 0.004$ ; 6-months:  $p < 0.0001$ ) and contralateral hemispheric volume (1-month:  $p = 0.0006$ ; 6-months:  $p < 0.0001$ ), as well as total brain volume (1-month:  $p = 0.0001$ ; 6-months:  $p < 0.0001$ ) was observed.

**INTERPRETATION:** The volumetric data provides evidence that SND occurs as early as 1-month post-stroke, and is the first report of post-stroke SND in a clinically-relevant large animal model. This dataset will have utility in understanding the timeline of SND progression, although volumetric data of other known SND sites (e.g., hippocampus, basal ganglia), in conjunction with histology to examine neuronal number and cell death markers are essential next steps.

**FUNDING:** NeuroSurgical Research Foundation

## AUS-mTBI: Designing and Implementing Novel Health Informatics Approaches to Improve Outcomes For People with Mild TBI Across Australia

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**Background:** In Australia, there are ~180,000 mild traumatic brain injuries (mTBI) each year. Mild TBI can have long-lasting negative impacts on individuals, their families, and society. Management of people with mTBI is hindered by poor prediction of those at risk of delayed recovery and inconsistent treatment and care.

**Methods:** The AUS-mTBI national consortium is establishing a multi-faceted online platform to gather data to allow reliable prediction of outcome and improved management of mTBI. AUS-mTBI will collect self-reported data via online platforms, and more detailed data from participants recruited via three inner-metropolitan Emergency Departments (Melbourne; Brisbane; Perth). Demographic, injury circumstance, health status, mTBI symptomatology and care management data will be collected for all participants, with follow-up for 12 months or until symptom resolution. Participants recruited from Emergency Departments will provide

blood and saliva samples within 12 hours of injury, and MRI scan, vestibular/ocular motor screening, cognitive assessment and balance assessment within four days of injury; with follow-up via telephone at 1 week, 2 weeks, 1, 3, 6 and 12 months after injury. The presence of persistent post-concussion symptoms will be assessed using the Rivermead Post-Concussion Questionnaire (adults), or the Post-Concussion Symptom Inventory (< 18 years). Specific methodologies are also being developed by and with Aboriginal and Torres Strait Islander people to better understand outcomes.

**Interpretation:** The data will be used to develop comprehensive online platforms that will identify a suite of predictors of outcomes after mTBI, use machine learning models to predict those at risk of poor outcome and identify improved care pathways for people after mTBI.

**Funding:** This project is funded by the MRFF.

## Evaluation of Diffuse Axonal Injury in a Paediatric Gyrencephalic Ferret Model of TBI.

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Diffuse axonal injury (DAI) drives most functional deficits following traumatic brain injury (TBI). Mechanical force causes biochemical and structural axonal changes which initiate secondary injury cascades, leading to ongoing axonal damage. Although the immature brain is known to respond differently to the adult brain due to differences in brain water content and myelination amongst other factors, how age at injury may modulate DAI is unclear. Most DAI mechanisms are studied in lissencephalic models, lacking the gyri found in humans. Such that, the present study sought to characterise paediatric DAI in a clinically relevant gyrencephalic TBI ferret model, with increased white matter DAI hypothesised.

TBI was induced using the CHIMERA (Closed-Head Injury Model for Engineered Rotational Acceleration). Paediatric males (2-3 months; 0.71kg [0.49-0.89kg]) were randomly allocated to sham or TBI (n=5-6/group) and were perfused after 24hr. Multiplex immunofluorescence was used to assess the injury response. DAI was assessed through impaired axonal transport (APP) and neurofilament compaction (RMO-14) as was colocalization with phosphorylated tau (AT180). Microglial changes were assessed with IBA-1.

Paediatric animals showed a varied response to injury and were thus assessed via non-parametric statistical tests. In the corpus callosum TBI produced significant increases in APP+ axons (TBI: 16.848 [8.01-47.29] vs Sham: 1.97 [0.07-2.38]; p=0.0043) but not RMO-14+ axons (p>0.99). AT180 was observed in approximately 50% of all APP+ and RMO-14+ axons irrespective of injury. Microglia showed no changes in area-standardised counts, microglial surface area, or immunofluorescence intensity.

Axonal injury in paediatric animals was characterised by accumulation of APP, with minimal RMO-14 pathology, suggesting that acutely microtubule disruption, but not neurofilament compaction is key. Axonal calibre and neurofilament concentration increase with age, potentially explaining the poor utility of RMO-14. The mechanism behind varied AT180 response within injured axons is still unclear, prompting for further research into axonal injury mechanisms.

All research was funded with the James and Diana Ramsay foundation and was approved by the South Australia health and Medical Research Institute (SAM 21-074)

## Exploring the Use of iPSC-Derived Propriospinal Interneuron Transplants to Restore Function in a Mouse Model of Spinal Cord Injury

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**Background:** Traumatic spinal cord injury (SCI) causes irreversible damage to neural circuits and pathways. Therapeutic strategies involving neural stem cell transplants hold significant promise to replace lost tissue, restore continuity across the lesion and, in doing so, promote functional recovery from SCI. Propriospinal interneurons may be ideal for transplantation purposes as these cells naturally connect distant segments of the spinal cord through both ascending and descending projections, and they are also thought to have crucial roles in motor control.

**Methods:** This study explored the feasibility of generating V2a propriospinal interneurons from mouse-induced pluripotent stem cells (iPSCs), subsequently transplanting these cells syngeneically into SCI mice. We successfully developed a 6-day differentiation protocol to guide iPSCs towards a Chx10+ V2a interneuron fate; this included the precise temporal addition of specific morphogens: retinoic acid, sonic hedgehog, and a notch signalling inhibitor. A Chx10- Blasticidin S deaminase (BSD) selectable cell line that constitutively expressed the reporter tdTomato was used for purification and identification of Chx10+ transplants.

**Findings and interpretation:** A significant downregulation of the pluripotency gene Oct4 was observed by day 4 in iPSCs following differentiation, along with the induction of the neural markers Nestin and NCAM. Expression analysis of the transcription factor Chx10, and also Tuj1 as a more general neuronal lineage marker, confirmed that V2a interneurons were indeed generated from mouse iPSCs (~17% efficiency). Transplantation of purified V2a interneurons into SCI mice demonstrated long-term survival, extensive neurite outgrowth, and integration of these cells within the injured spinal cord. Importantly, the transplantation of iPSC-derived V2a interneurons led to a significant return of locomotor function and reversed hindlimb paralysis by 1 month post-transplantation. Our ongoing experiments are focused on assessing the longer-term therapeutic potential of these cells as neuronal relays, and also to demonstrate the direct causative effects of these cells on restoring function after SCI.

**Funding:** Perry Cross Spinal Research Foundation

## Behavioural and Histological Outcomes Following 50% Compression of The Spinal Cord in Adult and Infant Rats.

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**Background:** Non-traumatic spinal cord injury (SCI) arising from conditions such as compression due to tumours or bony growths, vascular malformations and infections account for about 20% of all spinal cord injuries. This is much higher (~66%) in infants and in the elderly. There is relatively little research and few animal models available to study the progression and potential treatments for non-traumatic spinal cord injury, especially in the very young.

**Methods:** We have developed a solid 3D printed spacer model of chronic spinal cord compression that can be modified for animals of different ages, to compress the spinal cord by different amounts and can remain in situ for different recovery times. 32 (16M:16F) adult and 16 (mixed sex) PNday 9 infant Sprague Dawley rats were used. Age-appropriate spacers were inserted under the T10 vertebrae to compress the spinal cord by 50% in half the rats while the other half underwent sham surgery only. Weekly locomotor tests were undertaken using an open field and horizontal error ladder. At 6 weeks spinal cords were dissected for histological examination. The study had Ethics approval from UTS ACEC. Animals were randomised to experimental groups and, within each age group, behavioural scoring and histological analysis were conducted blind as to group.

**Findings:** Adult rats showed an early deficit ( $P < 0.05$ ) and gradual recovery in locomotor function from D1 to W6. There were no sex differences noted using two-way ANOVA tests. Semi-quantitative tissue injury scores were higher for the compression groups, compared to the sham groups ( $p < 0.05$ ). Infant rats, by comparison did not display marked locomotor changes or altered histology after 50% spinal cord compression.

**Interpretation:** This model has the potential to be modified and adapted to include decompression and to test treatments options for non-traumatic SCI in animals of different ages.

**Funding:** None

## Quantitative Changes of Femur Trabecular and Cortical Bone in A Concomitant TBI and SCI Rodent Model.

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The mechanisms of a spinal cord injury (SCI) can often result in a concomitant traumatic brain injury (TBI), which has impacted 60% of 20, 8000 individuals diagnosed with an SCI in Australia. In both injury types, cases have reported changes in the skeletal microarchitecture, often displaying a decline in trabecular and cortical bone volumes. However, research regarding bone loss after a concomitant TBI and SCI lacks within the field of neurotrauma. Therefore, our study aims to understand bone loss in the femur of male Sprague Dawley rats post concomitant TBI (450g Marmarou weight drop model from 1 meter) and left C5 hemi-contusion SCI (laminectomy + 200kd). The right femur bones from TBI+SCI (n=2), TBI only (n=2), and SCI only (n=1), contralateral from the SCI site were scavenged for quantitative micro computed tomography ( $\mu$ CT) imaging (85kV, 235uA, 35mV).  $\mu$ CT images were reconstructed and measurements of bone volume fraction (BV/TV), average cortical area (Ct.Ar), average cortical thickness (Ct.Th), and average trabecular thickness (Tb.Th) within the femur were quantified for trabecular and cortical bone loss. Bone analysis revealed minor variation of BV/TV and Ct.Ar between the groups. Although, a decrease in Ct.Th and an increase in Tb.Th was shown in SCI only, compared to TBI+SCI and TBI. Though, further data analysis is currently underway, including naïve and sham animals. Therefore, it is hypothesised that all injury groups will have trabecular and cortical bone loss, and TBI+SCI models will exhibit a pronounced decline in trabecular and cortical bone, compared to the distinctive groups described. Thus, this research will demonstrate the outcome of trabecular and cortical bone within the femur following a concomitant injury, warranting further investigation to better understand the alterations of bone inherent to neurological damage.

## Divergent Trajectories of Serum GFAP and NFL are Associated with Loss of Consciousness After Sport-Related Concussion.

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**Context:** Despite global efforts to identify objective tools for informing decisions such as return to play in sport-related concussion (SRC), clinical decisions continue to heavily depend on subjective measures. In this prospective study, we investigated two blood biomarker candidates, GFAP and NfL, aiming to conduct the most comprehensive analyses of their temporal profiles after SRC to date, and to use these profiles to identify distinct classes of biomarker patterns that may be indicative of neurobiological recovery.

**Methods:** One-hundred and thirty-seven amateur Australian footballer players (81 SRC, 56 controls) had blood collections, symptom and cognitive assessments at 24-hours, 1-, 2-, 4-, 6-, 8-, 12-, and 26-weeks post-SRC/match. Serum GFAP and NfL levels were quantified with Simoa assays. Growth mixture models were used to identify sub-classes of biomarker trajectories within the SRC group and linear mixed models to investigate outcomes over time.

**Findings:** At a group level, GFAP was elevated in the SRC group at 24-hours and 4-weeks compared to controls. Two GFAP trajectory classes were identified: one displaying an extreme and prolonged elevation persisting out to 6-weeks, and a second with a moderate increase at 24-hours only. For NfL, there was a delayed but prolonged elevation in the SRC group compared to controls. Three NfL trajectory classes were identified: the first class demonstrated an extreme and prolonged rise, a second class showed a moderate and prolonged rise, and a third class exhibited minimal or no change compared to controls. Notably these divergent biomarker trajectories were similarly present when categorizing SRC participants by the presence or absence of loss of consciousness (LOC).

**Interpretation:** A subset of SRC cases appear to have enduring neurobiological changes for at least 6-weeks post-SRC. Additionally, the presence of LOC emerges as a conspicuous and immediately translatable indicator for identifying SRC cases likely to exhibit such neurobiological alterations.

**Funding:** This study was funded by a grant (2020/GNT2002689) awarded to SM by the NHMRC, and internal funding through Monash University.

## Investigating the Pathological Signature of Chronic Traumatic Encephalopathy Using Multiplex Immunohistochemistry

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**Background:** Chronic Traumatic Encephalopathy (CTE) is a neurodegenerative disease associated with repetitive head trauma and is characterised by the perivascular accumulation of hyperphosphorylated tau in the depths of cortical sulci. Besides the accumulation of tau, very little is known about the pathological changes that occur in CTE and how they compare to other neurodegenerative tauopathies like Alzheimer's disease. We hypothesise that the brains of former athletes diagnosed with CTE contain a spectrum of molecular pathologies associated with brain injury and dementia. In this study we sought to identify the anatomical signature of pathology in CTE, compared to Alzheimer's disease and neurologically normal aging using postmortem human brain tissue.

**Methods:** We examined superior frontal gyrus sections from nine CTE, nine neurologically normal, and eight Alzheimer's disease cases. Multiplex immunohistochemistry was performed to label each section with 35 antibodies that identify reactive gliosis, neuronal subtypes, axonal proteins, vasculature, pathological protein aggregates and protein degradation pathways. This process involves iterative cycles of antibody labelling, imaging, and antibody stripping. To identify differentially expressed markers, the images from each section were aligned and split into 10  $\mu\text{m}^2$  pixel bins, within which the mean fluorescence intensity was measured for each marker. The proportion of total pixel bins coded as positive for each marker was compared between CTE, Alzheimer's disease and normal cases.

**Results:** Markers associated with aggregated protein pathology and reactive gliosis were differentially expressed in CTE compared to Alzheimer's disease and normal cases. Qualitatively, we observed a localised concentration of reactive astrocyte and microglia markers around the tau lesion vessels in CTE, compared to a more ubiquitous distribution in Alzheimer's disease.

**Conclusion:** The distribution of glial reactivity markers may be a neuropathological hallmark of CTE and suggests that repeated head trauma leads to a chronic and localised neuroinflammatory environment around blood vessels in the sulci.

## An Investigation Of Electrophysiological Activity In The Spinal Cords Of Rats To Develop Biomarkers Of Injury

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**Background:** The underlying cause of functional deficits in spinal cord injury is damage to the neural circuitry that transmits motor and sensory information between the brain and the periphery. Characterisation of injury in terms of neural activity is limited due to the difficulty in recording this information. We have developed a bioelectronic implant that can record neural activity from the surface of the spinal cord and are investigating the potential to develop electrical biomarkers that characterise injury.

**Methods:** Two groups of 2-3 month old female Sprague-Dawley rats ( $n = 8-10$ ) had bioelectronic implants, containing 24 recording electrodes 60  $\mu\text{m}$  diameter, inserted into the subdural space between the T10-T12 spinal processes. One group received a 175 kdyn contusion at T11 using an Infinite Horizons Impactor, while the other group received no injury. Electrode position was validated with micro-CT imaging. Electrical recording of neural activity was performed weekly for 12 weeks and processed for spike detection using the SpykingCircus algorithm. Within each detected neural unit, the propagation velocity of individual spikes was analysed to calculate the speed and direction of propagation.

**Findings:** We demonstrate the ability to extract unique units of neural activity, likely originating from white matter tracks in the spinal cord. Extracted units are spatiotemporally distinct and correlate with animal behaviour. Action potential propagation velocities were between 20 and 90  $\text{ms}^{-1}$ , primarily in the afferent direction. The velocity of neural units remained stable for two weeks.

**Interpretation:** Preliminary data suggests that recordings of neural activity have the potential to characterise spinal cord injury in terms of spinal neural activity. Current data is limited by degradation to the quality of electrical recordings over time.

**Funding:** CatWalk Trust, Health Research Council NZ, Neurological Foundation

## A Rodent Model Of Intimate Partner Violence: Perinatal Hypoxic And Traumatic Brain Injury Combined With Early Life Stress Increases Offspring Susceptibility To Pain Sensitivity

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**Background:** Approximately 25% of women have experienced intimate partner violence (IPV), with incidence often escalating during pregnancy. Given that adversity rarely occurs in isolation, IPV is frequently connected to other adverse childhood experiences (ACEs), such as neglect. The heightened neuroplasticity associated with early life exposure to perinatal trauma may increase ones' risk for persistent pain. Therefore, the aim of this study was to examine the effect of combined perinatal IPV and postnatal neglect on pain sensitivity following injury.

**Methods:** Sprague Dawley rat dams were randomly allocated to a control or IPV condition (TBI+60s hypoxia). Litters were then randomly assigned to early life neglect (maternal separation (MS)), or no stress, and further into an injury or sham group (n's>8/group). All pups were tested for pain and emotional processing. At euthanasia, tissue was taken for telomere length analysis. Four-way ANOVAs were run with sex, IPV, MS, and injury as factors.

**Findings:** The cold plate examined thermal nociceptive sensitivity, whereby the female and IPV groups had shorter latencies to react ( $p$ 's<.001). The vonFrey measured mechanical nociception, where smaller filament weights were observed for the IPV and MS groups ( $p$ 's<.001). The elevated plus maze measured comorbid anxiety-like behaviour, whereby in the female MS group, IPV animals spent more time in the closed arms than the No IPV animals ( $p$ <.001). Lastly, in the female No MS+Sham group, IPV animals had shorter telomere length than No IPV animals ( $p$ =.048).

**Interpretation:** Generally, we found that IPV increased both thermal and mechanical sensitivity, and interacted with sex, MS, and injury to increase anxiety and reduce telomere length. Additionally, female sex increased thermal sensitivity and MS increased mechanical sensitivity. This study provides invaluable insight into offspring outcomes associated with IPV and MS, as we demonstrate that cumulative early life trauma increases risk for pain.

**Funding:** This research was supported by the National Health and Medical Research Council (NHMRC; RM-AP1173565) of Australia.

## **A New Animal Model To Evaluate Concomitant Traumatic Brain Injury And Its Role In Developing Neuropathic Pain Following Spinal Cord Injury**

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**Background:** Neuropathic pain develops in ~75% of people with spinal cord injury (SCI) and is associated with altered sensory signalling pathways in the spinal cord and brain. SCI commonly co-occurs with a traumatic brain injury (TBI), however the effect of concomitant injury on the development of neuropathic pain is not known, exacerbated by a lack of clinically relevant animal models to recapitulate a co-occurring SCI and TBI injury. Accordingly, this study aims to develop a novel model of concomitant TBI and SCI to evaluate development of neuropathic pain post-injury.

**Methods:** Male Sprague Dawley rats (n=41) were assigned to study groups; Naïve, sham, SCI only (mild/moderate), TBI only or SCI (mild/moderate)+TBI. Mild TBI (Marmarou weight drop model [1m, 450g]) and/or hemicontusion SCI at C5 (Infinite horizons [100kdyne: mild; 200kdyne: moderate]) were induced, following which motor, cognitive and neuropathic pain were assessed (6 weeks post-injury). Statistical analysis was not performed due to sample size of preliminary data (presented as mean).

**Findings:** Preliminary data (n=1-3/group) show decreased locomotor activity at 1-week post-injury for the mod-SCI group (-53.09%baseline), with no additive effect of TBI (TBI+mod-SCI = -54.16%baseline), compared to shams (-11.21%baseline). Preliminary thermal hyperalgesia data (n=5-6/group) demonstrates lower sensitivity in the mild-SCI concomitant group at 3- (68.83%baseline) and 6-weeks (59.1%baseline) compared to all other injury groups. However, initial data assessing supraspinal responses to pain (Place Escape Avoidance Paradigm [n=2-4/group]) demonstrates that injury groups with TBI are more likely to experience pain compared to SCI groups alone.

**Interpretation:** Our preliminary results demonstrate feasibility of a novel clinically relevant animal model of concomitant SCI+TBI. We further highlight an additive effect of TBI on neuropathic pain outcomes, however completion of the study (increase samples sizes) is required to determine statistical significance.

**Funding:** Neurosurgical Research Foundation.

## Abnormal Default Mode Network Functional Connectivity Is Associated With Poorer Attention In Children With Acquired Brain Injury: A HD-EEG Study.

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**Introduction:** Traumatic brain injury (TBI) is the most common type of acquired brain injury (ABI) in children, where 691 per 100,000 children present in emergency departments annually. Attention problems are commonly reported as an ongoing problem following ABI, thought to arise due to brain network dysfunction particularly in the default mode network (DMN).

**Methods:** We investigated differences in functional connectivity in n=15 children with ABI (age: mean 12.7 (SD 3.33) years; males: n=11 (73%); injury type: n=12 (80%) TBI (predominantly mild); time post-injury: 3.8 (SD 2.7) years) compared to n=15 similar healthy controls using high-density electroencephalography (HD-EEG). Participants completed 5 minutes of resting eyes open (RO) and resting eyes closed (RC) HD-EEG. Reaction time (RT) was measured using the flanker task. Following pre-processing in EEGLAB, Brainstorm was used to extract source-based envelope correlation, and Network Based Statistics (NBS) was used to compare connectivity between ABI and HC groups.

**Results:** Increased slow wave connectivity was seen in ABI participants (RO delta and theta bands) across fronto-parietal interhemispheric brain networks. Increased fast wave connectivity (increased RC gamma and decreased RC alpha) was seen in the fronto-parietal DMN in ABI participants. Increased slow wave connectivity was moderately associated with slower RT in ABI participants.

**Discussion:** In keeping with previous literature, our results indicate ongoing DMN and fronto-parietal network dysfunction in association with attention in chronic, symptomatic ABI in children. Our findings encourage the use of HD-EEG as a portable and affordable prognostic tool in children.

No funding was received for this research.

## Home Based tDCS In Children With Acquired Brain Injury: Preliminary Results From The hrtDCS-Attention Trial.

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**Background:** Following acquired brain injury (ABI), 25% of children experience chronic attention difficulties. Daily transcranial direct current stimulation (tDCS) may improve attention after ABI; however, daily clinic visits are burdensome, limiting treatment access. Home-based, remotely-supervised tDCS may improve access and adherence to tDCS treatment.

**Methods:** This clinical trial ('hrtDCS-Attention') was a randomised, single-blind, dose-controlled feasibility study involving ten days of home based, remotely-supervised tDCS in n=10 children aged 7-18 years with ABI. Patients were randomised to receive 1 mA or 2 mA tDCS for 10 consecutive weekdays (20 minutes, anode: left dorsolateral prefrontal cortex (dlPFC), cathode: right dlPFC, 5x5cm) during gamified attention training. Feasibility, tolerability, and changes in attention performance over time (according to mean flanker reaction time (RT)) were measured.

**Results:** All patients attended ten sessions. Due to tolerability problems, n=1 patient was moved from the 2mA to the 1mA group, and n=1 patient (1mA) completed only 4/10 sessions (1mA group: n=5, mean age 11.1 (SD 3.2) years; n=4 (66%) male; 2mA group: n=4, mean age 13.7 (1.7) years; n=1 (25%) male). The 2mA group experienced greater itching (p=0.016), tingling (p=0.014), and burning (p<0.001) sensations. Dosage significantly affected post-tDCS RT, where the 2mA-group became significantly faster (F=8.6, p=0.02; controlling for pre-tDCS RT). Over 10 days, there was a significant effect of session (F=12.3,

$p < 0.001$ ), dosage ( $F=7.4$ ,  $p=0.03$ ), and session\*dosage ( $F=2.7$ ,  $p=0.004$ ), where 2mA-group became faster than 1mA-group.

**Discussion:** Ten days of home-based, remotely-supervised tDCS (1-2mA) is safe, feasible and tolerable in most children with ABI, and may improve attention.

This research was funded by a HIRF Pilot Scheme grant and UQ CHRC small equipment grant.

## Using NODDI To Characterise Longitudinal Changes In Free Water In Children With Chronic Mild Traumatic Brain Injury.

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**Introduction:** Approximately 300 to 550 children per 100,000 sustain a mild traumatic brain injury (mTBI) each year, where about 25-30% have long-term cognitive problems. Following mTBI, diffuse axonal injury (DAI) causes blood-brain-barrier disruption, gliosis and chronic neuroinflammation, causing movement of cerebrospinal fluid (CSF) into the extracellular space and leading to free water (FW) accumulation in frontal white matter (WM) tracts. Diffusion tensor imaging (DTI) can measure structural integrity following mTBI but is not sensitive to FW. Neurite Orientation Dispersion and Density Imaging (NODDI) metrics such as orientation dispersion index (ODI) and fraction of isolated free water (FISO) allow deeper insight into microstructural damage.

**Methods:** Whole-brain and tract-specific differences in ODI and FISO were investigated in children with persistent symptoms after mTBI (n=80) and children with clinical recovery (n=32) at 1- and 2-3 months post-mTBI compared to healthy controls (HCs; n=21). Two-way repeated-measure ANOVA and voxel-wise two-sample t-tests were conducted to compare whole-brain and tract-specific diffusion across groups. Results were corrected at pFDR<0.05. The association between NODDI metrics and clinical outcomes, and the sensitivity of NODDI metrics in predicting future recovery from mTBI were investigated.

**Results:** Whole-brain ODI was significantly increased in symptomatic participants compared to HCs at both one and two-months post-injury, particularly in the uncinate fasciculus (UF) and inferior fronto-occipital fasciculus (IFOF). ODI (e.g., diffusion) was highest in symptomatic participants, lower in asymptomatic participants, and lowest in HC participants. No changes in FISO were found across groups or over time. With 87% predictive power, ODI (1 month post-injury) significantly predicted recovery at 2-3 months post-injury, with more sensitivity than FA. FISO could not predict recovery at 2-3 months post-injury.

**Discussion:** Our results show evidence of ongoing microstructural reorganisation or neuroinflammation between 1- and 2-3 months post-injury, further supporting delayed return to play in children who remain symptomatic.

No funding received for this research.

## Cortical Electric Field Predicts tDCS-Related Attention Improvement In Children With Acquired Brain Injury: A Sham-Controlled Clinical Trial.

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**Background:** Approximately 25% of children experience long-term attention problems following acquired brain injury (ABI). Transcranial direct current stimulation (tDCS) can improve attention after adult ABI but response to treatment varies due to injury differences. We investigated whether simulated cortical electric field (E-field) was associated with tDCS-related attention improvement in children with ABI.

**Methods:** In a randomised, single-blind, counterbalanced, sham-controlled clinical trial, n=15 children with ABI (age: mean 12.7 (SD 3.33) years) and n=15 healthy controls (HCs) of similar age and sex received three single tDCS sessions during attention training (Go/No-Go and continuous performance): 20 minutes, 1 mA, 5x5cm, bilateral (1) dorsolateral prefrontal cortex (dlPFC), (2) inferior frontal gyrus (IFG), and (3) sham. Flanker reaction time (RT) was measured immediately pre- and post-tDCS. Individual head models were created from T1 and/or T2 scans taken prior to tDCS (simNIBS.4.0.1). Mean normalised E-field magnitude was estimated in 10mm or 25mm spherical ROIs over bilateral dlPFC or IFG and correlated with RT change post-tDCS.

**Results:** RT change was strongly associated with cathodal E-field in right dlPFC (25mm-ROI; Spearman's  $r=-0.71$ ,  $p=0.01$ ); or anodal E-field in left dlPFC (10mm-ROI;  $r=-0.60$ ,  $p=0.04$ ), where participants with higher E-field became faster (mean dlPFC-tDCS RT change: -0.08s (SD 0.2); mean normalised E-field anodal 10mm-ROI: 0.21V/m (0.05); cathodal 25mm-ROI: 0.20V/m (0.06)). RT change following IFG-tDCS was not associated with E-field.

**Discussion:** Results indicate a dose-response relationship between E-field and tDCS-related attention improvement following bilateral dlPFC, but not IFG, tDCS in children with ABI, supporting the personalisation of tDCS dosage.

This research was funded by a HIRF Pilot Scheme grant and UQ CHRC small equipment grant.

## Investigating The Association Between Brain Network Connectivity and Attention Following Acquired Brain Injury: A Systematic Review Of Structural And Functional Measures.

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**Introduction:** Globally, traumatic brain injury (TBI) and stroke are the most common causes of acquired brain injury (ABI), affecting 69 million and 15 million people each year, respectively. Cognitive complaints are common following ABI and have been linked to alterations in brain network connectivity following injury. However, the relationship between brain network disruption and common cognitive issues such as attention dysfunction is heterogenous. This systematic review examined the relationship between brain connectivity, measured across multimodal forms of neuroimaging (diffusion tensor imaging, functional magnetic resonance imaging, electroencephalography, functional near infrared spectroscopy), and attention following ABI.

**Methods:** Following the initial search (4693 results) and full text screening, we systematically reviewed 43 studies which reported a correlation between attention and brain connectivity.

**Results:** Following TBI, greater attention was associated with greater structural global and local efficiency within and between the executive network (ECN), salience network (SN) and default mode network (DMN); or greater fc within and between ECN and DMN. Following stroke, poorer attention was associated with lower structural connectivity within ECN; or greater fc between task positive networks. Attention-related structural connectivity differed across pediatric (n=7 studies) and adult (n=36 studies) populations, whereas fc trends were similar across the lifespan. Following TBI, recovery of attention over time was associated with normalisation of DMN activity; following stroke, with greater network modularity, particularly in DAN.

**Conclusions:** These results indicate that structural and functional connectivity changes in ECN, DMN, DAN and VAN are associated with attention following ABI across the lifespan and during recovery. The use of portable techniques such as EEG and fNIRS demonstrates potential for use at the point-of-care. Limitations include the predominant utilisation of ROI-based analyses, therefore biasing brain regions examined. Future research is needed using standardized analysis pipelines to limit heterogeneity, as well as long term follow-up to better understand recovery trajectories.

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## ROCK2 Regulates Microglia Proliferation And Neuronal Survival In A Mouse Model Of Traumatic Brain Injury.

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**Background:** Traumatic brain injury (TBI) results in prolonged and non-resolving activation of microglia. Forced turnover of these cells during the acute phase of TBI aids recovery, but the cell-intrinsic pathways that underpin the pro-repair phenotype of these repopulating microglia remain unclear. Previously, we found STAT3 activity upregulated within repopulating microglia. Molecular regulators of STAT3 includes the Rho-associated coiled-coil kinase, ROCK2. In the present study, we used KD025, a selective ROCK2 inhibitor aiming to investigate whether ROCK2 signalling influences microglia function(s) and outcomes after TBI.

**Methods:** Experiments were conducted approval from UQ Animal Ethics Committee. We used a unilateral controlled cortical impact model of TBI. Adult female C57Bl/6J mice or CX<sub>3</sub>CR1<sup>creERT2</sup> x iDTR mice (n=7-8/group) were used, the later for microglia repopulation, wherein tamoxifen was administered 6 weeks prior to surgery and repopulation of CX<sub>3</sub>CR1-expressing microglia induced using diphtheria toxin (IP). KD025 (200mg/kg, oral gavage) was used to inhibit ROCK2 signalling after TBI; control mice recovered a vehicle. Hippocampal function (spatial learning and memory) was assessed using the active place avoidance task. Brains were processed for immunohistology to assess microglial proliferation and survival of hippocampal neurons quantified using stereological methods.

**Findings:** We show that acute KD025 treatment (1-3 days post-injury) impairs the proliferative response of microglia after TBI as well as during genetically induced turnover of microglia. Acute KD025 treatment abolished the substantial neuroprotective and cognitive benefits conferred by repopulating microglia, preventing these cells from replenishing the depleted niche during the early critical time window post-injury. Delaying KD025 treatment to the subacute phase of TBI (4-6 days post-injury) allowed microglial repopulation to occur, but this did not enhance the benefits conferred by repopulating microglia.

**Interpretation:** Taken together, our data indicate that ROCK2 mediates neuronal survival and microglial population dynamics after TBI, including the emergence of repopulating microglia with a pro-repair phenotype.

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## Turnover Dynamics of CNS Border-Associated Macrophages in Adult Mice

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**Background:** Border-associated macrophages (BAMs), including meningeal, perivascular and choroid plexus macrophages, play essential roles in neuroimmune surveillance and responses in homeostasis and neuropathology. However, compared with microglia, the innate parenchymal macrophages in the central nervous system (CNS), the ontogeny and functions of BAMs in health and disease remain relatively unknown. This project focuses on the turnover of the macrophages by circulating monocyte-derived immune cells at the CNS border.

**Methods:** We adopted a transgenic model, ScfCreERT2 x R26-ZsGreenfl/fl mice, by which only bone marrow-derived BAMs rather than resident BAMs will be irreversibly marked when the mice are treated with tamoxifen. It prevents the drawbacks of many previous studies based on the bone marrow chimeric model (whole-body irradiation followed by bone marrow transplantation), which can induce strong immune responses and affect the normal dynamics of immune cells.

**Findings:** We found that macrophages in dura mater underwent 70% turnover in a span of four months. By contrast, only about 20% of the macrophages in pia mater and choroid plexus were replenished by circulating monocytes. Surprisingly, ageing did not affect the turnover rate. We established a reliable, non-invasive model to study the turnover dynamics of BAMs and determined the turnover rates under homeostatic and ageing conditions.

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