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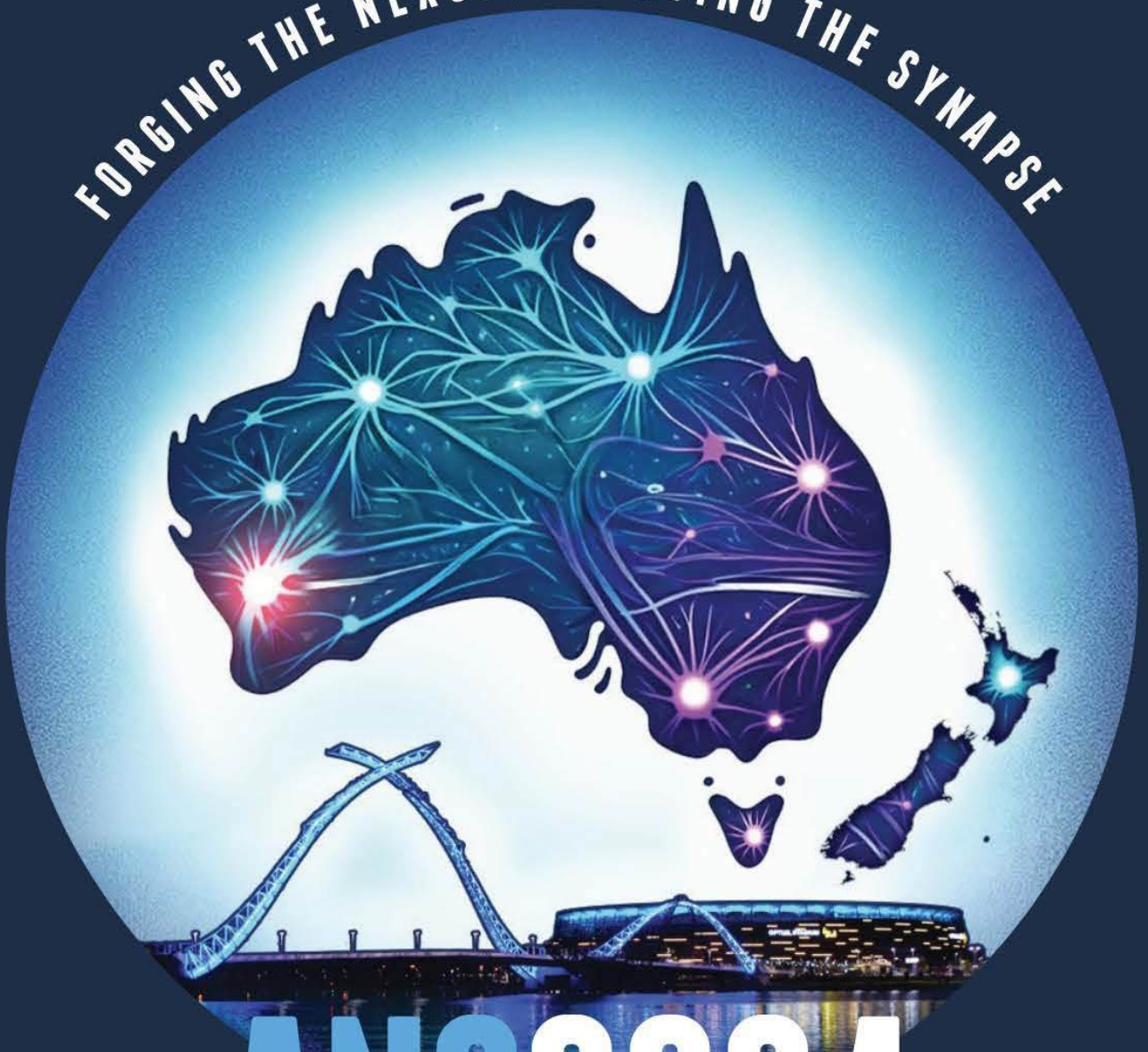

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id #21760

Actin dysregulation in sporadic ALS

Cyril Jagaraj[1], Sayanthooran saravanabhavan[1], Sonam Parakh[1], Sina Shadfar[1], Sara Assar Kashani[1], JULIE ATKIN[1]

1. Macquarie University, Marsfield, AUSTRALIA, Australia

Pathological forms of TAR-binding protein 43 (TDP-43) are present in frontotemporal dementia (FTD), Alzheimer's disease, and almost all amyotrophic lateral sclerosis (ALS) cases. Hence, identifying mechanisms that induce TDP-43 pathology are central to neurodegeneration and developing new therapeutic strategies. Actin has important roles in neurons, and it exists in dynamic equilibrium between monomeric G-actin and polymeric F-actin. Cofilin is an important actin-binding protein regulating actin dynamics. Its activity is controlled by phosphorylation (Ser3), which renders it inactive.Here, we demonstrate that dysregulated actin dynamics induces TDP-43 pathology in sporadic ALS. Actin dynamics was dysregulated in sporadic ALS, rNLS8 mice and neuronal cells expressing cytoplasmic TDP-43. Increased F-actin and enhanced cofilin phosphorylation were detected in spinal cord motor neurons of sporadic patients and mice with TDP-43 pathology. Pharmacological actin polymerization using Jasplakinolide induced TDP-43 pathology and recruitment to stress granules. TDP-43 interacted with actin and co-localised with cofilin in motor neurons. Cofilin peptides containing Ser3 rescued TDP-43 pathology, implying that cofilin phosphorylation is a therapeutic target for neurodegeneration.This is the first study to demonstrate that dysregulation of actin dynamics and the LIMK1/2-cofilin pathway are associated with TDP-43 pathology in ALS/MND. Moreover, we confirmed these defects are present in sporadic ALS/MND patients, thus are applicable to most cases. This study therefore identifies novel therapeutic targets for TDP-43 pathology in ALS/MND.

1. {Jagaraj, 2023 #1955} Dysregulated actin dynamics and cofilin correlate with TDP-43 pathology in sporadic amyotrophic lateral sclerosis Cyril Jones Jagaraj, Prachi Mehta, Sonam Parakh, Sina Shadfar, Shafi Jamali, Alexandra K Suchowerska, Jessica Sultana, Thomas Fath, Julie D Atkin

id #22017

Direct In Vitro Reprogramming of Reactive Astrocytes Using NeuroD1 Promoting Cre-FLEX AAV-5

Mikayla G Compton[1, 2], Alan R Harvey[1, 2], Isabella Drew[1, 2], Zara Van Zijl[1, 2], Stuart I Hodgetts[1, 2]

1. School of Human Sciences , The University of Western Australia , Crawley, WA, Australia

2. Perron Institute for Neurological and Translational Science, Perth , WA, Australia

Neural transcription factors are important for the differentiation and integration of neurons. Reprogramming neural cells to increase expression of the transcription factor NeuroD1 is a potential therapeutic approach to treat neurodegenerative diseases through increased local neurogenesis. Using direct reprogramming to transdifferentiate astrocytes into neurons results in downregulation of astrocyte specific promoters such as GFAP and subsequent loss of vector-related fluorescent markers. Dual AAV Cre-FLEX vectors lock their target genes in an 'activated' orientation, providing clearer evidence on whether reported effects on neurogenesis result from AAV reprogramming or spared neuronal populations. Using a serotype-five AAV (AAV-5) Cre^{GFAP}-FLEX $NeuroD1\text{-}GFP$ system that specifically links to the GFAP promoter of reactive astrocytes, NeuroD1 reportedly directs astrocyte transdifferentiation towards neuronal phenotypes. We used supposedly pure fetal human astrocytes or neural progenitor cell (NPC) cultures to determine the specificity of the Cre^{GFAP}-FLEX $NeuroD1\text{-}GFP$ AAV-5 system for astrocytes, and whether it causes transdifferentiation towards a neuronal phenotype. After one and six days there was no obvious GFP-fluorescence or consistent change in cellular morphology in either 'astrocyte' or NPC cultures. Instead, after plating commercially available fetal cell lines, a surprising heterogenous cell culture was identified by fluorescent protein markers in control and experimental trials, indicating limitations of the Cre-FLEX AAV-5 system. Namely, that the CRE-FLEX system requires both the Cre and FLEX AAVs to transfect individual cells to be effective, that the AAV-5 serotype may not be suitable for transfecting astrocytes, and that the presence of heterogeneous cell cultures may generate misleading result interpretation.

id #21508

The impacts of childhood stress on human cortical neuroglia in stress-associated psychopathology

Natalie Matosin[1]

1. The University of Sydney, Camperdown, NSW, Australia

Early life stress is a major societal problem due to the unacceptably high prevalence that transdiagnostically increases risk to psychopathology. Neuroglia, meaning "nerve glue," are crucial for holding the brain together, shaping its intricate structure and function. Consequently, neuroglia are implicated in various psychopathologies that involve global dysfunction, including schizophrenia, depression, and bipolar disorder. Animal and cell studies have shown that neuroglia are highly stress-responsive, and alterations to their function generates psychiatric-like symptoms. However glia are relatively understudied in humans, presenting a critical knowledge gap given glia are often poorly conserved between rodents and humans. My lab has been combining single-cell RNA sequencing, spatial transcriptomics, and advanced histological approaches to characterise human neuroglia and how they are impacted by stress and in psychopathology. We examine postmortem samples from individuals across a range of psychiatric disorders exposed to childhood versus adulthood stress, as well as 2D and 3D human pluripotent stem cell-derived glia treated with dexamethasone, a cortisol analogue, to mimic the stress response in a dish. In this presentation, I will give an overview of our work focusing on characterising astrocytes and microglia in the human prefrontal cortex. Our studies have shown, for the first time, that there are many subtypes of molecularly-defined astrocytes. Furthermore, these astrocyte subtypes in combination with previously defined microglia subtypes, are overrepresented in individuals with transdiagnostic psychiatric disorders exposed to stress in childhood. Lastly, our data suggests that

changes in astrocytes and microglia may culminate in neuropathologies related to neuroinflammation and the blood brain barrier.

id #21764

Insights into neurological immune-related adverse events associated with immune checkpoint inhibitor cancer therapy

Magdalena Lerch[1, 2], Ming-Wei Lin[3], David McDonald[3], Markus Reindl[4], Sarosh Irani[5], David Brown[3], Martin Stockler[6, 7], Matthew Cook[8, 9], Sudarshini Ramanathan[1, 2, 10]

1. Translational Neuroimmunology Group, Kids Neuroscience Centre, Children's Hospital at Westmead, Sydney, NSW, Australia

2. Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia

3. NSW Health Pathology-ICPMR, Centre for Immunology and Allergy Research, Westmead Institute for Medical Research, University of Sydney, Sydney, NSW, Australia

4. Clinical Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria

5. Mayo Autoimmune Neurology Group, Department of Neurosciences, Mayo Clinic, Jacksonville, Florida, United States

6. Department of Medical Oncology, Concord Cancer Centre, Concord Hospital, Sydney, NSW, Australia

7. Department of Medical Oncology, Chris O'Brien Lifehouse, Sydney, NSW, Australia

8. Division of Immunology and Infectious Diseases, John Curtin School of Medical Research, Australian National University and Canberra Clinical Genomics, Canberra, ACT, Australia

9. Cambridge Institute of Therapeutic Immunology and Infectious Disease, Department of Medicine, University of Cambridge, Cambridge, United Kingdom

10. Department of Neurology, Concord Hospital, Sydney, NSW, Australia

Aims: Cancer is a leading cause of morbidity and mortality worldwide. The development of immune checkpoint inhibitors (ICI) has revolutionised cancer therapy, and patients that were previously incurable can now have complete responses. However, their use can lead to the development of immune-related adverse events, which can induce severe disability, interruption of cancer therapy, and even death. Neurological autoimmune sequelae occur in 1-10% of patients treated with ICI and can be fatal. They can be associated with antibodies binding to neuronal or glial proteins. Here, we studied the presence of such autoantibodies in the serum of ICI-treated cancer patients, to define patients at risk and help with enabling a more personalised therapy. Methods: We analysed 169 serum samples of cancer patients before and after ICI treatment for the presence of neurological autoantibodies binding to nine different targets using gold standard live cell-based assays.Results: We found autoantibodies specific for multiple neuronal and glial targets in our ICI-treated cohort. Interestingly, while some patients developed these autoantibodies following ICI treatment, the majority of patients were already seropositive prior to ICI treatment initiation.Discussion: Our findings highlight the importance of testing for autoantibodies associated with neurological autoimmunity in cancer patients prior to receiving ICI. This can help with evaluating the individual risk for developing neurological immune-related adverse events associated with ICI, thereby creating a better personalised risk profile. Ultimately, a deeper understanding of the underlying immunological processes will contribute to minimising complications from ICI treatment and improving patient survival and quality of life.

id #22020

Opioid and Dopamine interaction in the amygdala-striatal transition zone regulate fear learning.

Roger Wang[1], Muskaan Kalra[1], Gabrielle Gregoriou[1], Elena Bagley[1]

1. School of Pharmacy, Faculty of Medicine and Health, Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia

Specificity in learning is crucial for our survival, ensuring knowledge is applied when needed, especially in the context of fear learning. Dysfunctions in these learning mechanisms can lead to undesirable responses and behaviours such as addiction or anxiety. Studies in both humans and rodents highlight opioids as a key neuromodulator inhibiting fear learning, while dopamine signalling in the amygdala is thought to induce synaptic plasticity, strengthening synapses and driving fear learning. The amygdalo-striatal transition zone (AST) is an enkephalin rich region receiving input from the medial geniculate nucleus (MGM) during fear learning and also activated by foot shock. The AST containing neurons with D1 or D2 receptors positions it as a potential site of opioid dopamine interaction. However, how endogenous opioids are released and interact with dopamine during fear conditioning remains unknown. Using electrophysiology and optogenetics in brain slices from male Sprague-Dawley rats, we asked whether MGM activity induces endogenous opioid release in the amygdala and whether the AST is the source. We observed that the opioid antagonist naloxone increased the synaptic responses following a train of stimuli (28.5±11.1%, n=6) whereas a single stimulus did not (4.55±3.98%, n=8), suggesting opioid release is driven by MGM activity. Furthermore, data from the fluorescent opioid sensor δ-light suggests the AST as the source, with opioid release modulated by dopamine. Knocking down proenkephalin in vivo further indicates that opioids in the AST are critical for fear learning. These findings highlight the importance of endogenous opioid release in the AST in the fear learning process.

id #22021

Structure and function of the human NALCN channelosome

Han Chow Chua[1]

1. The University of Sydney, Camperdown, NSW, Australia

The sodium leak channel (NALCN) conducts a neuronal background Na^+ current that plays a crucial role in the regulation of breathing, sleep and movement. To perform these functions, NALCN has to associate with auxiliary subunits including UNC79, UNC80, and FAM155A. Functional disruptions of one of these

evolutionarily conserved proteins have debilitating consequences ranging from apnoea, reversed circadian cycle to locomotor deficits. Despite its physiological significance, fundamental aspects regarding the organisation, composition and gating of the NALCN channelosome remain obscure, precluding a meaningful structure-function analysis. We determined the structure of this one megadalton complex, and discovered several unanticipated features. Contrary to previous speculations, the giant subunits UNC79 and UNC80 (2635 and 3258 amino acids, respectively) are highly structured, intertwined and anchored intracellularly to NALCN. We identified key regions in the interdomain linkers of NALCN that interact with UNC79 and UNC80, and showed that they are important for channel function using truncation and mutagenesis. Calmodulin (CaM), a previously uncharacterised component, copurifies with the channelosome. The intracellular activation gate of NALCN remains tightly shut even in the presence of all necessary components, incongruent with the expectation that a non-inactivating leak channel would have a constitutively open gate. However, single-channel recordings revealed an intrinsically low open-channel probability for NALCN, rationalising the non-conductive gate conformation captured. Together, these unprecedented glimpses into the architecture of the NALCN channelosome enabled us to decipher the contribution of individual component to function and to explore the gating mechanism of this machinery.

id #21510

Can threatening virtual reality protocols induce acute anxiety sufficient to cause physiological responses consistent with noradrenergic system activation?

James H O'Loughlin[1], Christopher Latella[1], Janet L Taylor[2]

1. Neurophysiology Research Laboratory, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia

2. Neuroscience Research Australia, Randwick, New South Wales, Australia

The noradrenergic system is engaged by many stimuli, including acute anxiety (AA), with widespread influence on autonomic systems. This study aimed to create AA using threatening virtual reality protocols (VRP) and determine whether physiological responses were consistent with noradrenergic system activation.

Naïve participants (n=10), underwent nine 3-min trials comprising six different threat VRP (overt threat [OT], implied threat [IT1-IT5]), two relaxing control VRP, and one mental arithmetic task (MA). Participants reported anxiety scores after each trial (1-5 Likert-scale [1=not anxious at all, 5=extremely anxious]). Pupil diameter (PD), galvanic skin response (GSR) and heart rate (HR) were recorded with means calculated from 0.5-2.5min in each trial. Pressure pain threshold (PPT) was assessed in the last 30s. After repeated measures ANOVAs, post-hoc paired t-tests compared each anxiety-producing protocol to the averaged relaxing protocols (RAVG). Exploratory repeated measures correlations (Rmcorr) were performed between outcome measures across protocols.

Several threat VRP induced mild AA (median=2.5/5) and significant increases in PD (IT5, $p<0.001$, mean difference [95%CI lower,upper], 12.4% [8.5,16.4]; IT2, $p=0.003$, 8.1% [4.4,11.9]; OT, $p=0.003$, 11.4% [6.1,16.8]) and PPT (OT, $p=0.014$, 119.2mN [43.9,194.4]; IT5, $p=0.019$, 108.2mN [32.4,183.9]; IT4, $p=0.021$, 152.3mN [38.6,266]) from RAVG. MA (AA: median=3/5) resulted in significant changes in PD ($p=0.016$, 10.6% [3.8,17.3]), PPT ($p=0.026$, 195.9mN [42.7,349]), GSR ($p<0.001$, -35.7mV [-45.7,-25.7]) from RAVG. Furthermore, PD correlated with PPT ($r_{rm}=0.27$ [0.029,0.49], $p=0.03$), PPT with GSR ($r_{rm}=-0.3$ [-0.5,-0.075], $p=0.01$), and GSR with HR ($r_{rm}=-0.36$ [-0.57,-0.17], $p=0.00095$).

These findings indicate that VRP can successfully create AA, sufficient to cause physiological responses consistent with noradrenergic system activation.

id #21512

A novel perfusion circuit enabling long-term in-vitro electrophysiological analysis and neuromodulation.

Azin Azadi[1], Andrew Doherty[1], Forough Habibollahi[1], Kwaku Dad Abu-Bonsrah[1], Brett Kagan[1]

1. Corticallabs, Melbourne, VIC, Australia

Advanced in-vitro tools have emerged to address ethical and environmental concerns in bio-related research[1]. Traditional methods maintain in-vitro cultures in incubators with manual media changes and monitoring. We developed a perfusion circuit for nearly intervention-free long-term neural cell culture maintenance and electrophysiological measurement. It incorporates an adaptable media supply system, a gas-exchange unit, a temperature controller, a filtering system and cell-hosting compartment. This device, integrated with a reader-stimulator for electrophysiology and neuromodulation, permits significant insights into synaptic plasticity, essential for learning and memory[2], along with detailed tracking of cell-culture maturation.

The automated closed-loop perfusion circuit is driven by multiple peristaltic pumps to ensure systematic media refreshment, enabling us to correlate neuronal activity with the consumption rate of media components. Additionally, the multiple pumps allow differential pressures to optimize a filtration system designed to reuse cleaned media for extended periods. The counter-flow filtration system, used in the perfusion circuit, provides an effective mechanism for neuronal media filtration within the perfusion circuit throughout the experiment. These features make the perfusion circuit ideal for neuroscientific research, disease modelling, and drug testing.

Neural cultures were grown on glass planar multielectrode arrays and integrated into the perfusion circuit[3]. Spent media and measured electrophysiological activity was measured over time. Our results confirmed that the perfusion circuit supports neural cell maintenance for over six months. With further development, we believe the perfusion circuit will become a valuable in-vitro tool, enhancing sustainability in neuronal studies and reducing animal testing in early-stage neuronal disease modelling and drug testing.

id #21768

The role of a cortico-hypothalamic pathway on hunger and metabolism

Vasilios Drakopoulos[1], Alex Reichenbach[1], Zane B Andrews[1]

1. Monash University, Clayton, VIC, Australia

The neural mechanisms controlling food intake and body weight have traditionally been ascribed to neuroendocrine and nutrient feedback. Within this homeostatic framework, hunger-sensing agouti-related peptide (AgRP) neurons increase food intake and bodyweight in response to feedback signals of energy deprivation. However, recent studies show that AgRP neurons receive significant sensory input from other brain regions, which regulates food intake. This sensory input provides important learned external information about food availability and palatability, which is integrated with mood and stress levels, to affect current and future motivated food-seeking and foraging. Although these neural circuits transmitting sensory information to hypothalamic feeding centres remain unknown, they may have important roles to override the homeostatic control of feeding behaviour and drive excessive consumption in the absence of hunger or suppress feeding in the presence of hunger. Indeed, overriding this homeostatic control of feeding may underlie different pathological states characterised by abnormal feeding behaviour such as obesity or anorexia nervosa. Recently a novel stress-sensitive neural pathway from the medial prefrontal cortex (mPFC) to lateral hypothalamus (LH) was identified to suppress feeding behaviour. Hence, we hypothesised an interaction between the mPFC-LH pathway and AgRP neurons that may affect food intake, feeding behaviour, and body weight. We show that chemogenetic activation of the mPFC-LH pathway supressed baseline and ghrelin-induced AgRP activity assessed using in vivo calcium imaging. Furthermore, via optogenetics and viral techniques, we also demonstrate its effect on bodyweight, food intake, and metabolism. These studies establish a top-down, novel cortico-hypothalamic network controlling food intake and metabolism.

id #22026

Unlocking the Gut-Brain-Axis: A Pilot Study Protocol on Faecal Microbiota Transplantation and Cognitive Function

Sara Alaeddin[1], Yanna Ko[1], Genevieve Steiner[2], Tara Roberts[1], Slade Jensen[1], Vincent Ho[1]

1. School of Medicine, Western Sydney University, Campbelltown, NSW, Australia

2. NICM Health Research Institute, Western Sydney University, Westmead, NSW, Australia

Faecal Microbiota Transplantation (FMT) is an emerging, gut-microbiome altering therapy showing promising results for various gastrointestinal and neurological disorders (Bakker & Nieuwdorp, 2017). Previous research suggests that FMT might improve cognitive function in neurodegenerative diseases (Chen et al., 2023; Cheng et al., 2023; Kim et al., 2024; Park et al., 2022). Additionally, studies on other microbiome altering techniques, such as probiotics, indicate effects on cognitive functions in healthy adults (Cannavale et al., 2023; Chung et al., 2016). However, to the best of our knowledge, no clinical trials have yet investigated the efficacy of FMT on cognition in cognitively unimpaired adults. To address this gap, we are conducting a pilot study to explore the impact of FMT on cognitive function in adults with Irritable Bowel Syndrome (IBS).

In this randomised, controlled, double-blinded trial, participants have received a single dose of FMT or placebo, following comprehensive baseline testing. The primary outcome is cognitive function, while secondary outcomes include IBS symptom severity and mood. Additionally, microbiome composition and blood biomarkers, potentially reflecting changes in the microbiota-gut-brain axis, are assessed as tertiary outcomes. Outcomes are being measured at baseline and at one, three, six and twelve months post-intervention.

This pilot study aims to explore potential changes in cognitive function following FMT or placebo. To assess a broad range of cognitive domains, including reaction time, verbal memory, working memory, visual memory, and problem-solving, we are using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Data collection is set to conclude in December 2024.

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id #22027

The vasopressin receptor antagonist SRX246 alters expression and extinction of conditioned avoidance in rats.

Fergus Scott[1], Elise Cleary[1], John Lin[1], James Crane[1], William Connolly [1]

1. School of Medicine , University of Tasmania , Hobart, Tasmania, Australia

Vasopressin is an ancient neuropeptide with key physiological roles, but it is also involved in behaviour, particularly fear and anxiety. We aimed to test if endogenously released vasopressin is involved in the expression and/or extinction of conditioned avoidance, a key symptom of fear and anxiety. Male Sprague Dawley rats were trained for 10 days in a platform mediated avoidance protocol in which rats learn to avoid a 2s foot shock (0.4mA) that co-terminates with a 30s tone (4 kHz, 75 dB) by stepping onto a plastic platform. On day 11, rats were administered the V1a receptor antagonist SRX246 (2.5mg/kg; n = 8; i.p.) or vehicle (n = 9; i.p.) 30 min prior to an extinction procedure consisting of 9 tone presentations without shock. On day 12, the rats were re-exposed to the tone alone to test for avoidance extinction. On day 11, there was a significant main effect ($p<0.001$) of SRX246, increasing the time rats spent on the platform during inter-tone periods. However, there was no significant effect of SRX246 treatment on avoidance, compared to vehicle, during the 30 second tones. On day 12, SRX246 treated animals spent significantly more time on the platform during the tones ($p<0.001$) and periods separating the tone ($p<0.05$) when compared to vehicle treated rats. These results suggest that endogenous interaction with the V1a receptor during PMA could act to promote risk taking in threatening contexts and could be involved in extinction learning. This challenges the traditional assumption surrounding vasopressin as an anxiogenic.

id #22028

Association of elevated Trimethyl amine and its metabolites with Gut microbial dysbiosis in Parkinson's Disease.

Divya Mondhe[1], Katherin E Roper[1], Nanthini Jayabalan[1], Zizheng Xian[1], Helen M. Woodhouse[1], Robert J. Dr Adam[2], John D. Dr O'Sullivan[2], Anumantha G. Dr Kanthasamy[3], Richard Dr Gordon[1]

1. Queensland University of Technology, Wooloongabba, QLD, Australia

2. University of Queensland, UQ Centre for Clinical Research, Brisbane, Queensland, Australia

3. University of Georgia, Athens , Georgia, United States of America

Multiple cohort studies indicate that gut microbiota composition is significantly altered in people with Parkinson's disease (PD). Despite this, pathological significance of the gut microbial dysbiosis remains elusive. Gastrointestinal dysfunction, often preceding clinical PD diagnosis, is commonly observed in PD patients. In this study, we evaluated the changes in gut microbial metabolites in PD patient biofluids and used functional metagenomics to determine changes in microbial composition and pathways in PD patients compared to an age-matched healthy cohort. Our analysis identified notable shift in multiple microbial metabolites, including Trimethylamine (TMA), a brain-permeable metabolite of bacterial origin with a positive association with the rate of PD progression and cognitive decline. TMA and its metabolites were also altered in both PD patient blood and urine in a cohort of healthy (n =31) and PD (n = 31) patients. We also uncovered elevated levels of Formaldehyde (FA), in our PD cohort. Our functional metagenomics studies demonstrate for the first time that TMA-generating bacteria, as well as bacterial enzymatic pathways responsible for its generation to be increased in PD. We also uncovered a trend towards loss of beneficial bacteria which produce anti-inflammatory metabolites, such as butyrate, in PD. Our results provide novel insights into how gut dysbiosis and microbial metabolism can drive PD.

id #21517

Characterising the role of the locus coeruleus norepinephrine system in the zebrafish model

Rebecca Lee[1], Marcello Lepre[1, 2], Cheryl Lee[2], Elizabeth O'Brien[1], Raphael Ricci[2], Carlie Cullen[2], Itia Favre-Bulle[1, 3]

1. Queensland Brain Institute, Brisbane, Queensland, Australia

2. Mater Research Institute-UQ, Brisbane, Queensland, Australia

3. School of Mathematics and Physics, The University of Queensland, Brisbane, Queensland, Australia

The locus coeruleus (LC) is an anatomically small, yet functionally powerful nuclei that is the main source of norepinephrine (NE) to the central nervous system and is essential for several key cognitive processes. Despite its small size, current understanding of the circuitry underlying the LC-NE system remains limited due to its positioning within the brainstem. In the zebrafish, the LC is evolutionarily conserved and composed of approximately 10 neurons. This low number of neurons alongside the advantage of optical transparency in zebrafish offers the opportunity to comprehensively characterise the LC-NE system. In this study, we have established a series of transgenic zebrafish lines that target the LC-NE system using genetically encoded calcium indicators and opsins. Previous work has established that dopamine beta hydroxylase (DBH) selectively labels NE neurons. Therefore, using the strengths of the Gal4/UAS system, we established transgenic lines through the injection of dbh:Gal4. Collectively, these transgenic lines characterise the LC-NE system in terms of its structural, functional, and circuitry implications. Preliminary work using these zebrafish lines have seen heterogeneity in the number of LC-NE neurons both between fish and within each fish bilaterally. Additionally, the calcium activity of LC-NE neurons also appears to show differing response profiles across individual cells. This provides supporting evidence for the emergent theory that LC neurons act independently to achieve specific cognitive goals. Through manipulation of the LC-NE system with sensory stimulation and optogenetics, this study will provide key insight into the level of influence the LC has on sensory processing.

id #22029

Maternal immune activation disrupts LINE-1 mobile DNA regulation during neurodevelopment

Jose de Los Rios Barreda[2, 1], Marie-Jeanne H.C. Kempen[2], Francisco J. Sanchez-Luque[2], Adam Ewing[2], Geoffrey J. Faulkner[2, 1], Gabriela O. Bodea[1]

1. Queensland Brain Institute, University of Queensland, St Lucia, QLD, Australia

2. Mater Research Institute-University of Queensland, Woolloongabba, QLD, Australia

Maternal immune activation (MIA) during pregnancy is a recognised environmental risk factor for psychiatric diseases, such as schizophrenia. MIA triggers various pathophysiological processes, including inflammation in the mother and the developing offspring. However, the precise molecular mechanisms through which MIA influences brain development are not well understood.

To gain insights into the early impact of MIA on neurodevelopment, pregnant dams were injected with Poly (I:C), a mismatched double-stranded RNA mimicking viral infection, at embryonic day 9.5. To identify gene expression changes triggered by MIA, we performed RNA sequencing on embryonic brains at 3 hours (peak immune response) and 72 hours (response subsided) post-MIA.

Our results reveal significant changes in genes associated with proliferation and differentiation, alongside a marked reduction in Long Interspersed Element 1 (LINE-1) expression 3 hours post-MIA. LINE-1 is a repetitive, mobile DNA element that can affect gene expression, and its dysregulation has been previously linked to schizophrenia. Using Oxford Nanopore long-read DNA sequencing, we identified genome-wide increased LINE-1 methylation in MIA compared to controls. Further, LINE-1 protein expression was reduced in embryonic brains at 72 hours, a finding confirmed through an orthogonal approach in neuronal cultures.

These observations are intriguing, as recent research (Toda et al., Cell Rep, 2024) suggests that LINE-1 may play a role in maintaining neural progenitor pools and regulating neuronal differentiation. Our ongoing work aims to determine whether LINE-1 downregulation persists at later stages in the MIA model, identify the specific epigenetic mechanisms at play, and understand its contribution to pathogenesis.

id #22031

Investigating the Relationship Between Neuroinflammation and Synaptic Loss

Thomas Alexakis[1], Bill Zhu[1], Bryce Vissel[1]

1. Centre for Neuroscience and Regenerative Medicine, St. Vincent's Centre for Applied Medical Research , Darlinghurst, NSW, Australia

Alzheimer's Disease (AD) is a progressive neurodegenerative disease that has been established as the leading cause of dementia, primarily affecting the elderly population. One of the best correlates of cognitive decline in AD, is the loss of synapses within the hippocampus. In addition to this, we also know that neuroinflammation is a prominent feature of AD that is also shared with numerous other neurodegenerative conditions. As such, we hypothesise that there may be an association between neuroinflammation and the loss of synapses and therefore, for this study we aim to determine if neuroinflammation has an effect on the loss of synapses. To determine if there is an association between neuroinflammation and synaptic loss, we will first aim to establish an in-vivo model of neuroinflammation and subsequently determine if there is a loss of synapses. To accomplish this, we will be investigating the effect of age and the administration of various pro-inflammatory stimuli such as LPS and poly(I:C) in isolation and in combination, on neuroinflammation and synaptic loss. We are currently investigating this via the intrahippocampal administration of these various pro-inflammatory stimuli in mice at various ages. Hippocampal tissue will then be assessed for the presence of neuroinflammation and analysed to determine if there are any changes in synaptic density. We hope that by establishing a model of neuroinflammation with associated synaptic loss, it will contribute to gaining a better understanding of how synapses are lost within the context of AD pathology.

id #21520

Microglia make direct contact with the cerebral vasculature through gaps between adjacent astrocyte endfeet

Gary P Morris[1], Catherine G Foster[1, 2], Brad A Sutherland[1], Soren Grubb[3]

1. Tasmanian School of Medicine, University of Tasmania, Hobart, TASMANIA, Australia

2. Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, Washington, United States of America

3. Center for Translational Neuromedicine, Faculty of Health Sciences DK-2200 , University of Copenhagen, Copenhagen , Denmark

Publish consent withheld

id #22032

Plasma soluble TREM2 and its association with other biomarkers in cognitively normal older adults at risk of Alzheimer's disease

Prita Asih[1], Cameron Morris[2], Wang Hong[2], Steve Pedrini[3], Kathryn Goozee[1], Kevin Taddei[3], Hamid Sohrabi[4], Simon Laws[3], Stephanie Rainey-Smith[4], Pratishtha Chatterjee[5], Ralph Martins[3, 1]

1. Macquarie University, Macquarie Park, NSW, Australia

2. Eli Lilly and Company, Indianapolis, IN, USA

3. Edith Cowan University, Perth, WA, Australia

4. Murdoch University, Perth, WA, Australia

5. The Florey Institute, Melbourne, Victoria, Australia

Objective: Investigate the association of plasma sTREM2 levels with Neocortical Amyloid-β Load (NAL) and other AD biomarkers in cognitively normal elderly at risk of AD (high NAL) compared to those with low NAL. Also, to investigate the effect of AD-linked TREM2 variants on plasma sTREM2 levels and preclinical

AD features.

Methods: Plasma sTREM2 concentrations were measured employing the Meso Scale Discovery (MSD) assay in the KARVIAH cohort, aged 65–90 years. Genetic data, levels of microglial serum kynurenine pathway (KP) metabolites, plasma GFAP, total tau, phosphorylated tau, and NFL were included for correlation analysis. Based on NAL SUVR (low NAL (n = 65) and high NAL (n = 35), clinical plasma sTREM2 levels, APOE and TREM2 genotypes, subgroup analysis was carried out. **Results**: No significant difference in plasma sTREM2 was observed between high NAL and low NAL participants; however, among APOE ε4 carriers, higher NAL levels were observed in individuals with higher sTREM2 concentrations. Furthermore, plasma sTREM2 positively correlated with NAL and plasma NFL in high NAL participants only. Additionally, plasma sTREM2 positively correlated with plasma KP metabolites, pTau181, and pTau231, albeit independently of NAL status. Interestingly, plasma sTREM2 in high NAL with R47H carriers positively correlated with NAL and negatively with cognitive performance.

Conclusions: Plasma sTREM2 is closely associated with amyloid-β positivity, neuroinflammation, and tau-induced neurodegeneration in cognitively normal older adults at risk of AD. This study also unveiled a critical genetic association between APOE and TREM2 which may synergistically affect brain amyloid-β load at the early stage of AD.

id #21521

Brain criticality analysis reveals excitation-inhibition balance and network stability in the primary visual cortex network

Han Wang[1], Jason Jung[1], Steven Prawer[1], Michael Ibbotson[1], Wei Tong[1]

1. University of Melbourne, Parkville, VIC, Australia

A functional balance between excitation and inhibition (E-I) in neural circuits is crucial, as its disruption is often linked to neurological disorders like epilepsy. Brain criticality theory suggests the brain operates optimally near a critical state when E-I balance is achieved. The branching ratio (BR) describes the brain's proximity to this optimal state and the E-I balance. A BR value close to 1 indicates a more critical brain. This study uses brain criticality analysis to explore the role of different neurons in E-I balance and network stability.

We analysed single-unit activity recorded from cat's primary visual cortex (V1) driven by white Gaussian noise, classifying neurons by waveforms. BRs were estimated using the MR estimator toolbox. Results show significant differences in BRs between excitatory and inhibitory neurons (BR_e=0.978±0.007, BR_i=0.925±0.052. T-test P=0.011). Excitatory neurons (BR closer to 1) play a major role in maintaining the overall E-I balance. Furthermore, excitatory neurons exhibit less variable BRs than inhibitory (Std=0.007), indicating their cohesive role in network stability. In contrast, inhibitory neurons display greater BR variability (Std=0.052), reflecting their diverse functions. Silencing most neurons in V1 via a GABA agonist, muscimol, significantly reduced the BR (P=0.012), further underscoring the major role of excitatory neurons in maintaining the E-I balance.

This study demonstrates the effectiveness of brain criticality analysis in revealing the roles of excitatory and inhibitory neurons in the E-I balance and underscores the critical role of excitatory neurons in maintaining network stability. These results could offer insights for developing therapeutic strategies for neurological disorders.

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id #21777

A new angle on transcranial magnetic stimulation coil orientation: A targeted narrative review

Andris Cerins[1], Elizabeth Thomas[1], Tracy Barbour[2], Joe Taylor[2], Shan Siddqi[2], Nicholas Trapp[3], Alexander McGirr[4], Kevin Caulfield[5], Josh Brown[6], Leo Chen[1]

1. Brain Stimulation Laboratory, Dept. Psychiatry, Monash University, Melbourne, Victoria, Australia

2. Harvard Medical School, Boston, Massachusetts, USA

3. Dept. Psychiatry, University of Iowa, Iowa City, Iowa, USA

4. Dept. Psychiatry, University of Calgary, Calgary, Alberta, Canada

5. Dept. Psychiatry, Medical University of South Carolina, Charleston, South Carolina, USA

6. Brain Stimulation Mechanisms Laboratory, Division of Depression and Anxiety Disorders , McLean Hospital, Belmont, Massachusetts, USA

Background: Repetitive transcranial magnetic stimulation (rTMS) for treatment-resistant depression is effective in approximately half of those treated. Response may be related to stimulation parameters including intensity, pattern, stimulation site, and brain network engagement. Another parameter, the orientation or yaw angle of the figure-of-eight rTMS coil may also be relevant because it is thought to influence the intensity and site of stimulation. However, coil orientation has remained largely unchanged since rTMS was first used to treat depression.

Methods: We performed a targeted narrative review of coil orientation in experimental, clinical and computational TMS studies.

Results: We provide an illustrated overview of the TMS electric-field and its orientation-dependent interaction with underlying head tissues. Current evidence suggests that changes to coil orientation can alter the TMS electric-field and change the site of stimulation, which may not be under the coil center. Additionally, computational research suggests that coil orientation might influence brain network targeting. We therefore canvass generalized and individualized approaches to selecting rTMS coil orientation. We suggest that rTMS induced current could be directed perpendicular to local sulcal axes, as determined by average or individual anatomy. We highlight that modelling of the TMS-induced electric-field can be used to estimate the coil position and orientation for stimulating a chosen target. We further note possible implications for brain-network engagement.

Conclusions: Coil orientation is an under-explored aspect of rTMS treatment. Informed adjustments to coil orientation might contribute to accurate dosing and targeting, suggesting a potential role in future research directed at improving rTMS treatment outcomes.

id #22033

Our Moon's Mission: Brain organoids as a platform to advance AAV9 gene therapy to clinical trials: a hereditary spastic paraplegia 56 case study

Hannah Leeson[1], Connie Petroeschevsky[2], Dominik Froehlich[3], Elizabeth Gillam[2], David Coman[4], Ernst Wolvetang[1]

1. Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, QLD, Australia

2. School of Chemistry & Molecular Biosciences , The University of Queensland, St Lucia, Qld, Australia

3. School of Biomedical Sciences, The University of New South Wales, Sydney, NSW, Australia

4. School of Medicine, The University of Queensland, St Lucia, QLD, Australia

Unreliable mouse models have been the downfall of many promising therapies for neurological disease. Here we conduct safety and efficacy trials of an AAV9 gene therapy for hereditary spastic paraplegia 56 (SPG56) in patient-derived brain organoids to more accurately recapitulate this neurological disorder and with the added objective of determining disease mechanisms. SPG56 is characterised by early onset spasticity and axonal neuropathy and is caused by mutations in CYP2U1, a CYP450 enzyme involved in long chain fatty acid metabolism. We generated iPSCs from two SPG56 patients and their heterozygous parents, and used these lines to generate neuronal and brain organoid cultures. We subsequently treated brain organoids with EF1A-CYP2U1-AAV9 and measured CYP2U1 enzymatic activity, mitochondrial function, and conducted transcriptomic, proteomic and metabolomic analysis. Mouse trials were performed in parallel to explore the potential of organoids as a pre-clinical platform. We found that CYP2U1^{mut} organoids and neuron cultures had impaired neuronal morphology and electrical function, reduced energy generation (altered Kreb cycle metabolites and down regulated fatty acid β-oxidation pathways), and impaired mitochondrial properties, including respiration. Treatment with AAV9 gene therapy increased CYP2U1 expression, restored neuronal processes and significantly altered levels of Krebs cycle metabolites and transcriptomic pathways. By comparing brain organoid data to data obtained from CYP2U1-AAV9 mouse trials conducted in parallel, we not only provide the data required to progress to clinical trials in a small number of patients, but are also validating the use of brain organoids for pre-clinical gene therapy.

id #21522

A new immunocompetent brain organiod model to study demyelinating diseases

Soumya Garawadmath[1], Huiwen Zheng[1], Selin Pars[1], Eduardo Albornoz Balmaceda[1], Merja Joensuu[1], Trent Woodruff[1], Jessica Mar[1], Ernst Wolvetang[1], Giovanni Pietrogrande[1]

1. University of Queensland, Brisbane, QLD, Australia

We have harnessed the potential of immunocompetent brain organoids to study neurodegeneration, particularly in the context of demyelination and multiple sclerosis. By developing a novel protocol, these organoids now intrinsically generate various neuronal types, myelinating oligodendrocytes, microglia, and astrocytes (MMBOs), effectively representing a complete model of the human brain. By treating the organoids with lysolecithin to induce demyelination, we gain valuable insights into the relationship between this process, inflammatory responses of microglia and alterations in neuronal activity, which ultimately leads to neuronal degeneration. Our groundbreaking approach allows us to examine the intricate dynamics between demyelination, inflammation, and neuronal activity within the brain organoids. With microglia serving as key players in neurodegenerative processes, we can now assess their role in real time and the impact on neuronal function. This comprehensive brain organoid model opens new avenues for understanding neurodegenerative diseases and identifying potential therapeutic targets. Adopting this advanced model, we plan to uncover critical mechanisms underlying neurodegeneration and facilitate the development of innovative treatments to combat these debilitating conditions and promote remyelination.

id #21778

Biological models of perception, conscious perception and decision at cellular scale

Artemio Soto-Breceda[1], Nathan Faivre[2], João Barbosa[3, 4], Michael Pereira[1]

1. Grenoble Institut des Neurosciences, INSERM, Université Grenoble Alpes, Grenoble, AUVERGNE-RHôNE-ALPES, France

2. Univ. Savoie Mont Blanc, CNRS, , LPNC, Université Grenoble Alpes, Grenoble, France

3. Cognitive Neuroimaging Unit, Université Paris-Saclay, NeuroSpin center, Gif/Yvette, France

4. Institut de Neuromodulation, GHU Paris Psychiatrie et Neurosciences, Centre Hospitalier Sainte-Anne, Université Paris Cité, Paris, France

Perceptual decision-making is the cognitive process of interpreting sensory information to guide behaviour, which is accompanied by confidence, a subjective assessment of certainty. While neural circuits for perceptual decision-making are well-characterised, those for confidence are not. Moreover, the cellular and subcellular mechanisms related to perception and decision-making remain poorly understood and research suggests that mechanisms driving perception may differ from those underlying decision-making. Our study aims to uncover neural mechanisms correlating with perceptual decision-making.

We present an approach extending Wang's (2002) attractor network model, which uses leaky integrate-and-fire neurons to replicate drift diffusion behaviour for two-choice decision-making. Our recurrent neural network model adapts the leaky integrate-and-fire equations to rate-based neurons and builds upon this biophysical framework, expanding it to incorporate confidence through a four-layer architecture: sensory, integration, decision, and confidence layers. This allows us to investigate whether detection can occur without conscious perception, and to study the neural basis of confidence judgments.

We validated our model against behavioural data, successfully replicating key metrics including detection accuracy, reaction times, confidence ratings, and perceived stimulus duration across various conditions. This framework offers a cellular-scale tool for investigating perceptual decision-making processes.

Future research will involve calibrating the model using intracranially recorded neural activity from humans. Our work presents a novel application of Wang's model, adapted to incorporate perceptual experience and confidence. This provides a biologically plausible approach to understanding cognitive process dynamics and examining neuronal-level variations in perceptual decision-making, opening the door for investigating potential biomarkers for psychiatric associated with perception and decision.

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id #21267

Seeking into the pathogenesis of Parkinson's disease and the development of the therapeutics

Kensuke Ikenaka[1]

1. Osaka University Graduate School of Medicine, Suita, OSAKA, Japan

Parkinson disease (PD) is characterized by the progressive loss of dopaminergic neurons caused by alpha-synuclein (αSyn) aggregation. Although the toxicity of the existing αSyn has been studied extensively, the upstream of the αSyn aggregation hasn't been elucidated so far. Previously, our team has revealed by FT-IR spectroscopy that the lipid content is rich in the Lewy body inclusion in the brain of PD patients and hypothesized that the aggregation of αSyn can be initiated by the accumulation of some lipids in the neuron. In accordance with this hypothesis, it has been known that about 10 % of PD patients possess the mutation in glucocerebrosidase (GBA), leading to the accumulation of Glucosylceramide; however, in the remaining 90 % of the sporadic cases, the upstream cause of the αSyn aggregation has not been elucidated. Therefore, we performed the screening of the lipids interacting with αSyn and inducing the aggregation and found that phosphatidylinositol-3-phosphate (PIP3) strongly affects αSyn aggregation. Interestingly, the αSyn fibril created with PIP3 showed similar structural features to the fibrils amplified from the PD patient's brain, indicating that PIP3 plays a role in the PD brain. We confirmed that PIP3 is indeed accumulated in the PD brain and the accumulation of PIP3 induces αSyn aggregation in cultured cells, primary cortical neurons, and C. elegans. We are currently performing drug development targeting the aberrant interactions between PIP3 and αSyn as a preventive therapy against the development of PD.

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id #21523

Combined administration of D-cycloserine (DCS) and MK-801 disrupts threat memory reconsolidation in mice and reduces pERK-expressing neurons in the lateral amygdala

sayed koushik ahamed[1, 2], **Sanket Raut**[3, 2, 4], **Fionnghuala James**[5, 2], **Vanni Caruso**[6], **Sukhwinder S. Sohal** [7], **Luke Johnson**[2, 8, 9]

1. Pharmacy and pharmacology, PhD Candidates, Hobart, TASMANIA, Australia

2. School of Psychological Sciences , University of Tasmania, LAUNCESTON, Australia

3. School of Medicine, University of Queensland, , Research Fellow , Brisbane, Queensland, Australia

4. Gallipoli Medical Research , Research Fellow, Brisbane, Qu

5. Pharmacy and pharmacology, PhD candidates, Hobart, Tasmania, Australia

6. School of Pharmacy and Pharmacology, University of Tasmania, Hobart, TASMANIA, Australia

7. Health Sciences, University of Tasmania, LAUNCESTON, TAS, Australia

8. School of Medicine, , University of Queensland, Brisbane, Queensland, Australia

9. Center for the Study of Traumatic Stress, USU School of Medicine, Bethesda, MD, USA

Background and Objective: Post-traumatic stress disorder (PTSD) is characterized by persistent threat-related memories with limited effective treatment options. The N-methyl-D-aspartate (NMDA) receptor dependent extracellular signal-regulated kinase (ERK) pathway in the lateral amygdala (LA) is necessary for synaptic plasticity and memory processes, including threat memory consolidation and reconsolidation. Reconsolidation requires memories to be brought into a labile state. Disruption of these memories during the reconsolidation window has been proposed as a therapeutic strategy for PTSD. We investigated the effect of the NMDA receptor agonist D-cycloserine (DCS) and antagonist MK801 on threat memory reconsolidation in male C57BL/6 mice.

Method: Using auditory threat conditioning, DCS or a vehicle was administered one hour before memory reactivation to facilitate labialisation of a recalled threat memory, followed by MK-801 or a vehicle after reactivation to disrupt the memory. Experimental groups included vehicle, DCS 15 mg/kg, MK-801 0.06 mg/kg, and MK-801 0.12 mg/kg alone and combined. The effect on phosphorylated ERK (pERK) expressing neurons was analysed using neuron counting and topographical mapping in the lateral amygdala (LA).

Results: MK-801 alone (P = 0.0421 and 0.0228) and in combination with DCS (P = 0.0040 and 0.0007) significantly reduced the number of pERK-expressing neurons in the LA. Freezing behaviour was reduced by high dose MK801 when combined with DCS.

Conclusion: These findings suggest that DCS and MK-801 together effectively disrupt pERK expressing neurons and disrupt fear memory reconsolidation, offering new MDMA receptor-based treatment strategies for PTSD.

id #22035

Clearing the fog: The potential of plasmalogens in mitigating chemotherapy-induced cognitive impairment.

Isobel T Thomas-Bland[2, 1], John Mamo[2, 1], Ryu Takechi[2, 1], Yu Yu[1, 3], Tim Rosenow[4], Virginie Lam[2, 1]

1. Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia, Australia

2. Curtin School of Population Health Perth, Curtin University, Perth, Western Australia, Australia

3. Curtin School of Medicine, Curtin University, Perth, Western Australia, Australia

4. National Imaging Facility at Centre for Microscopy Characterisation and Analysis, University of Western Australia, Perth, Western Australia, Australia

Chemotherapy-induced cognitive impairment, often called 'chemobrain', is a devastating neurocognitive sequela of chemotherapy that adversely impacts up to 70% of breast cancer survivors. Pre-clinical investigations postulate that chemobrain arises from several neurotoxic mechanisms, including exaggerated circulating pro-inflammatory cytokines, elevated oxidative stress, chronic neuroinflammation, and, subsequently, neurovascular breakdown, impairing myelin homeostasis and neuroplasticity.

The recognition of interventional adjunct therapeutics with pleiotropic effects to target the implicated neuropathological effects remains unfulfilled and is critical in preventing chemobrain. Plasmalogens are neuroprotective in several neurodegenerative conditions, likely attributed to their pivotal role in cell signalling and potent antioxidant and anti-inflammatory properties.

To induce chemobrain, C57BL/J6 mice were administered 2.0 mg/kg of doxorubicin once weekly for four weeks. Mice received either standard or 2%w/w of plasmalogen-supplemented chow for four weeks. Post-chemotherapy, cognitive function was assessed, followed by in vivo magnetic resonance imaging to determine volumetric alterations and white matter anomalies. Ex vivo confocal immunomicroscopy was used to determine blood-brain barrier permeability, neuroinflammation, oxidative stress and neuronal death.

Doxorubicin-treated mice developed significant spatial memory and learning impairments, accompanied by reduced hippocampal atrophy, heightened neuroinflammation and substantive neuronal loss. Plasmalogen co-supplementation in doxorubicin-treated mice substantially restored cognitive function in conjunction with 'restoration' of volumetric alterations in specific regions of interest, compared to controls. Revival of cognition was associated with a marked reduction in blood-brain barrier permeability, microglia and astrocyte activation, and oxidative stress. Overall, our findings identify plasmalogens as an effective therapeutic agent for preventing and reversing chemobrain and restoring brain health, enabling potential clinical translation.

id #21524

Distribution of vasoactive intestinal polypeptide (VIP)-expressing interneurons across the marmoset cortex

Sadaf Teymornejad[1], Katrina H. Worthy[1], Nafiseh Atapour[1], Marcello G. P. Rosa[1]

1. Department of Physiology and Neuroscience Program, Monash University, Clayton, VIC 3800, Australia

Vasoactive intestinal polypeptide (VIP) is known to be present in a subclass of GABAergic cortical interneurons. Here, using stereological analysis of VIP-stained sections, we investigated the distribution of VIP-positive (VIP+) interneurons across areas and layers of the marmoset cortex. Estimates of mean VIP neuronal density revealed an increasing trend from posterior to anterior cortex with prefrontal and frontal cortices having the highest VIP densities. Visual, posterior parietal and inferior temporal areas were characterised by lower VIP densities. VIP+ interneurons were more abundant in the supragranular layers and constituted ~2-8% of the total cortical neuronal population. These interneurons had different morphologies including bipolar, double bouquet and bitufted cells. Interestingly, we also found VIP to be present in the giant layer 5 pyramidal (Betz) neurons which are characteristic of the limb and axial representations of the marmoset primary motor cortex (cytoarchitectural area 4ab). The selective expression of VIP was indicated by the lack of VIP staining in smaller layer 5 pyramidal cells present in the primary motor facial representation (cytoarchitectural area 4c), or in the premotor cortex (e.g. the caudal subdivision of the dorsal premotor cortex, A6DC). Our results suggest that VIP interneurons are most abundant in the anterior cortical areas and demonstrate the expression of VIP in the largest excitatory neurons of the primate cortex, which may offer new functional insights into the role of VIP in the brain and provide opportunities for genetic manipulation of Betz cells.

id #22036

Machine learning-based segmentation and classification of axons following optic nerve injury

Parth Patel[1], Sarah Hellewell[1, 2, 3], Brittney Lins[1, 2], Terry McGonigle[1], Andrew Warnock[1], Naing Lin[1], Enoch Teo[1], Carole Bartlett[1], Melinda Fitzgerald[1, 2], Chidozie Anyaegbu[1, 2]

1. Curtin University, Canning Vale, WA, Australia

2. Perron Institute for Neurological and Translational Sciences, Sarich Neuroscience Research Institute, Nedlands, Western Australia, Australia

3. Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Crawley, Western Australia, Australia

Changes to axonal ultrastructure provide important pathological insight into secondary degeneration following manual and semi-automated methods for quantifying axonal morphometrics require significant user input, making them labour-intensive. AxonDeepSeg is a convolutional neural network for automatic morphometric feature analysis of micrographs. We hypothesised that AxonDeepSeg could be used to derive both traditional and new axon and myelin metrics while retaining high fidelity to manual measurements. We trained an AxonDeepSeg model to segment axons in transmission electron micrographs of optic nerves from female Piebald Virol-Glaxo rats following a partial transection injury (n=5) or sham surgery (n=5). Our model segmented 68 images with a 94.7% pixel-wise accuracy and was comparable to manual measurement of axon diameter. Using AxonDeepSeg measurements, we found that injury increased the g-ratio ($p<0.03$), solidity ($p<0.02$), and axon area/fibre area ratio ($p<0.02$). Unsupervised spectral clustering revealed several clusters of axons with unique

defining metrics. Injury significantly altered these cluster metrics, resulting in a larger g-ratio (p=0.02), solidity (p=0.03) and axon area/fibre area ratio (p=0.03) in cluster 1 and larger g-ratio (p=0.02), axon area (p<0.001), axon diameter (p=0.03), solidity (p=0.02), and axon area/fibre area ratio (p=0.02) in cluster 2. We conclude that AxonDeepSeg accurately reproduced manual measurements of axon morphology, and enabled discovery of novel morphometrics that distinguish injured axons from uninjured axons, allowing charting with injury. AxonDeepSeg will be a valuable tool for objective and high-throughput analysis of axonal morphology, facilitating future investigations in neurotrauma and neurodegeneration.

id #21525

Moderate coffee and tea consumption is associated with slower cognitive decline: data from UK Biobank

Kelsey Sewell[1], Belinda M Brown[2], Ralph N Martins[3], Stephanie R Rainey-Smith[2], Samantha L Gardener[3]

1. AdventHealth Research Institute, Neuroscience, Orlando, Florida, USA

2. Centre for Healthy Ageing, Health Futures Institute, Murdoch University, Murdoch, Western Australia, Australia

3. Edith Cowan University, Nedlands, WA, Australia

Objectives – Worldwide, coffee and tea are two of the most popular beverages. Studies suggests they have a protective role and reduce the risk of cognitive decline. We aimed to assess the relationship between their intake and cognitive decline.

Methods – Cognitively unimpaired (n=8,451) from UK BioBank (≥60 years) with at least 2 follow up visits (average follow up time 8.83 years) were included. Participants completed baseline self-report coffee and tea intake, and cognitive assessment including Pairs Matching test, Reaction Time test, Numeric Memory test, and Fluid Intelligence.

Key findings – Linear mixed models (including potential confounders) showed those both never consuming coffee and those with moderate coffee consumption (1-3 cups) had slower declines in Fluid Intelligence compared to those with high coffee consumption (³4 cups, β=0.06, *SE*=0.02, p=.005; β=0.07, *SE*=0.02, p<.001, respectively).

Those who never drank tea showed a greater decline in Fluid Intelligence compared to those who had moderate and high tea consumption (β=0.06, *SE*=0.02, p=.009; β=0.06, *SE*=0.02, p=.003), respectively).

Conclusions - Coffee and tea drinking may be a protective factor against cognitive decline, particularly for maintaining Fluid Intelligence. There is an upper limit for beneficial effects of coffee intake (maximum 3 cups per day), and no coffee intake is more beneficial than 4 or more cups. Conversely, high tea consumption provided positive effects on cognition in addition to moderate consumption, with high tea consumption being more beneficial than no tea consumption. Additional longitudinal observational and intervention studies are required to validate our findings and confirm this hypothesis.

id #21781

Pharmacological treatment of brain copper deficiency

Anne A Li[1], Benjamin G Trist[1], Tzongtyng Hung[2], Michael P Gotsbacher[3], Carl Power[2], Rachel Codd[3], Kay L Double[1]

1. Brain and Mind Centre and School of Medical Sciences (Neuroscience), , The University of Sydney, Sydney, New South Wales, Australia

2. Biological Resources Imaging Lab & Biospecimen Services Mark Wainwright Analytical Centre, Lowy Cancer Research Centre University of New South Wales Sydney, Sydney, New South Wales, Australia

3. Molecular Biomedicine Research Theme, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia

Maintaining physiological levels of copper is crucial for cellular function. Imbalances in cellular copper levels lead to a range of disorders, including rare life-threatening genetic diseases such as Menkes disease and Wilson's disease, and common neurogenerative disorders, such as Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS). Copper dyshomeostasis represents an early change in PD and ALS, with restoration of physiological copper levels in the central nervous system (CNS) proposed to slow disease progression. The copper-containing ionophore diacetylbis(N(4)-methylthiosemicarbazonato)copper(II) (CuATSM) delivers copper to the CNS and may restore physiological brain copper levels in these, and other, disorders characterised by CNS copper deficiency. However, the dosage and treatment frequency required to achieve CNS copper restoration using CuATSM is unknown. We employed copper-deficient $Ctr1^{+/-}$ mice to model brain copper deficiency and orally treated these animals with 8 mg/kg of CuATSM for either 10, 15 or 21 days. Treatments were performed daily, or 4 days per week, using micropipette-guided drug administration. Copper levels in the cerebellum, cortex, brainstem, deep brain nuclein, serum, muscle, and liver of CuATSM-treated $Ctr1^{+/-}$ mice were compared with untreated $Ctr1^{+/-}$ mice and wild-type C57BL6 mice. We confirmed untreated $Ctr1^{+/-}$ mice have significantly lower levels of copper compared to wild-type C57BL6 mice in all CNS regions studied. Twenty-one days of 8 mg/kg daily CuATSM treatment restored $Ctr1^{+/-}$ mice copper levels in all CNS regions studied to equivalent levels observed in wild-type mice. Data from this study could inform the design of copper supplementation in preclinical research and clinical trials.

id #22038

Impact of chronic repetitive transcranial magnetic stimulation on the retinocollicular pathway in ephrin-A2 knockout mice: a retrograde tracing approach

Liz Jaeschke-Angi[2, 1], Deondra Ong[3, 1], Marissa Penrose-Menz[2, 1], Sarah Etherington[3], Jennifer Rodger[2, 1]

1. School of Biological Sciences, The University of Western Australia, Perth, WA, Australia

2. Perron Institute of Neurological and Translational Sciences, Perth, WA, Australia

3. School of Medical, Molecular and Forensic Sciences, Murdoch University, Perth, WA, Australia

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive therapy for neuropsychiatric conditions, yet its cellular mechanisms are not well understood, limiting its optimization. Our lab uses transgenic mice lacking ephrin-A2 to study the effect of low-intensity (LI) rTMS on neural circuitry. Ephrin-A2-/- mice have abnormal supernumerary projections in the retinocollicular pathway, and we previously showed that chronic LI-rTMS removes ectopic terminations in the superior colliculus (SC) in these mice. However, anterograde tracing cannot determine if ectopic projections in the transgenic mice stem from incorrectly projecting retinal ganglion cells (RGCs) or aberrant branching, limiting our understanding of LI-rTMS mechanisms. To address this, we employed dual-colour retrograde tracing from the SC to test whether (1) ectopic projections are due to abnormal branching (double-labelled RGCs) and (2) if LI-rTMS reduces these ectopic branches. Adult ephrin-A2-/- mice were assigned to LI-rTMS or control groups, receiving 10 daily sessions of LI-rTMS or no treatment. Post-stimulation, animals underwent stereotaxic injection of Hoechst 33342 and DiI into the rostral and caudal SC to retrogradely label RGCs. Retina wholemounts were analysed 1 week after the injections. All control animals (n=4) had double-labelled RGCs (0.84% ± 0.68 of total labelled RGCs), indicating ectopic projections arise from abnormal branching. Preliminary data from an LI-rTMS-treated mouse suggest LI-rTMS does not reduce double- labelled RGCs (0.33% double-labelled RGCs) compared to controls. Our results contradict previous findings, suggesting ectopic projections in ephrin-A2-/- mice may originates from both aberrant branches and incorrectly projecting RGCs, with LI-rTMS affecting only the latter.

id #21528

Behavioural Effects of High Doses of Psilocybin in Female Rats

Larissa LR Larissa[1], Bart BE Ellenbroek[1]

1. Victoria University of Wellington, Wellington, WELLINGTON, Brazil

Depression and anxiety are two of the most predominant mental disorders and leading causes of disability. The World Health Organization estimated that 3.8% of the world's population suffers from depression, and between 0.4% and 3.6% have an anxiety spectrum diagnosis. Psychedelics, particularly psilocybin, have been extensively studied as new and promising treatments, showing long-lasting positive results when combined with psychotherapy for patients with depression and anxiety, among other disorders. However, pre-clinical trials investigating the precise neurobiological mechanisms underlying these results are still scarce, especially with female subjects. Additionally, emerging evidence suggests that hormonal fluctuations may influence the drug's efficacy, potentially providing a protective effect. This study examined the behavioural effects of high doses of psilocybin on depression and anxiety-like measures in adult female rats. SERT knockout animals were tested with 4 or 8 mg/kg doses of psilocybin using the affective disorders test (ADT). A secondary analysis in a small group of animals assessed the impact of the oestrous cycle phase when the animals were injected. Results showed no robust effects on depression and anxiety-like measures with either dose. However, trends were observed in anxiety-like measures at 4 and 8 mg/kg. Evaluation indicated an interaction between treatment and the oestrous cycle phase, suggesting female hormones might protect against the drug's effects on depression-like measures.

id #21784

Retinol supplementation improves locomotion and reduces anxiety-like behavior in male mice fed a high-fat diet

Abdur Rahman[1], Abrar Al-Shahabi[1], Irina Al-Khairi[2], Jehad Abubakr[2], Mohammad Al-Onaizi[3, 2], Mohamed Abufarha[2]

1. College of Life Sciences, Kuwait University, Kuwait, KUWAIT

2. Biochemistry and Molecular Biology, Dasman Diabetes Institute, Kuwait, Al-Asima

3. Department of Anatomoy, Faculty of Medicine, Kuwait University, Kuwait, KUWAIT

Feeding high-fat diet (HFD) to rodents has been shown to induce anxiety-like behavior by exacerbating amygdala plasticity and amygdala-dependent aversive memory. Vitamin A (VA) deficiency has been shown to be associated with impaired cognition. We investigated if VA supplementation could ameliorate anxiety-like behavior in mice fed HFD. Eight weeks old C57BL/6J mice were divided into two groups. Group 1 (HFD-control) was fed an HFD diet (60% fat) for 8 weeks, and group 2 (Retinol-HFD) was supplemented with VA (10,000 IU retinol/Kg/day) by gavage for 8 weeks along with HFD. Exploratory activity and anxiety-like behavior were measured by an open-field test. Distance traveled and time spent in the center zone by each animal was recorded for 30 min with a video-imaging system. Body weight was significantly higher in the Control-HFD group ($p<0.05$) in both male and female. In the male mice, the number of entries to the central zone, the time spent, and the distance covered in the central zone were all significantly higher ($p< 0.05$) in the Retinol-HFD group compared to the control-HFD group. In addition, the total distance covered in the open field was significantly higher in the Retinol-HFD group compared to the control-HFD group. In the female mice, VA supplementation did not show any effect on any of these parameters of the open-field test. These data suggest that VA supplementation improves locomotion and reduce anxiety-like behavior in an HFD-fed male mice only. The mechanism by which VA produce these effects is currently under investigation.

id #22043

Quantitative electroencephalography reveals regional brain dysfunction in mild traumatic brain injury

Caerwen S Beaton[1, 2], Andre Avila[1, 2], Melissa Papini[1, 2], Jacinta Thorne[1, 2], Aleksandra Gozt[1, 2], Francesca Buhagiar[3], Elizabeth Thomas[4, 5], Alexander Ring[6, 7], Glenn Arendts[8, 9], John C Iliff[10, 11, 12, 13], Antonio Celenza[14, 15], Sjinene Van Schalkwyk[16], Philip Brooks[5, 10, 17], Dan Xu[4, 10, 18],
19, 20 1, 4, 10 1, 3 9, 21, 22 23 24 25

Stephen Honeybul , Gill Cowen , Carmela Pestell , Daniel Fatovich , Ben Smedley , Ashes Mukherjee , Michael Bynevelt , Melinda Fitzgerald[1, 2], Sarah Hellewell[1, 2]

1. Faculty of Health Sciences, Curtin Health Innovation Research Institute, Perth, WA, Australia
2. Perron Institute of Neurological and Translational Science, Perth, WA, Australia
3. The University of Western Australia, School of Psychological Science, Perth, WA, Australia
4. Curtin University, School of Population Health, Perth, WA, Australia
5. School of Medicine, The University of Western Australia, Division of Pathology and Laboratory Medicine, Perth, WA, Australia
6. Murdoch University, Institute for Immunology and Infectious Diseases, Perth, WA, Australia
7. Faculty of Health Sciences, Curtin University, School of Physiotherapy and Exercise Science, Perth, WA, Australia
8. Fiona Stanley Hospital, Emergency Department, Perth, WA, Australia
9. Harry Perkins Institute of Medical Research, Centre for Clinical Research in Emergency Medicine, Perth, WA, Australia
10. Curtin University, Curtin Medical School, Perth, WA, Australia
11. Saint John of God Hospital Murdoch, Emergency Department, Perth, WA, Australia
12. Royal Perth Hospital, Emergency Department, Perth, WA, Australia
13. Western Operations, Royal Flying Doctor Service, Perth, WA, Australia
14. Sir Charles Gairdner Hospital, Emergency Department, Perth, WA, Australia
15. School of Medicine, The University of Western Australia, Division of Emergency Medicine, Perth, WA, Australia
16. Joondalup Health Campus, Emergency Department, Perth, WA, Australia
17. Saint John of God Midland Public Hospital, Emergency Department, Perth, WA, Australia
18. Sun Yat-Sen University, The First Affiliated Hospital, Guangzhou, China
19. Government of Western Australia, Department of Health, Perth, WA, Australia
20. Royal Perth Hospital and Fiona Stanley Hospital, Sir Charles Gairdner Hospital, Perth, WA, Australia
21. Royal Perth Hospital, Emergency Medicine, Perth, WA, Australia
22. The University of Western Australia, Perth, WA, Australia
23. Rockingham General Hospital, Emergency Department, Perth, WA, Australia
24. Armadale Health Service, Emergency Department, Perth, WA, Australia
25. Sir Charles Gairdner Hospital, The Neurological Intervention & Imaging Service of Western Australia, Perth, WA, Australia

Objectives: Mild traumatic brain injury (mTBI) can cause physical, psychological, cognitive and sleep-related symptoms, though it is unclear how symptoms relate to brain dysfunction. This study used quantitative electroencephalography (qEEG) to detect regional brain dysfunction and determine correlation with symptoms post-mTBI.

Methods: 24 participants with mTBI (36.56±12.87y; 10F) and 30 matched controls (33.88±12.15y; 14F) were included in the Concussion REcovery STudy subanalysis. Participants completed the Post-Concussion Symptom Scale (PCSS) and Depression-Anxiety-Stress Scale (DASS-21) 5-days post-injury. qEEG scans were acquired in Neuroguide across 19 channels in resting-state with eyes open. NeuroNavigator generated Brodmann areas (BA) current source density (CSD). T-tests were used to determine the effects of mTBI on CSD across BAs. Pearson correlations evaluated the relationship of BA CSD findings to PCSS and DASS-21 scores.

Findings: Substantial bilateral CSD increases in mTBI participants were detected within the cingulate and retrosplenial cortices compared to controls. In the mTBI group, delta CSD within the left cingulate correlated with PCSS total score ($r= -0.48$, $p<0.05$) and severity ($r= -0.53$, $p<0.05$). Beta band bilateral CSD of cingulate (BAs 23, 31) and retrosplenial cortices (BAs 26, 29) correlated with DASS-21 anxiety and stress scores (all $r>0.44$; $p<0.05$), while unilateral CSD correlated with depression scores: right-BA 23, $r=0.47$, $p<0.05$; left-BA 26, $r=0.52$, $p<0.05$; right-BA 29, $r=0.63$, $p<0.001$.

Conclusion: These findings suggest that the cingulate and retrosplenial cortices are vulnerable to mTBI. Early CSD alterations may drive emotional symptoms post-injury, as they correlate with emotional states linked to BAs involved in anxiety, depression, and stress-related memory.

id #21532

The cuprizone-autoimmune encephalomyelitis (CAE) model of inflammatory demyelination causes immune cell infiltration, oligodendrocyte loss and gliosis in the adult mouse brain

Thomas S Lewis[1], Roisin A Moloney[1], Surbhi Agarwal[1], Alastair J Fortune[1], Kalina Makowiecki[1], Jessica L Fletcher[1], Kaylene M Young[1]

1. Menzies Institute of Medical Research, Hobart, TAS, Australia

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system (CNS). Unlike other demyelinating mouse models, cuprizone autoimmune encephalitis (CAE) has the potential to recapitulate key elements of the MS lesion environment specifically within the brain. To examine this possibility, mice received 2 weeks of cuprizone (0.2% w/w) in standard chow to biochemically alter brain myelin, after which they receive complete Freund's adjuvant and Pertussis toxin to activate the peripheral immune system. Brain tissue was collected 21 days later. By performing immunohistochemistry, we determined within the CAE brain there was $CD3^+$ T cell infiltration of the corpus callosum ($p=0.0001$), and reduced myelin basic protein (MBP) expression ($p=0.0001$). This demyelination was associated with decreased oligodendrocyte densities ($p=0.0001$). We also detected evidence of gliosis, including microgliosis ($p=0.01$), astrogliosis ($p=0.0001$) and an increase in oligodendrocyte progenitor cell densities ($p=0.01$). To examine if these OPCs can differentiate into new premyelinating or myelinating oligodendrocytes within the CAE lesion environment, we have fluorescently labelled and fate mapped OPCs in young adult *Pdgfra-CreER^{TM} :: Rosa26-YFP* transgenic control and CAE mice. We hypothesise that new oligodendrocyte survival will be significantly impaired within CAE lesions. We conclude that the CAE model of inflammatory demyelination recapitulates key elements of MS pathophysiology, providing insight into how the this condition may affect key cells within the CNS.

id #22045

Resilience and coping as defenders or drivers of symptom severity following mild traumatic brain injury.

Melissa G Papini[2, 3, 1], Jacinta Thorne[2, 3, 4], Aleksandra Gozt[5], André Avila[2, 3, 1], Caerwen Beaton[2, 3, 1], Geena Gill[6], Elizabeth Thomas[7, 8], Francesca Buhagiar[9], Alexander Ring[4, 10], Glenn Arendts[11], Antonio Celenza[12, 13], Dan Xu[1, 14], Gill Cowen[2, 3], Carmela Pestell[4, 9], Daniel Fatovich[15, 16], Michael Bynevelt[17], Melinda Fitzgerald[2, 3], Sarah C Hellewell[2, 3]

1. Curtin Medical School, Curtin University, Bentley, Western Australia, Australia

2. Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University, Perth, Western Australia, Australia

3. Perron Institute of Neurological and Translational Science, Nedlands, Western Australia, Australia

4. School of Allied Health, Faculty of Health Sciences, Curtin University, Bentley, Western Australia, Australia

5. Connectivity, Traumatic Brain Injury Australia, Nedlands, Western Australia, Australia

6. Curtin University, Nedlands, WA, Australia

7. Centre for Clinical Research Excellence, School of Population Health, Curtin University, Bentley, Western Australia, Australia

8. Division of Surgery, School of Medicine, University of Western Australia, Nedlands, Western Australia, Australia

9. School of Psychological Science, The University of Western Australia, Nedlands, Western Australia, Australia

10. Institute for Immunology and Infectious Diseases, Murdoch University, Murdoch, Western Australia, Australia

11. Emergency Department, Fiona Stanley Hospital, Murdoch, Western Australia, Australia

12. Emergency Department, Sir Charles Gardiner Hospital, Nedlands, Western Australia, Australia

13. Division of Emergency Medicine, School of Medicine, The University of Western Australia, Nedlands, Western Australia, Australia

14. The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, Guangdong Province, China

15. Emergency Medicine, Royal Perth Hospital, Perth, Western Australia, Australia

16. Centre for Clinical Research in Emergency Medicine, Harry Perkins Institute of Medical Research, Nedlands, Western Australia, Australia

17. The Neurological Intervention & Imaging Service of Western Australia, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia

Objectives: Resilience and adaptive coping strategies may mitigate the impact of psychological distress on symptom severity after mild traumatic brain injury (mTBI). This study investigates how resilience and coping styles moderate the relationship between acute psychological distress and symptom severity at 3-, 6-, and 12-months post injury.

Methods: 31 participants (13F, 36.6±13.5y) recruited ≤7d post-mTBI as part of the Concussion REcovery STudy completed self-reports: Depression Anxiety and Stress Scale-21 (total: psychological distress), Brief Resilience Scale (low: ≥$\bar{x}$-1SD, high: ≥$\bar{x}$+1SD) and Utrecht Coping List (coping styles: active, passive, avoidant, palliative, emotion, social, reassuring thoughts). Post-Concussion Symptom Scale total scores (PCSS) at 3-(n=30), 6-(n=29) and 12-months (n=28) determined severity.

Findings: Elevated distress at ≤7d post-injury predicted greater PCSS scores at 3-(β=0.754, p<0.001), 6-(β=0.486, p=0.022) and 12-months (β=0.438, p=0.026). Hayes PROCESS assessed moderating influences of resilience and coping style. Resilience moderated distress effects at 3-months (b=0.0538, p<0.05); low resilience increased PCSS scores (b=0.734, p<0.001), high resilience mitigated these effects. At all levels, resilience moderated increased 6-month PCSS scores (b=0.4054, p<0.05). Lower passive coping increased influence of distress, worsening 6-month PCSS scores (b=1.233, p=0.008). Higher emotion-based coping augmented effects of distress intensifying 3-month PCSS scores (b=1.141, p<0.001). Neither resilience nor coping style moderated relationships at 12-months.

Conclusions: Psychological distress at the time of injury increases vulnerability to elevated long-term symptoms. Resilience and coping style may buffer or exacerbate this relationship depending on recovery stage. Implementing early interventions focused on fostering adaptive coping strategies could be critical in attenuating symptom severity and improving long-term outcomes.

id #22047

Dopamine and Acetylcholine release dynamics in the pDMS during the acquisition and updating of goal-directed behaviour

Thomas J Burton[1], Genevra Hart[1], Christopher R Nolan[1], Bernard W Balleine[1]

1. University of New South Wales, Randwick, NSW, Australia

Plasticity in the posterior dorsomedial striatum (pDMS) is critical to the acquisition and updating of goal-directed behaviour and requires the precise timing and patterning of coincident dopamine (DA) and acetylcholine (ACh) release. Recently, we demonstrated that pDMS DA release at the time of the action reflects the long-term action-outcome relationship, the associative substrate for goal-directed behaviour. For example, stable profiles of action-related DA release are transiently disturbed by action-outcome reversal. However, it remains to be determined how DA release during the consumption of the outcome changes during such reversal learning. Further, the real-time dynamics of ACh have not been directly assessed under such conditions. Here we aimed to characterise real-time DA and ACh release during action-outcome learning and reversal. Using dLight (DA) or AChSnFR (ACh) biosensors in separate subjects, fiber photometry recordings were taken from the pDMS as hungry rats learned to press two levers (actions) for distinct food rewards (outcomes) and as these contingencies were subsequently reversed. Replicating our previous findings, we confirm that DA release at the time of the action is perturbed by reversal but recovers with further training. However, no change in release was observed during outcome consumption. While we describe a novel profile for ACh release during goal-directed behaviour, we did not observe any obvious changes in ACh release after the contingency manipulation. Therefore, pDMS DA release appears to be sensitive to contingency manipulations during the action but not the outcome. Real-time ACh release profiles during goal-directed behaviour appear to be stable once acquired.

id #21792

Brain computer interface for tetraplegic patients using EEG signals of motor imagery

Jessica Hall[1], Carl Xing[1], Helena Peng[1], Adeline Trieu[1], Praghashkmar Thanasegeren[1], William Chen[1]

1. Melbourne Bioinnovation Student Initiative (MBSI), Parkville, VIC, Australia

Modern day wheelchairs lack accessibility for individuals with tetraplegia. Brain computer interfaces (BCIs) can use motor imagery (MI) decoded from motor cortical activity to control external devices, offering means of control for individuals with few options to access independence.

We have designed a minimally interactive BCI-controlled wheelchair that only requires the user to have control over eye movement and a functioning motor cortex. The proposed system combines gaze tracking technology for directional commands, MI for stop/start initiation, and observational error related potentials (OErrp) for maintaining safety in the event of an error from the user or the BCI.

MI experiments were recorded via wireless electroencephalography (EEG) using only channels C3, Cz and C4. A linear discriminant analysis (LDA) model was used to classify the presence/absence of MI based on power spectral density (PSD) features of the signal between the Mu band of 7-30Hz. Offline decoding accuracies were obtained using 5-fold cross-validation. Using publicly available OErrP datasets, we developed a principal component analysis (PCA) and support vector machine pipeline to decode OErrPs. Using leave-one-out cross-validation, we showed that using PCA could improve the decoding accuracy of OErrP from ~80% to >90%.

Preliminary results indicate the possibility to command a BCI-controlled wheelchair with a high level of accuracy. Future work will involve continuous development of BCI systems by recording pilot OErrP experiments and implementing parallel online decoding of MI and OErrPs.

id #22050

Chronic fatigue syndrome is accompanied by lower persistent inward current activity of the tibialis anterior muscle

Christopher Latella[1], James L Nuzzo[1], Anthony Blazevich [1], Greg Pearcey[2], Janet L Taylor[1, 3]

1. School of Medical and Health Sciences, Edith Cowan University , Joondalup, Australia

2. Memorial University, Newfoundland, Canada

3. Neuroscience Research Australia, Randwick, Australia

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex condition characterised by persistent fatigue amongst other symptoms. Potentially, altered motoneuron behaviour could contribute to the muscle weakness and increased effort in performing motor tasks that are sometimes reported(1). In particular, motoneuron persistent inward current (PIC) activity is essential to produce strong muscle contractions and depends on monoamine neuromodulation, which may be disrupted in ME/CFS(2). Hence, this study compared PIC activity in individuals with and without ME/CFS. Individuals with ME/CFS (n=7, 3F, 45±11y) and healthy controls (n=6, 2F, 37±9y) performed ankle dorsiflexor submaximal voluntary ramp contractions (20-s duration) at 10% and 30% maximum voluntary force (MVF) while high-density electromyography recorded motor unit activity from tibialis anterior. After decomposition, ΔF was calculated as an indicator of PIC activity level. A 10-point OMNI scale assessed perceived effort during contractions. A mixed-effects model compared motoneuron data between groups. Individuals with ME/CFS reported greater perceived effort during submaximal contractions than healthy controls (10%MVF contractions: 5.2±2.2 vs. 1.3±0.5 [p=0.003], 30%MVF contractions: 5.7± 2.0 vs. 3.2±1.0 [p=0.017]). Overall, ΔF was ~24% lower (3.26 vs. 4.27 pulses/s, p=0.01) in people with ME/CFS. ΔF appeared higher in 30%MVF than 10%MVF contractions for healthy controls (4.83 vs. 3.79 pulse/s) but not individuals with ME/CFS (3.41 vs. 3.11 pulses/s). These preliminary results suggest that impaired motoneuron behaviour in ME/CFS is accompanied by greater perceived effort to perform muscular contractions. Less increase in ΔF at higher force levels may indicate impaired monoaminergic input to motoneurons in individuals with ME/CFS.

1. 1. Institute of Medicine, 2015. doi.org/10.17226/19012
2. 2. Pereira et al. 2021. Front Syst Neurosci 15:698240

id #21795

Do statins enhance cerebral collateral angiogenesis?

Fern Williams[1], Kristy Martin[1], David Clark[1], Merce Fuentes Amell[1], Neil Spratt[3, 2, 1], Daniel Beard[2, 1], Kirsten Coupland[2, 1]

1. School of Biomedical Sciences and Pharmacy, The University of Newcastle, Newcastle, NSW, Australia

2. Hunter Medical Research Institute, Newcastle, NSW, Australia

3. Department of Neurology, John Hunter Hospital, Hunter New England Local Health District, New Lambton Heights, NSW, Australia

Background: Statins are cholesterol lowering drugs that improve stroke outcome in pre-clinical models. Clinical trials of statin use after stroke have not replicated this effect. We hypothesise that statins enhance collateral vessel blood flow due to the growth of new leptomeningeal collateral vessels (LMC) via angiogenesis (neo-collateral formation, NCF). If NCF, which can take weeks to occur, is a key protective mechanism of statins, this would explain why prescribing statins after stroke has failed to improve outcome.

Aim: Determine whether simvastatin treatment reduces collateral flow resistance and if so, is this because of increased LMC number and/or increased LMC diameters.

Methods: Two mouse strains, one LMC-rich (C57BL/6) and one LMC-poor (BALB/c), were given 10 mg/kg of Simvastatin orally daily for four weeks. Animals

were euthanised and a yellow silicone cast (Microfil™) of the cerebral vasculature made. Number, diameter, and length of LMCs were measured and resistance to LMC blood flow was estimated.

Results: Simvastatin treated BALB/c mice had lower estimated resistance to LMC blood flow compared to controls [statin: 0.023±0.017, vehicle: 0.10±0.09; p=0.037] and had higher number of LMC [statin: 4.0±3.16, vehicle: 1.55±1.63; p=0.037]. There was no significant difference in estimated LMC flow resistance between C57BL/6 treatment groups, however there was a region-specific increase in LMC number [statin: 9.45±2.29, vehicle: 7.69±1.84; p=0.0401] and decrease in LMC diameter [statin: 14.0±1.42 µm, vehicle: 16.3±1.75 µm, p= 0.0083].

Conclusion: Simvastatin stimulates NCF in collateral poor and rich mouse strains. In collateral poor mice this results in a decrease in estimated collateral flow resistance.

id #22051

The tryptamine-derivatives, 5-methyltryptamine and 5-methoxytryptamine, increase cell viability in a Parkinsonian cell culture model.

Kayla Carruthers[1], Nicole Jones[1], Mourad Tayebi[2], Sam Merlin[2], Teri Furlong[1]

1. School of Biomedical Sciences, UNSW, NSW

2. School of Science & Health, Western Sydney University, Campbelltown, NSW, Australia

5-methyltryptamine and 5-methoxytryptamine are part of the tryptamine family of chemicals. Like tryptamine itself, they interact with serotonin receptors in the brain, and are likely to influence mood and perception, but are rarely investigated. The current study examined whether these tryptamine derivatives altered cell viability of the brain-derived SH-SY5Y-cell line. This cell line is often used to model Parkinson's disease as these cells share many features with dopamine neurons, such as generating enzymes that produce and breakdown dopamine. In this study, tryptamine, 5-methyltryptamine and 5-methoxytryptamine were each applied to SH-SY5Y cells separately (for 24 hours) at a range of concentrations (10 µM, 30 µM, 100 µM, 300 µM, 1 mM, 3 mM, and 10 mM). Cell variability was measured using the widely used MTT assay, where MTT by-product is proportional to the number of viable, metabolically active cells. Compared to vehicle-control, it was found that tryptamine had no effect on MTT at low concentrations, but MTT was reduced at high concentrations (3mM and 10mM), and thus tryptamine was neurotoxic. In contrast, both 5-methyltryptamine and 5-methoxytryptamine both increased MTT at low concentrations (10 uM and 30 uM) and thus increased cell viability (and were also toxic to cells at high concentrations). Thus, this study demonstrated for the first time that 5- methyltryptamine and 5-methoxytryptamine increase cell viability, and may therefore be neuroprotective of dopamine neurons and possibly serve as a treatment for Parkinson's disease.

id #21797

Characterising maladaptive axonal and intrinsic plasticity in the sub-acute phase post stroke

Emily King[1, 2], Jamie L Beros[1, 2], Hakuei Fujiyama[3, 5, 4], John N.J Reynolds[6], Alex D Tang[1, 2]

1. The university of Western Australia, Crawley, WESTERN AUSTRALIA, Australia

2. Perron Institute for Neurological and Translational Sciences, Nedlands, Western Australia

3. School of Psychology, Murdoch University, Perth, WA, Australia

4. Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, WA

5. Centre for Healthy Ageing, Health Futures Institute, Murdoch University, Perth, WA, Australia

6. Department of Anatomy and the Brain Health Research Centre, The University of Otago, Dunedin, New Zealand

Stroke is a leading cause of disability and stroke-induced changes in cortical excitability are thought to impede functional recovery. Identifying cellular targets that contribute to maladaptive excitability holds great potential for developing new therapeutic interventions to improve stroke outcomes.

One potential target is the axon initial segment (AIS), the site of action potential initiation in neurons. In the acute phase post-stroke, neurons in the peri-infarct zone display abnormal AIS structural properties which may contribute to altered neuronal excitability. However, whether this continues into the sub-acute phase post-stroke, a period with heightened plasticity and when physical rehabilitation typically begins, is unknown.

Here, we induced an ischemic stroke to the motor cortex alongside virus labelling of pyramidal neurons in the peri-infarct zone and contralesional hemisphere in mice. Immunofluorescence and patch clamp electrophysiology measurements were made 28-days later to assess changes in AIS structure and function. Additionally, we investigated hemispheric-, layer-, and sex-dependent differences in AIS and intrinsic membrane properties.

We found AIS structure and function was preserved in the sub-acute phase post-stroke. However, input resistance was reduced across both hemispheres and evoked spike firing frequencies were reduced in the peri-infarct zone in both sexes. In addition, we found stroke reduced evoked firing frequencies in the contralesional hemisphere of male mice only, suggesting sex-dependent differences in neuronal function post-stroke.

These findings highlight intrinsic excitability as a contributor to the dysregulation of cortical excitability observed in the sub-acute phase post-stroke and supports the need to develop sex-specific treatments to improve stroke outcomes.

id #21542

A Novel GFP-Reporter Mouse Model for Functional Analysis of the Epilepsy Gene Pcdh19

Stefka Mincheva-Tasheva[1], Michaela Scherer[1], Louise Robertson[1], Sandra Piltz[1], Julien Bensalem[2], Daniel Pederick[1], Paul Thomas[3, 1]

1. School of Biomedicine and Robinson Research Institute, The University of Adelaide, Adelaide, South Australia, Australia

2. School of Medicine and Robinson Research Institute, University of Adelaide, Adelaide, South Australia, Australia

3. Genome Editing Program, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, South Australia, Australia

Mutations in the X-linked gene *PCDH19* are the cause of PCDH19-clustering epilepsy, an infantile-onset disorder characterized by seizures and intellectual disabilities. Although several intra and extracellular functions of PCDH19 have been identified, the spatiotemporal impact of *Pcdh19* deletion *in vivo* is poorly understood. To investigate the consequences of eliminating *Pcdh19* in specific cell and brain regions, we generated a novel *Pcdh19* floxed mouse with a GFP reporter (*Pcdh19*-cKO-GFP). Using *Pcdh19*-cKO-GFP and *Syn1*-Cre mouse lines we demonstrated that *Pcdh19* elimination in neurons leads to abnormal hippocampal neurogenesis and impaired mouse behaviour. To assess the impact of region-specific elimination of *Pcdh19* on brain physiology we used a *Gfap*-Cre mice line. Specific *Pcdh19* deletion in the hippocampus resulted in increased neurogenesis and decreased memory formation. Finally, we assessed the feasibility of using our conditional mouse model for stage-specific *Pcdh19* elimination during embryogenesis using a Dox-inducible Cre-deletor line. Taken together, these results demonstrate the utility of our unique *Pcdh19*-cKO-GFP mouse model to investigate PCDH19 function in brain physiology and pathology.

id #22055

Uncovering novel interacting partners for the NLR family protein NLRX1 in Parkinson's disease

Aishwarya Mary Johnson[1], Katherine E. Roper[2], John D. O'Sullivan[2], Robert J. Adam[2], Alexander E. Lehn[1], Richard Gordon[1]

1. QUT, BRISBANE, ACT, Australia

2. UQ, Brisbane

Nucleotide-binding oligomerization domain, leucine-rich repeat-containing X1 (NLRX1) is a novel NLR family member and a crucial regulator of inflammation across various diseases. Its expression in the CNS positions NLRX1 as a promising target for making it a potential target for neuroinflammation. Parkinson's disease (PD) is characterized by progressive dopaminergic neuronal loss, mitochondrial dysfunction, and neuroinflammation. NLRX1's ability to mitigate inflammation makes it as a potential target for therapeutic intervention in PD, with NLRX1 agonists, such as NX-13 (Landos Biopharma) being developed for inflammatory diseases such a IBD and MS. NLRX1 has been shown to suppress inflammasome activation in peripheral inflammation and infectious diseases through various mechanisms, such as binding to proteins involved in mitochondrial function and inflammasome oligomerization. The specific binding partners of NLRX1 in neuroinflammation, however, remain to be fully characterized. This study sought to identify proteins that interact with NLRX1 in immune cells and neurons under PD conditions using in vitro models of neuroinflammation and neuronal death. Using THP-1 macrophages, we identified novel NLRX1 interactions with TRIM21, PRDX3, PSMD4, and TOMM70, along with the known interaction of NLRX1-TUFM. Notably, there was a significant enrichment of NLRX1 interaction with PSMD4 and TOMM70 in activated macrophages. PSMD4, a component of the 26S proteasome, suggests a mechanism for the proteasomal degradation of NLRX1, while TOMM70's role in mitochondrial protein import indicates a direct link to mitochondrial function. Our results provide new insights into the mechanisms by which NLRX1 could regulate neuroinflammation in immune cells during PD.

id #22056

Temporal profile of the development of cellular hallmarks of Parkinson's disease following repeated mild traumatic brain injury

Chidozie C Anyaegbu[1, 2, 3], Anastazja M Gorecki[1, 2, 3], Giuseppe Verdile[1], Sarah C Hellewell[1, 2, 3], Brittney R Lins[1, 2, 3], Melinda Fitzgerald[1, 2], Kinta Pinchin-Yamada[1]

1. Curtin Health Innovation Research Institute, Curtin University, East Perth, WESTERN AUSTRALIA, Australia

2. Perron Institute for Neurological and Translational Sciences, Nedlands, WESTERN AUSTRALIA, Australia

3. Centre for Neuromuscular & Neurological Disorders, University of Western Australia, Crawley, WESTERN AUSTRALIA, Australia

Repeated mild traumatic brain injury (rmTBI) is prevalent in contact sports and increases risk of developing neurodegenerative conditions including Alzheimer's and Parkinson's disease (PD). However, definitive links have not been established between primary injury and cellular hallmarks of neurodegenerative disease. While the significance of alpha synuclein (α-syn), tau protein and neuroinflammation in secondary neurodegeneration is widely acknowledged, the onset and progression of these pathologies are unknown. Using a rat model of closed-head rmTBI, where sham (no injury) or two injuries were delivered 24h apart, this project investigated increases in phosphorylated α-syn, tau, activated CD8+ T cells (Granzyme B+) and microglia density (Iba1+) in the brain in a temporal manner, with the goal of identifying the onset of each pathology and their relationships. Whole-brain sections collected at 4 days, 2 weeks, 3 months and 6 months post-injury were assessed by multiplexed fluorescence immunohistochemistry, imaged on a Zeiss AxioScan Z.1 Digital Slide Scanner and analysed using the Zeiss Zen Blue software. (Phosphorylated Tau, Phosphorylated alpha-synuclein, and Tyrosine Hydroxylase), was performed via multiplexed immunohistochemistry and whole slide scans were imaged using the Zeiss AxioScan Z.1 Digital Slide Scanner at CHIRI. Although analysis is ongoing, preliminary analysis indicates increased cytoplasmic accumulation of phosphorylated tau and alpha-synuclein in dopaminergic neurons (Tyrosine Hydroxylase+) in the substantia nigra, a hallmark of PD. Increased numbers of activated CD8+ T cells was also observed. Data from this study will establish, for the first time, a biological link between rmTBI in young adulthood, neuroinflammation and neurodegenration later in life.

id #21801

Transthalamic pathways in decision-making

Mitchell Riley[1], Lindsay Shaw[1], Nicole Martin[1], Christina Mo[1]

1. The Florey Institute, University of Melbourne, Melbourne, VIC, Australia

The brain processes sensory decisions by activating primary sensory cortex followed by higher order cortical areas. Yet data in the last few years has shown that it is not the direct corticocortical projections that are important but rather the cortico-thalamo-cortical pathways, challenging the cortex-centric view of perception. We have found that these *transthalamic* pathways between sensory cortices propagate reward-relevant information for perceptual decisions. However, it is unknown if these pathways also link other brain regions to support decision-making in frontal cortex.

We used rabies trans-synaptic tracing to establish the first evidence of a transthalamic pathway linking secondary sensory cortex to frontal cortex, providing an alternative feedforward route for frontal cortex activation during perception.

To understand the function of transthalamic pathways during decision processing, we trained water-restricted mice on a head-fixed whisker perceptual task and recorded GCaMP6m-expressing neuronal activity using a 'miniscope' attached to a lens in higher order thalamus. Optogenetic tagging of corticothalamic inputs allowed identification of thalamic cells that receive input from the transthalamic pathway.

Importantly, between the sensory stimulation and choice epochs of the task, there was a 'delay' period during which preparatory choice activity is maintained. Surprisingly, on correct trials, 17% of higher order thalamic cells showed persistent activity across the delay period suggesting that higher order processing in frontal cortex could be driven by higher order thalamus.

Transthalamic pathways thus support the maintenance of choice or preparatory information for correct sensory decisions and challenge the textbook 'map' of decision-making, which should include signalling via the thalamus.

id #21802

Can marmosets maintain the balance between response execution and action inhibition?

Ranshi Samandra[1], Marcello G.P Rosa[1], Farshad A Mansouri[1]

1. Department of Physiology , Monash University, Melbourne, Victoria, Australia

To adapt to novel socio-dynamic events, a balance between response execution and action inhibition is imperative. Even though accumulated evidence indicates inhibitory control deficits in neurodevelopmental and substance abuse disorders, the neural underpinnings remain unknown. To address this, comparative studies across primate species are crucial. Although common marmosets (New-world monkeys) are among the most suitable animal models for translational research on neurodevelopmental disorders, their inhibition ability (in the context of a Stop-task paradigm), have not been thoroughly investigated. Therefore, we trained 4 marmosets in a specifically designed marmoset Stop-signal task, in which response time was measured at millisecond resolution.Marmosets performed many repeated Go trials with high accuracy (³70%) and learned to inhibit their response when it was explicitly instructed. Using a performance-dependent tracking procedure, we estimated marmosets' stop signal reaction time (SSRT) at millisecond resolution. The mean SSRT values across sessions ranged between 677 ms to 1464 ms across the 4 marmosets. We also examined the effects of intranasal oxytocin on response execution and inhibition. Our results indicated that oxytocin enhanced both response execution and inhibition, as marmosets exhibited a faster RT in Go trials and a shorter SSRT in oxytocin sessions compared to control (saline) sessions. This marmoset model, for assessing the balance between response execution and inhibition, will further facilitate exploring the neurodevelopmental alterations and the effects of contextual and environmental factors on executive functions.

id #21547

Anti-fibrotic drugs reduce the extent of cochlear implant-induced fibrosis in an *in vivo* guinea pig model of cochlear implantation.

Kady J Braack[1], Tylah Miles[2], Kelly Short[3], Jafri Kuthubutheen[4, 5], Marcus D Atlas[6, 7], Lee Yong Lim[8], Wilhelmina H.A.M Mulders[1], Cecilia M Prêle[2, 3]

1. School of Human Sciences, University of Western Australia, Crawley 6009, WA, Australia

2. Institute for Respiratory Health, University of Western Australia, Crawley 6009, WA, Australia

3. School of Medical, Molecular and Forensic Sciences, Murdoch University, Murdoch 6150, WA, Australia

4. Medical School, University of Western Australia, Crawley 6009, WA, Australia

5. Department of Otolaryngology Head and Neck Surgery, Sir Charles Gairdner Hospital, Nedlands 6009, WA, Australia

6. Ear Science Institute, Subiaco 6008, WA, Australia

7. Centre for Ear Science, Medical School, University of Western Australia, Crawley 6009, WA, Australia

8. School of Allied Health, Pharmacy, University of Western Australia, Crawley 6009, WA, Australia

Cochlear implants allow restoration of sound perception in individuals with severe to profound hearing loss; however, there is still significant variability in patient outcomes. One potential factor that may account for this unexplained variability is the formation of fibrosis within the cochlea after implantation. The current study investigated the effect of a small molecule inhibitor, with known antifibrotic properties, on the extent of fibrosis after cochlear implantation. The drug was delivered in a novel hydrogel formulation to allow for sustained release of the drug into the cochlea. The therapeutic potential of the drug was assessed using a guinea pig model of cochlear implantation, wherein the drug (n=9) or vehicle (n=13) was applied locally to the round window membrane immediately following insertion of a dummy cochlear implant via a cochleostomy. The extent of intracochlear tissue reaction was quantified using micro–CT before and after contrast staining with osmium tetroxide. Compound action potential thresholds of the auditory nerve were used to assess the effect of drug/implant on hearing thresholds. Drug treatment had a statistically significant effect on the sum of total tissue reaction, with drug treated animals showing a 54% (pre-osmium) and 42% (post-osmium) decrease when compared to controls. This effect was localised to areas close to the round window membrane, where the drug was applied. The drug treated group showed no significant changes in hearing thresholds compared to controls. In summary, we have developed an effective and novel anti-fibrotic drug-hydrogel formulation that reduces the extent of the cochlear implant-induced tissue response.

id #21548

Genome-wide CRISPR activation screen reveals human genetic modulators of TDP-43-mediated toxicity and aggregation.

Matisse T Jacobs[1], Rebecca San Gil[1], Adam K Walker[1]

1. Queensland Brain Institute, University of Queensland, Brisbane, Queensland, Australia

Objective:

To identify potential therapeutic strategies for motor neuron disease (MND) by identifying human genes that modulate TAR DNA-binding protein 43 (TDP-43) - mediated aggregation and toxicity.

Methods:

We conducted a pooled human genome-wide CRISPR activation screen in HEK293T cells expressing cytoplasmic-targeted TDP-43 protein mimicking pathology found in disease. This assessed the capability of over 18,000 genes to, when activated, modulate protein aggregation and confer cytoprotection in HEK293T cells with TDP-43 pathology. Top modulators of toxicity and TDP-43 aggregation were selected for investigation. Biochemical, microscopy and flow cytometry techniques were used to investigate the proposed influence top modulators have on TDP-43 pathology *in vitro*.

Results:

Subsets of genes were identified that when activated significantly protected from, or sensitised cells to, toxicity (601 'Protect' genes; 733 'Sensitise' genes. LFC > 1) and inhibited or enhanced TDP-43 aggregation (832 'Inhibit' genes; 37 'Enhance' genes. LFC > 1). This revealed that activation of a NF-KB signalling pathway modulator, which belongs to the leucine-rich repeat containing protein family, sensitised TDP-43 mutant cells to death. Further investigations confirmed that overexpression of this target sensitised cells to toxicity in the context of TDP-43 aggregation. Flow cytometry techniques revealed that target overexpression significantly increased the percentage of cells with insoluble aggregates (P=0.0196). Target knockdown using shRNA inhibited this effect on aggregation.

Conclusions:

This CRISPR activation screen identified hundreds of potential targets that modulate TDP-43 pathology. Manipulation of these target gene pathways may promote clearance or refolding of pathological TDP-43, supporting development of therapies for those living with MND.

id #21804

Novel Small Molecule Strategies for Enhancing Proteostasis in Alzheimer's Disease

Ajish Ariyath[1], Binosha fernando[1], Ralph Martins Martins[1, 2], Prashant Bharadwaj[1]

1. Centre of Excellence for Alzheimer's disease Research and Care, School of Medical and Health Sciences, Sarich and Patricia Neuroscience Research Institute, Edith Cowan University, Joondalup, Western Australia, Australia

2. School of Biomedical Science, Macquarie University, Sydney NSW, Australia , Macquarie University, Sydney, NSW, Australia

Background: Autophagy is over activated in Alzheimer's disease (AD) brain, but still activating autophagy has received most attention, however recent evidence suggests that ubiquitin-proteasome system can clear aggregate proteins and a potential therapeutic target for AD and protein misfolding diseases.

Methods: We developed an assay using the MC65 AD cell model to screen library of small molecule proteostasis modulators and identified IU1, a USP14 inhibitor, that improved cell survival and promoted Aβ clearance. Screening 71 novel small molecule proteostasis inhibitors revealed better neuroprotective analogues of IU1, AA10 and AA51. The effects of these compounds on autophagy, proteasome activity, and APP-C99/Aβ clearance, neurodegeneration and behavior were analyzed using fluorescence microscopy, western blotting, proteasome studies, neurodegeneration, toxicity and lifespan assay by AD cell and worm models.

Results: Our data on MC65 and *C. elegans* AD models have shown promising results with compounds AA10 and AA51 from the screened library of proteostasis modulators. In cell studies, these compounds alleviated AD proteostasis dysfunction, reduced protein aggregation, and regulated autophagy clearance. Cell survival improved from 40% with IU1 to 55% with AA10 and AA51. Initial studies on the worm model support these findings, showing a 55% reduction in neurodegeneration with AA10 and 53% reduction with AA51.

Conclusions: Ours, is the first report of using IU1 in AD models as USP14 inhibitor. The novel IU1 analogues AA10 and AA51 looks more promising and hold potential as candidates for pre-clinical validation in AD. The next steps involve testing therapeutic efficacy and brain bioavailability in AD mice model.

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id #22060

Exploring the Alteration of Firmicutes and Bacteroides in Preclinical Alzheimer's Disease

Sithara Dissanayaka[1, 2], Thilini Jayasinghe[3], Hamid Sohrabi[4, 5, 2], Stephanie Rainey-Smith[5, 2], Karen Scott[6], Ralph Martins[1, 4, 2], Binosha Fernando[1, 2]

1. Centre of Excellence for Alzheimer's Disease Research and Care , School of Medical and Health Sciences , Edith Cowan University, Nedlands, WA, Australia

2. Alzheimer's Research Australia, Nedlands, WA, Australia

3. The University of Sydney, Camperdown, NSW, Australia

4. Department of Biomedical Sciences, Macquarie University , Sydney, NSW, Australia

5. Murdoch University, Murdoch, WA, Australia

6. University of Aberdeen, Aberdeen, UK

Alzheimer's disease is a progressive neurodegenerative disorder characterized by memory loss and significant behavioral changes. Recent studies emphasize the pivotal role of gut dysbiosis in AD pathogenesis, with notable alterations in the gut microbiome observed in individuals with AD or mild cognitive impairment. However, the specific changes in microbiota during the preclinical stages of AD, defined by cerebral amyloidosis without cognitive impairment, are not well understood. This study seeks to address this critical gap in knowledge.

All study participants (17 CU Aβ+ and 54 CU Aβ-) were selected from highly characterised cohorts and underwent Pittsburg compound B-positron emission tomography. The taxonomic composition of faecal samples was examined through metagenomic analysis.

The analysis revealed differences in microbial taxa abundances between the CU Aβ+ and CU Aβ- groups. At the phylum level, Bacteroidetes were more abundant in the CU Aβ+ group, while Firmicutes predominated in the CU Aβ- group. At the genus level, Bacteroides (phylum Bacteroidetes) showed high abundance in both groups. At the species level, Faecalibacterium prausnitzii and Bacteroides vulgatus had higher relative abundances in the CU Aβ- group, whereas Bacteroides copri (phylum Bacteroidetes) was more prevalent in the CU Aβ+ group.

These findings suggest that dysbiosis in Firmicutes and Bacteroides phyla may indicate an imbalance of beneficial and harmful gut bacteria in preclinical AD. Identifying these microbiota shifts may provide insights into early therapeutic targets for managing AD progression and may assist in preclinical AD diagnosis. A deeper understanding of the relationship between gut microbiota and AD is needed.

id #22061

Affinity purification mass-spectrometry of tau pT217 implicates the CTLH E3 ubiquitin ligase as an early interactor

Tomas Kavanagh[1], Manon Thierry[2, 3], Kaleah Balcomb[1], Jackeline Ponce[4], Glenda Halliday[1], Beatrix Ueberheide[4], Thomas Wisniewski[2, 3, 5, 6], Eleanor Drummond[1]

1. Brain and Mind Centre, School of Medical Science, The University of Sydney, Sydney, NSW, Australia

2. Center for Cognitive Neurology, Grossman School of Medicine, New York University, New York, NY, USA

3. Department of Neurology, Grossman School of Medicine, New York University, New York, NY, USA

4. Proteomics Laboratory, Division of Advanced Research Technologies and Department of Biochemistry and Molecular Pharmacology, Grossman School of Medicine, New York University, New York, NY, USA

5. Department of Pathology, Grossman School of Medicine, New York University, New York, NY, USA

6. Department of Psychiatry, Grossman School of Medicine, New York University, New York, NY, USA

Hyperphosphorylation of tau at threonine 217 (pT217) and threonine 231 (pT231) are proving to be excellent early biomarkers of Alzheimer's disease. However, it is not known how dysregulation of phosphorylation at these residues alters the role of tau in disease. Here we performed affinity purification mass spectrometry of two tau phosphorylated residues (pT217 and pT231) to determine how specific phosphorylation of tau alters its interactome in Alzheimer's disease post-mortem brain tissue (n=10). pT217 affinity purification mass spectrometry identified 23 interacting proteins (SAINT score > 0.65). pT217 enriched tau had a unique phosphorylation signature compared to PHF1 but shared significant overlap (Thierry et al., 2024, 15 interactors shared, Fisher's exact $p=2.3x10^{-14}$), suggesting pT217 tau was an earlier state of dysregulation but had similar interactomes. pT217 interacting proteins enriched for biological processes involved in protein catabolic process, most notably with the CTLH E3 ubiquitin ligase complex (5 interacting subunits: WDR26, ARMC8, GID8, RANBP9, MAEA). We validated the interaction between tau, p62 and the CTLH component WDR26 with co-immunoprecipitation (n=3 AD, n=3 CTRL, n=1 CBD, PiD and PSP) and immunofluorescent microscopy of fixed post-mortem brain tissue (n=3 AD, n=3 Control) in independent cohorts. These interactions were confirmed in both experiments and showed predominant interaction between tau and p62 and WDR26 in select types of tau aggregates. In conclusion, our results highlight the CTLH complex as an early interactor of tau in Alzheimer's disease that future studies can explore and potentially exploit to create early interventions for disease processes.

id #21806

Whole genome pleiotropy between Alzheimer's Disease and Autoimmune Disorders

Sarah K Davies[2, 1], Nesli Avgan[2, 1], Cue H Lee[3, 4], Bryce Vissel[2, 1]

1. St Vincents Centre for Applied Medical Research, Sydney, NSW, Australia

2. Centre for Neuroscience and Regenerative Medicine, Revesby, NSW, Australia

3. Department for Biomedical Sciences, BK21 Plus Biomedical Science Project, Seoul National University College of Medicine, Seoul, Jongno District, South Korea

4. Department of convergence Medicine, University of Ulsan College of Medicine, Asan Medical Centre, Seoul, South Korea

We aimed to determine pleiotropic risk genes mediating Alzheimer's (AD) and 7 autoimmune diseases using next generation multi-trait analysis tool, PLEIO. We analysed the largest and most recent GWAS from AD in combination with 7 autoimmune diseases including Inflammatory Bowel Disease (IBD), Crohn's Disease (CD), Ulcerative Colitis (UC), Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Type 1 Diabetes (T1D) and Vitiligo (Vi). We used the novel tool PLEIO to evaluate the influence of AD risk mutations in a multi-trait analysis of well-established autoimmune diseases. Unlike traditional meta analysis, PLEIO factors in the complex genetic architecture of traits such as correlations and heritabilities, and utilises a joint likelihood framework to increase power to detect pleiotropic single nucleotide polymorphisms (SNPs).

Autoimmune disease showed a higher percentage SNP heritability (H2 = 0.14 - 0.4) compared to AD (H2 = 0.07). Genetically, AD was positively correlated with CD, IBD, SLE, UC and Vi (rg = 0.002 - 0.01) and negatively correlated with RA and T1D (rg =-0.09 - -0.1). Pleiotropic analysis of disease associated loci for AD and 7 autoimmune diseases uncovered 40 novel SNPs, 29 of which are located on protein coding regions. Functional annotation identified 7 SNPs as expression quantitative trait loci (eQTLs) significantly associated with changes of expression for 10 genes. Gene prioritisation analysis identified 9 of the 10 genes as likely causal for disease outcomes.

Our study presents the largest pleiotropy study investigating the shared genetic etiology of AD and autoimmune disease with a newly developed analysis method.

id #22062

Lesions of the lateral hypothalamus reduce contextual fear renewal in rats

Sam Merlin[1], Asena Bingul[2], Simon Killcross[3], Teri Furlong[2]

1. Medical Science, Western Sydney University, Campbelltown

2. School of Biomedical Science, UNSW, Kensington

3. School of Psychology, UNSW, Kensington

The circuitry recognised to regulate conditioned fear is well-established. Central to this circuitry is the prefrontal cortex and amygdala which regulate both learning and expression of fear. In the current study, the lateral hypothalamus of rats was targeted with an AAV viral vector (Lesioned group) or sham lesioned with vehicle (Control group). Contextual control of fear learning was then examined using an ABA renewal design. Following tone-footshock pairings in a distinct context (context A), both groups of rats increased freezing behaviour across conditioning ($p<0.001$) with no difference between groups ($p>0.48$). Extinction then occurred in a separate distinct context (context B), where both groups of rats decreased freezing behaviour across tone-alone presentations ($p<0.001$) with no difference between groups ($p>0.48$). Rats were then tested in both the fear and extinction contexts (A and B contexts). Overall, there was no effect of context ($p>0.14$) and no difference between groups in total freezing ($p>0.7$). However, importantly, there was a significant interaction between context and group ($p<0.05$), with simple effects analysis revealing that freezing was higher in context A versus context B for the Control group ($p<0.02$), but not for the Lesioned group ($p>0.40$). This study shows that the lateral hypothalamus did not impact the acquisition, extinction, or expression of conditioned fear, but was important for distinguishing between contexts, and hence where fear was expressed. The lateral hypothalamus may therefore be important for distinguishing between safe and dangerous contexts, and thus regulate appropriate fear expression, which is known to be important for anxiety disorders.

id #21551

Exploring Kir4.2 as a Novel Therapeutic Target in Parkinson's Disease

Xiaoyi Chen[1, 2], Benjamin Garland[1, 2], zhiqiang shen[1, 2], Rocio Finol-Urdaneta[3], Mo Chen[2, 4], Alex Sykes[2], Bingmiao Gao [5], Jamila Iqbal[2], Des Richardson[1, 2], David J Adams[3], George Mellick[1, 2], Linlin Ma[1, 2]

1. School of Environment and Science, Griffith University, Nathan, QLD, Australia

2. Institute for Biomedicine and Glycomics, Griffith University, Nathan, QLD, Australia

3. School of Medical, Indigenous and Health Sciences, Molecular Horizons Research Institute University of Wollongong, Wollongong, NSW 2522, Australia

4. Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University,, Nathan, 4111, QLD, Australia

5. Engineering Research Center of Tropical Medicine Innovation and Transformation, Ministry of Education, Hainan Key Laboratory for Research and Development of Tropical Herbs, School of Pharmacy, Hainan Medical University , Haikou 571199, China

Parkinson's Disease (PD) is a notoriously difficult medical challenge due to its highly complex nature, involving multiple and incompletely characterized cellular and molecular mechanisms. Many critical breakthroughs toward understanding PD's etiology have emerged from studying rare families with inherited forms of the disease. These studies identified key proteins crucial in the disease process and have spurred the development of new drugs.

Through a genetic linkage study, we identified a genetic variant, *KCNJ15*$^{p.R28C}$, which strongly segregates with PD in a large family with familial PD. This variant was also found in other sporadic PD patients, underscoring the functional role of *KCNJ15*-encoded protein, the inwardly rectifying K^+ channel Kir4.2, in PD pathogenesis.

Our *in-silico* analyses employing multiple bioinformatics tools indicated that *KCNJ15*$^{p.R28C}$ (Kir4.2^{R28C}) is a deleterious variant. Indeed, patch clamp studies revealed that the mutation results in a complete loss of channel function with a strong dominant-negative effect. The loss of function in Kir4.2^{R28C} is partially due to reduced expression of the mutant protein. Further investigation into the underlying mechanisms showed that the Kir4.2^{R28C} mutant is unstable and more prone to lysosomal-mediated degradation with sacrificed plasma membrane trafficking capacity.

These findings are corroborated by our *in vivo* studies employing a newly developed *KCNJ15*$^{-/-}$mouse model, which exhibits Parkinson's traits, such as abnormal motor coordination, balance issues, and impaired special memory and learning capacity.

This study represents the first effort to explore the molecular mechanisms linking Kir4.2 and PD pathogenesis, highlighting Kir4.2 as a novel biological target for this devastating disease.

id #22063

Neurofeedback Intervention for Persistent Post-Concussion Symptoms: A Case Series on Functional Connectivity in the Salience Network

André Avila[1, 2], Caerwen S Beaton[1, 2], Melissa Papini[1, 2], Francesca Buhagiar[3], Melinda Fitzgerald[1, 2], Sarah C Hellewell[1, 2]

1. Curtin Health Innovation Research Institute, Curtin University, Perth

2. Perron Institute for Neurological and Translational Science , Perth

3. School of Pyschological Science, The University of Western Australia, Crawley

Objectives: Persistent post-concussion symptoms (PPCS) may occur in up to 50% of concussion cases. There is no effective treatment for PPCS. The salience network (SN), involved in shifting between internally and externally directed cognitive states, can be altered in PPCS. This pilot study explores the utility of Z-score neurofeedback to attenuate PPCS and restore changes in functional connectivity within the SN.

Methods: Two participants with PPCS aged 36 (16m post-injury) and 26 (13m post-injury), were randomised to two intervention conditions: (1) 18 sessions of personalised qEEG-informed neurofeedback targeting frontoparietal hyperactivity over 6-weeks, (2) placebo-neurofeedback of the same quantity and duration to delineate effects of environment. The post-concussive symptom scale (PCSS) was used to assess pre- and post-intervention symptom severity. NeuroNavigator was used to measure SN functional connectivity, expressed as Z-score coherence and averaged to examine percentage change pre- and post-treatment.

Findings: Neurofeedback was associated with improvements in the SN across all frequency bands: delta (49.99%), theta (69.71%), alpha (62.57%), beta (63.35%), and high-beta (28.16%). In contrast, the placebo participant had minimal changes, with delta worsening by 1.29%, and modest improvements in theta (9.40%), alpha (18.43%), beta (9.68%), and high-beta (9.74%). The neurofeedback participant demonstrated a full (100%) resolution of PCSS severity, while the placebo participant endorsed only a 22% reduction in symptom severity.

Conclusions: These pilot findings suggest that, compared to placebo, neurofeedback may improve SN functional connectivity and abrogate PPCS. This data supports a larger ongoing study to validate these findings and further investigate the mechanisms underlying neurofeedback.

id #22065

Extensive Splicing Dysregulation in Parkinson's Disease

Tomas Kavanagh[1, 2], Louise Cottle[2, 3], Lee Marshall[2], Jade Zhang[2], Fiona Weiss[1, 2], Kathryn Hill[4], Boris Guennewig[1, 2], Antony Cooper[4, 5]

1. Brain and Mind Centre, School of Medical Science, The University of Sydney, Sydney, NSW, Australia

2. Neuroscience Division, Garvan Institute of Medical Research, Sydney, NSW, Australia

3. Charles Perkins Centre, School of Medical Sciences, University of Sydney, Sydney, NSW, Australia

4. Australian Parkinson's Mission, Garvan Institute of Medical Research, Sydney, NSW, Australia

5. School of Clinical Medicine, Faculty of Medicine and Health, UNSW Sydney, Sydney, NSW, Australia

Parkinson's disease (PD) is the most common progressive neurodegenerative movement disorder. PD has exceedingly complex aetiology with ~85-95% of cases being idiopathic. To determine what molecular mechanisms might underpin regional vulnerability to PD, we employed total and targeted (capturing 45 Mbp of the PD-focused transcriptome) RNA sequencing. We sequenced post-mortem brain tissue across 3 regions with moderate (basal forebrain), minimal (superior frontal cortex) and no Lewy body pathology (superior occipital cortex) from individuals without PD (n=10) and PD patients (n=10) with both technologies. We assessed differential expression and alternative splicing (AS) of genes as well as gene co-expression networks. We found compensatory mechanisms in the pathologically unaffected regions, including upregulation of genes HSPA6 (log_2FC=4.5 SOC, log_2FC=4.1 SFC, molecular chaperone) and VAMP2 (log_2FC=7.63 SOC:BF). Splicing events were dramatically dysregulated, including mis-splicing of many PD familial and GWAS associated genes. Importantly these genes were not altered by differential expression at the gene level, highlighting complex dysregulation of the PD transcriptome. We used a combination of publicly available data to predict which regulatory splicing factors are driving the splicing dysregulation in PD. We show the addition of α-synuclein pre-formed fibrils to mouse primary hippocampal neurons is sufficient to cause mis-localisation of the predicted RNA binding proteins and that splicing factors are dysregulated in PD patient brains prior to pathology. Alternative splicing dysregulation in PD is substantially underappreciated and the identification of a program of regulatory splicing factors affected by α-synuclein could offer novel insights and treatment avenues for PD.

id #22066

The role of immediate early genes and complement system in synergistic pathology of epilepsy and Alzheimer's Disease

Anna Harutyunyan[1, 2, 3], Samantha LW Warren[4], Alison M Anderson[2, 3, 5], Patrick Kwan[2, 3, 5], Nigel C Jones[2, 3, 5]

1. Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia

2. Neuroscience, Monash University, Melbourne, Victoria, Australia

3. Medicine, Royal Melbourne Hospital, The University of Melbourne, Parkville, Victoria, Australia

4. Monash Micro Imaging, Central Clinical School, Monash University, Melbourne, Victoria, Australia

5. Neurology, Alfred Hospital, Melbourne, Victoria, Australia

Alzheimer's disease (AD) is a neurodegenerative disease affecting 50 million people worldwide. There is increased prevalence of epilepsy in patients with AD, and the two diseases are thought to have a bi-directional association, however, the mechanism underlying this association remains unknown. This study aimed to investigate potential mechanisms of synergy between recurrent seizures and already-present amyloid pathology in a well-established model of AD.

Transgenic 5xFAD mice (N=20) and WT littermates (N=22) underwent electrical amygdala kindling to induce epilepsy phenotype or were treated as sham (no epilepsy). Kindling rate, seizure severity and cognitive behavioural performance were compared across the kindled and sham 5xFAD and WT mice. Seven days after the last induced seizure, the hippocampal tissue was harvested for RNA sequencing (ipsilateral) and immunohistochemical (contralateral) analyses. The 5xFAD mice showed significantly impaired spatial memory ($p<0.05$) and increased susceptibility to kindling-induced seizures ($p<0.001$) compared to WT littermates. Transcriptomic profiling and differential expression analysis revealed profound overexpression of genes involved in reactive gliosis and neuroinflammatory pathways in the kindled-5xFAD group compared to sham-5xFAD and WT groups. Gene coexpression network analysis identified modules of immediate early genes (IEG) showing significant ($p<0.00001$) correlation with kindled-5xFAD group, but not shams. The regulatory hub genes of this module (Pcdh8, Nptx2) are activity-dependent synaptic plasticity-related genes, which are involved in synapse formation and maintenance in homeostatic conditions and lead to loss of dendritic spine density if deregulated.

Our results demonstrate a significant synergistic interaction between AD pathology and seizures, which may be mediated by activity-induced IEGs and reactive gliosis.

id #21555

A proteomics study of the prefrontal cortex following life-long exposure to environmental enrichment

Stephanie Huang[1, 2], Joyce Colussi-Mas[1, 2], Bart Ellenbroek[2], Lifeng Peng[1]

1. School of Biological Sciences and Centre for Biodiscovery, Victoria University of Wellington, Wellington, New Zealand

2. School of Psychology, Victoria University of Wellington, Wellington, New Zealand

Early-life environments play a critical role in shaping the trajectory of neurodevelopment and, therefore, affect life-long neurological outcomes. There is evidence that growing up in an impoverished environment is a risk factor for developing neuropsychiatric illnesses later in life, whereas growing up in a nurturing environment is a protective factor. In rodent studies, standard housing (SH) and environmental enrichment (EE) are analogous to impoverished and nurturing environments, respectively. EE is a housing condition with increased novelty and complexity where rodents experience richer cognitive, motor, and social stimulation. Many positive effects have been observed in both brain and behaviour following EE, yet the underlying molecular mechanisms remain unclear. Therefore, to holistically assess the impact of life-long EE and SH, we conducted a proteomics study on the medial prefrontal cortex of rodents raised in either EE (N = 30; 15 males & 15 females) or SH (N = 26; 13 males & 13 females) ranging from 84 to 124 days of age. We used liquid chromatography-tandem mass spectrometry (LC-MS^3) with tandem mass tagging (TMT) to identify the proteins and quantify their relative abundances to determine those differentially expressed between conditions. We identified a range of proteins of interest that are differentially impacted by EE and SH, mediated by sex and age. Our findings provide insights into the potential biological processes and molecular mechanisms that may explain how our surrounding environment shapes our brains.

id #21812

Studying approach-avoidance conflict in mice using the platform avoidance task

Chelsea Brown[1], Erin Campbell[1], Isabel Chew[1], Fay Waldrip[1], Maria Kutnetsova[1], Jacqueline Iredale[1], Lizzie Manning[1]

1. University of Newcastle, Callaghan, NSW, Australia

Shifts in decision making under approach-avoidance conflict play a central role in neuropsychiatric disorders, where biases towards avoidance [(e.g. anxiety, obsessive compulsive disorder (OCD)] or approach (substance use disorder) can manifest. In OCD, compulsive behaviours can be conceptualized as avoidance responses aimed to avoid the negative outcomes associated with the patient's obsessions, and therefore understanding how excessive avoidance can manifest and be extinguished may be helpful for understanding OCD and developing new treatments.

The platform avoidance task was developed in rats to study avoidance learning, approach avoidance conflict, and avoidance extinction, including extinction with response prevention relevant to the leading cognitive behavioural therapy for OCD. We are using this task to understand behavioural changes in an OCD mouse model and neurobiological mechanisms.

First, study 1 examined sex differences in approach avoidance conflict, given the extensive literature on sex differences in defensive behaviour across a variety of tasks. Study 2 describes changes in approach-avoidance conflict in a preclinical mouse model of OCD, Sapap3 knockout mice. Finally, study 3 will share preliminary data on effects of drugs targeting serotonin 5HT2C receptors, as 5HT2C receptors mediate the effects of first-line OCD antidepressant treatments on striatal dopamine, which is critical for expression of avoidance behaviour.

Together these studies highlight how the platform avoidance task can be used to study basic and maladaptive mechanisms of approach-avoidance conflict, and uncover mechanisms relevant to disrupted avoidance behaviour in OCD.

id #22068

The role of axon initial segment plasticity in sensorineural hearing loss.

Krisitn Barry [1], Helmy Mulders [1], Alex Tang [2], Jazmin Chalklen[3]

1. UWA , Auditory lab , Perth , WA, Australia

2. Plasticity lab, UWA, Perth , WA, Australia

3. UWA, South Perth, WA, Australia

Sensorineural hearing-loss (SNHL) is known to increase neuronal spontaneous firing rates (hyperactivity) in the ascending auditory system. Hyperactivity is proposed to be involved in the generation of tinnitus, a phantom auditory perception. However, the mechanisms underlying central hyperactivity remain unknown. A candidate mechanism is abnormal axon initial segment (AIS) structure and function. Action potentials are generated at the AIS which undergoes plasticity to maintain neuronal firing within physiological parameters. In the developing avian auditory system, SNHL results in AIS elongation and subsequent increase in neuronal excitability. Therefore, AIS elongation may be involved in the hyperactivity seen in the adult auditory system following SNHL. The present study investigated the effects of SNHL on AIS length in the auditory system of adult Sprague Dawley rats. Rats (n=13) were exposed to an acoustic trauma (AT) (n = 7) or sham procedure (n = 6). AIS length was quantified in several auditory nuclei using immunostaining for the AIS scaffolding protein Ankyrin-G. Preliminary analysis of 9 of 13 animals (5 = AT, 4 = sham) suggests AIS elongation in ipsilateral and contralateral dorsal (DCN) and ventral cochlear nucleus (VCN) 2-weeks after AT (Ipsilateral-DCN: AT = 12.0 ± 2.7 µm > Sham = 9.8 ± 3.4 µm; Contralateral-DCN: AT= 11.5 ± 3.0µm > Sham = 9.8 ± 3.7 µm; Ipsilateral-VCN: AT = 12.4 ± 3.3µm > Sham = 9.7 ± 2.5µm; Contralateral-VCN: AT= 11.2 ± 3.1µm > Sham = 9.8 ± 2.6µm). This suggests SNHL induces maladaptive AIS plasticity which could underlie neuronal hyperactivity.

id #21558

Lewy pathologies in sporadic versus *SNCA* mutation Parkinson's disease

Giselle Sagredo[1, 2], Hongyun Li[1, 2], Ping Wu[1, 2], YuHong Fu[1, 2], Glenda Halliday[1, 2]

1. Brain and Mind Centre and Faculty of Medicine and Health School of Medical Sciences, The University of Sydney, Sydney, NSW, Australia

2. Aligning Science Across Parkinson's (ASAP) Collaborative Research Network , Chevy Chase, MD, 20815

Aim: To compare the types of Lewy pathology (LP) in post-mortem cortical tissue between *SNCA* mutation and sporadic Parkinson's disease (PD).

Methods: Formalin-fixed paraffin-embedded post-mortem cingulate cortex from fifteen sporadic, two A53T, one G51D, and two E46K *SNCA* mutation cases were assessed (approved 2020/707) with multiplexed immunofluorescence labelling using scanner and confocal imaging. The colocalisation of proteins was determined and the proportion of the neurons with LP identified. Control cases had no discernable cortical LPs.

Results: LP were identified in neurons as puncta, or solid or mixed Lewy body (LB) deposits with all LBs colocalising p62. In all sporadic and *SNCA*-related cases, LBs were found in ~10% of neurons which concentrated in lower cortical layers. An additional 10% of cortical neurons in *SNCA*-related but not sporadic PD cases had a-syn puncta (50% colocalized p62 and a high proportion colocalised with RhoA, a small GTPase). Sporadic PD had mainly solid round LBs whereas nearly 50% of LBs in *SNCA*-related PD had mixed morphology. All LBs colocalised phosphorylated tau protein and over 50% of LBs in sporadic and *E46K*-mutant PD colocalised RhoA.

Conclusion: There are significant differences in the types of cortical LP in sporadic versus *SNCA*-related PD, with sporadic cases having a largely uniform population of matured solid LBs while *SNCA* mutation cases had a substantial amount of progressing LB types. Cortical LBs in A53T and G51D cases had limited RhoA colocalisation. These data suggest that the type and progression of cortical LB formation may differ between sporadic and *SNCA* cases.

id #22071

S-allyl Cysteine and Cannabidiol are Equally Effective as Metformin in Preserving Neurovascular Integrity, Retinal Structure, and Cognitive Function in db/db Type 2 Diabetic Mice

May MM Majimbi[1], John JM Mamo[1, 2], Virginie VL Lam[1, 2], Samuel SM McLenachan[3], Michael MN Nesbit[1], Emily EB Brook[1], Arazu AS Sharif[1], Fred FC Chen[3], Armin AM Mooranian[1], Hani HA Al-Salam[1], Ryu RT Takechi[1, 2]

1. Curtin Health Innovation Research Institute, Faculty of Health Science, Curtin University, Bentley, WA, Australia

2. Neurovascular and Metabolic Diseases, Perron Institute, Nedlands, WA, Australia

3. Lions Eye Institute Australia, Harry Perkins, Nedlands, WA, Australia

Type 2 diabetes mellitus (T2D) is associated with central nervous system (CNS) alterations marked by neurovascular, inflammatory, and oxidative damage, resulting in cognitive dysfunction and retinal neurodegeneration. This study explored the therapeutic potential of naturally derived S-allyl-cysteine (SAC) and cannabidiol (CBD) in LepR db/db mice, targeting blood-brain/retinal barrier (BBB/BRB) leakage, glial activation, and DNA oxidative damage to alleviate memory deficits and retinal neurodegeneration.

Diabetic mice underwent 23 weeks of dietary treatments: diabetic db/db controls, SAC, CBD, SAC+CBD, and metformin, with nondiabetic db/+ mice as negative controls. Memory function was assessed using novel object recognition and passive avoidance tests, while retinal thickness was measured via *in vivo* OCT imaging. Immunofluorescence imaging quantified neurovascular leakage (IgG extravasation), glial activation (GFAP and Iba1) and DNA oxidation (8OHdG) in brain sections and retinal whole-mounts.

In diabetic db/db mice with hallmark metabolic dysregulation, CNS alterations included BBB/BRB leakage, glial activation, and retinal neurodegeneration of the ganglion cell complex (GCC), correlating with memory impairments.

SAC and CBD exhibited significant therapeutic effects against CNS pathophysiologies, attenuating glial activation and synergistically preventing BBB leakage. In the retina, these compounds attenuated BRB leakage risk and reduced glial-mediated neuroinflammation. SAC and CBD preserved GCC thickness and

improved memory functions, proving to be as effective as or superior to metformin against diabetic-associated CNS pathophysiology.

id #21821

Impact of Neuropathic Pain on Fear Learning Systems Within the Brain

Kristen E Anderson[1], Chris W Vaughan[1], Bryony L Winters[1]

1. PMRI, Kolling Institute, Sydney, NSW, Australia

Background & Objectives:

Chronic neuropathic pain is a highly prevalent, disabling, and costly pain syndrome which is associated with debilitating psychological co-morbidities related to abnormal fear learning. The lateral amygdala has been shown to play a key role in integrating the sensory components of fear memory and both the acquisition and expression of conditioned fear. However, little is known about how fear learning and its associated brain regions are affected by animal neuropathic pain models.

Methods:

Adult male C57BL/6 mice underwent either chronic constriction injury (CCI) or control sham surgery. Mice were then subjected to a Pavlovian fear conditioning protocol, and the extent of fear-induced freezing behaviour was quantified to determine changes in fear learning and extinction. A separate cohort of CCI and sham mice was used in brain slice electrophysiology experiments to examine synaptic transmission onto lateral amygdala neurons.

Findings:

Following fear conditioning, CCI but not control mice displayed disrupted expression of fear learning and extinction. Unlike sham mice, evoked AMPAR-mediated excitatory postsynaptic currents (EPSCs) in CCI mice displayed inward rectification, indicating the expression of calcium-permeable, GluR2-lacking receptors. In addition, evoked EPSCs in CCI mice displayed a decrease in the AMPA paired-pulse ratio, indicating altered presynaptic excitability.

Conclusion:

These findings suggest that chronic neuropathic pain disrupts fear memory and is associated with pre- and postsynaptic modifications in the lateral amygdala, a crucial substrate for this learning process.

id #21836

Astrocyte-mediated Transregional Metaplasticity in the Hippocampus

Owen D Jones[1], Shruthi Sateesh[1], Anurag Singh[2], David Stellwagen[3], Wickliffe C Abraham[1]

1. Department of Psychology, University of Otago, Dunedin, New Zealand

2. Department of Anatomy, University of Otago, Dunedin, New Zealand

3. Department of Neurology and Neurosurgery, McGill University, Montreal, Canada

Long-term potentiation (LTP) is a memory mechanism that is a highly regulated process, including by mechanisms of metaplasticity. Recently, we discovered a unique transregional metaplasticity effect in the hippocampus whereby “priming” activity in stratum oriens of area CA1 selectively inhibits later LTP at synapses in stratum radiatum (SR) of CA1 but not in other CA1 lamina. Surprisingly, LTP at synapses in the middle molecular layer (MML) of the dentate gyrus is also inhibited. We hypothesized that this unusual transregional communication is accomplished by activation of the astrocyte network. We undertook intracellular astrocyte patch-clamping and extracellular field potential recordings in hippocampal slices taken from young adult rats and mice. In rat slices, Ca^{2+} was buffered in individually patched astrocytes by dialyzing EGTA while recording local synaptic potentials in SR (in the presence of D-serine) and MML (in the presence of glycine). Priming in control conditions inhibited LTP in both SR and MML while buffering astrocytic Ca^{2+} prevented the inhibition of LTP. Moreover, the priming effect was absent in IP3R2 knock-out mice. Finally, we found that the cytokine tumor necrosis factor-alpha (TNF), released by astrocytes and acting on TNF receptor-1, plus glutamate acting on GluN2BRs, are critical signaling molecules. Together, these data demonstrate a novel hippocampus-wide but synapse-selective regulation of LTP mediated by bidirectional astrocyte-neuron communication. We propose that such metaplasticity may play an important role in hippocampal information processing while also contributing to plasticity and memory impairments when aberrantly engaged under conditions of neuroinflammation.

Jones and Sateesh, Joint first authors

id #21839

Alterations in lipid droplet biology in Motor Neurone Disease

Yi Ling Clare Low[2, 1], Pawat Laohamonthonkul[2, 1], Emily Hart[2, 1], Samaneh Mirzaei[2, 1], Chien-Hsiung Yu[2, 1]

1. Florey Department of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia

2. The Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, Australia

Background

Lipid droplets (LDs), once seen solely as lipid storage organelles, are now recognised for their multifunctional roles in regulating cell physiology. They have also been increasingly implicated in neurodegenerative diseases. Aberrant accumulation of TAR DNA-binding protein 43 (TDP-43), a prominent feature of Motor Neurone Disease (MND), is associated with inflammation and lipid dyshomeostasis. This study investigates the emerging, yet unexplored, connection between LD biology and TDP-43-mediated neurodegeneration.

Methods

Neuroblastoma SH-SY5Y cells with doxycycline-inducible overexpression of TDP-43 wildtype, the disease-associated Q331K mutation, or empty vector control were treated with or without 100nM of JAK1 inhibitor Baricitinib. Following a 48-hour post-induction period, cells were analysed using immunofluorescence to assess lipid droplets.

Results

We observed a significant decrease in the total area of LD expression ($p<0.0001$; Mann-Whitney Test) and the average size of LDs in both our neuronal models of TDP-43 proteinopathy (i.e., TDP-43 WT ($p=0.0004$) and TDP-43 Q331K ($p<0.0001$)) compared to control cells. This reduction in LDs correlated with increased cytotoxicity as quantified by *lactate dehydrogenase* (LDH) assay. Notably, Baricitinib treatment significantly reverted the LD phenotype and mitigated neurotoxicity.

Conclusion

Our findings demonstrate phenotypic alterations in LDs in association with TDP-43 proteinopathy. The pronounced rescue of the LD phenotype to control levels with Baricitinib suggests that activation of the JAK/STAT signalling pathway driven by aberrant TDP-43 functions in neurons, may disrupt lipid homeostasis. This study lays the groundwork for new insights into the roles of LDs in neurodegenerative diseases.

id #21585

Increasing mTORC2 activity attenuates photoreceptor death in inherited retinal diseases

Alicia A Brunet[1, 2], Paula I Fuller-Carter[2], David M Hunt[1, 2], Alan R Harvey[1, 2, 3], Livia S Carvalho[1, 2, 4]

1. The University of Western Australia, Perth, WA, Australia

2. Lions Eye Institute, Perth, WA, Australia

3. Perron Institute for Neurological and Translational Science, Perth, WA, Australia

4. Department of Optometry and Vision Sciences, The University of Melbourne, Melbourne, VIC, Australia

Inherited retinal diseases (IRDs) are a group of conditions marked by progressive vision loss caused by rod and cone photoreceptor degeneration. Loss of cone photoreceptor function has the largest impact on the quality of life for people living with IRDs as they mediate daylight vision. However, the mechanisms of cone cell death in IRDs are yet to be fully elucidated, limiting the development of treatment approaches. Using the *Pde6c*^{cpfl1} mouse model of achromatopsia (cone dystrophy), and the *rd1* of retinitis pigmentosa (rod-cone dystrophy), this study aimed to investigate common disease mechanisms of primary and secondary cone degeneration, respectively, to identify novel therapeutic targets to prevent cone loss. Single-cell RNAseq (scRNAseq) was used to assess differential gene expression patterns between healthy and IRD cones. It was found that RICTOR, the major component of mTORC2, was inhibited in diseased cones. Increasing the activity of the mTORC2 signalling pathway *in vivo* through pharmaceutical interventions – treatment with either metformin, AICAR, or SC79 – led to therapeutic effects in photoreceptors. Cone numbers were recovered to wildtype levels in treated *Pde6c*^{cpf1} mice, while cone morphology was improved, and rod photoreceptor death was attenuated in *rd1* mice. These findings demonstrate that upregulation of mTORC2 pathway activity is neuroprotective to both cone and rod photoreceptor health and could become a clinically viable treatment approach for different types of IRDs.

id #21587

An injectable hydrogel improves transcription factor-based neuronal reprogramming

Negar Mahmoudi[2, 1], Alan Harvey[3], Clare Parish[4], Richard Williams[1, 5], David Nisbet[2, 1, 6]

1. The Graeme Clark Institute, The University of Melbourne, Melbourne, VIC, Australia

2. Department of Biomedical Engineering, Faculty of Engineering and Information Technology, The University of Melbourne, Melbourne, VIC, Australia

3. School of Human Sciences, The University of Western Australia, and Perron Institute for Neurological and Translational Science, Perth, WA, Australia

4. The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, , Melbourne, VIC, Australia

5. IMPACT, School of Medicine, Deakin University, , Geelong, VIC, Australia

6. Melbourne Medical School, Faculty of Medicine, Dentistry and Health Science, The University of Melbourne,, Melbourne, VIC, Australia

Regeneration of neural circuitry in mature mammalian brain or spinal cord after injury is limited. After trauma and degeneration, astrocytes become reactive and act as a physical and biochemical barrier to neuronal regeneration that retards the repair process. Potentially, developing methods that limit gliosis and permit the reestablishment of lost neural circuitry should aid recovery.

Recent advances have shown that somatic cells can be genetically modified to alter their phenotype and emerge as potential regeneration-based therapeutics for brain repair. Here, we used astrocytes as a large, endogenous cellular pool for ‘‘on-site’’ brain repair by reprogramming them into neurons, in and around an injury site in mouse cerebral cortex. To reprogram these glia, we used adeno-associated viral vectors (AAV) that encoded either the NeuroD1 or SOX2 transcription factor (TF).

Initial *in vitro* studies validated the effectiveness and efficiency of both of these vectors in reprogramming astrocytes to induced functional neurons, confirmed by morphological, histochemical, and electrophysiological analyses. *In vivo*, after needle stick injuries made in the cortex of anaesthetised adult mice, we found that a single dose delivery of these TFs can successfully converted reactive astrocytes to neurons and replenished the injury site while reducing glial scarring. Importantly, a 3D self-assembling peptide hydrogel was also used to create microenvironments that mimic cellular compartments and facilitate cell survival and

differentiation. This injectable hydrogel addresses multiple difficulties with viral vector delivery, reducing viral dosage at the target site, minimising off-target delivery, thus enhancing the vector's targeting and reprogramming efficiency.

id #21844

It's not all in our head: The autism-associated variant in Neuroligin-3 causes gastrointestinal dysfunction, microbial dysbiosis and resilience in a model of colitis.

Elisa L Hill-Yardin[2, 1], Samantha Matta[1], Chalystha Lee[1], Mohammed Alamoudi[1], Vic Lin[1], Angela Jimenez-Perez[1], Yuansong Li[1], Mitra Mohsenipour[1], Suzanne Hosie[3, 1], Peter J Crack[4], Anya Shindler[5], Ashley E Franks[5]

1. Department of Medical Sciences, RMIT University, Bundoora, VIC, Australia

2. Department of Anatomy & Physiology, The University of Melbourne, Parkville, VIC, Australia

3. Biomedical Engineering, The University of Melbourne, Parkville, VIC, Australia

4. Department of Biochemistry & Pharmacology, The University of Melbourne, Parkville, VIC, Australia

5. Department of Physiology, Anatomy & Microbiology, La Trobe University, Bundoora, VIC, Australia

Gastrointestinal dysfunction and inflammatory disorders are common in autism spectrum disorder (ASD; autism). Many ASD-implicated genes impact brain function including a missense variant in Neuroligin-3 encoding a cell adhesion protein expressed at neuronal synapses. We aimed to assess the impact of the $Nlgn3^{R451C}$ variation on gastrointestinal function, the microbiome and inflammatory response in mice.

We investigated gut permeability, microbial diversity/abundance and colitis-severity in $Nlgn3^{R451C}$ mice. Gut permeability to FITC4kDa dextran was assessed in ex vivo gut sacs from 16-18h-food-deprived mice using a fluorescent plate reader. Colitis was induced via 3% DSS in drinking water. Fecal microbial DNA was analysed using Illumina 16S-RNA sequencing via the QIIME2 pathway with comparisons for alpha/beta diversity and abundance (RStudio). Severity of colitis was assessed via disease-activity-index (DAI; body-weight, stool consistency, rectal bleeding).

$Nlgn3^{R451C}$ mice had increased small intestinal permeability. Microbial alpha diversity was unchanged in response to DSS-colitis. Colitis caused a shift in beta diversity in both WT and $Nlgn3^{R451C}$ mice. Relative abundance analyses indicated a shift in $Nlgn3^{R451C}$ communities with an increase in Akkermansiace genus in response to colitis. $Nlgn3^{R451C}$ mice showed reduced DAI scores.

The $Nlgn3^{R451C}$ variant impacts gut function, microbiome and the inflammatory response. Altered microbial diversity in $Nlgn3^{R451C}$ mice provides potential therapeutic targets to improve gut health in autism. $Nlgn3^{R451C}$ mice showed resilience to DSS-induced colitis. Given that gut health and the microbiome impact mood and behaviour, targeting the gut could improve quality of life for people with profound autism and gut problems.

id #21333

Cellular mechanisms of generation of synchronized rhythmic burst in the ventromedial hypothalamus.

Kamon KI Iigaya[1], Hiroshi HO Onimaru[1], Keiko KI Ikeda[2], Makito MI Iizuka[1], Masahiko MI Izumizaki[1]

1. Showa University School of Medicine, 1-5-8 Hatanodai, SHINAGAWAKU, Japan

2. Department of Oral Physiology, Showa University School of Dentistry, 1-5-8 Hatanodai Shinagawaku, Tokyo, Japan

目的　視床下部腹内側部（VMH）は摂食行動や交感神経系（SNS）の制御に重要な役割を果たしている　VMHには　同期した強い律動バースト発火（いわゆるVMH振動）を示すニューロン群が含まれている このニューロン群は グルコース抑制ニューロンで構成され 摂食関連ペプチドに応答する能力があり SNSの出力と機能的に結合しているという3つの特性がある しかし そのリズム生成と同期メカニズムの詳細は不明である 本研究では VMHを含む幼若ラットのスライス標本を用いて　電気生理学とカルシウムイメージングによって　この律動バースト活動の細胞メカニズムを調査した　方法　新生ラットの脳のスライス標本を作成し 電気生理学的特性とカルシウムイメージングを使用してVMH振動を検出した　主な結果 低Ca^{2+}/高Mg^{2+}シナプス伝達阻害溶液中で VMHの律動バーストが保存されていることを発見した　VMHの電気振動は　ギャップジャンクション阻害剤カルベノキソロン（100μM）または持続性ナトリウムチャネル阻害剤リルゾール（20μM）のいずれかを投与した後に消失した カルシウムイメージング研究により 細胞内カルシウムの増加によって検出された同期したリズム活動を示すすべてのVMH細胞が カルベノキソロンの投与後に沈黙していることが明らかになった　結論　これらの結果は VMH振動が持続性ナトリウムチャネルの活性化とギャップジャンクションを介した結合から生じることを示唆している

id #21590

Characterizing Developmental Myelination and Auditory Processing in Zebrafish Larvae

Marcello Lepre[1], Rebecca Lee[2], Cheryl Lee[1], Raphael Ricci[1], Rob Gasperini[3], Ethan K Scott[2, 4], Kaylene M Young[5], Itia Favre-Bulle[2], Carlie L Cullen[1, 5]

1. Mater Research Institute, University of Queensland, Brisbane, Woolongabba, Queensland, Australia

2. Queensland Brain Institute, University of Queensland , Brisbane, St. Lucia, 4067, Australia

3. School of Medicine, University of Tasmania, Hobart, Tasmania, Australia

4. University of Melbourne, Parkville, Victoria, Australia

5. Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia

Altered brain myelin content has been linked to neurodevelopmental disorders characterized by changes in network connectivity and information processing. Developmental myelination may be a key regulator of neural circuit formation and function. Our research aims to determine how altered myelin development affects sensory information processing.

To characterise the temporal pattern of myelination within the developing zebrafish central nervous system, we imaged fluorescently labelled myelin in live, intact tg(MBP:mScarlet) larvae from 3-12 days post fertilisation (dpf). Using IMARIS software to 3D reconstruct and quantify myelin we revealed a rostro-caudal pattern of myelination. Myelin was first present the spinal cord and hindbrain at 3dpf and continued to increase in volume until 12dpf. In the midbrain, myelination began at 5dpf, increased between 5-7dpf but remained consistent between 7-12dpf. No myelin was observed in the forebrain.

To determine the importance of appropriate myelination in neural network function we generated a model of delayed myelination by selectively ablating newly formed oligodendrocytes between 3-5dpf in tg(MBP:NTR:mCherry) larvae. We performed whole brain calcium imaging to record brain-wide neuronal activity in tg(MBP:NTR:mCherry); tg(HuC:H2B-GCaMP7c) or tg(MBP:mCherry); tg(HuC:H2B-GCaMP7c) double transgenic larvae at 6dpf . So far, we show that in control tg(HuC:H2B-GCaMP7c) larvae, neurons within the torus semicircularis, medial hindbrain and thalamus exhibit periodic bursting activity that corresponds to auditory stimulus presentation (30ms, 1kHz tone delivered every 30s).

Consistent with current literature, our early data, demonstrate a rostro-caudal pattern of myelin development and that the torus semicircularis, medial hindbrain and thalamus are key regions involved in auditory processing.

id #21847

Weakening of perineuronal nets following non-invasive brain stimulation

Maitri Tomar[1, 2], Jamie Beros[1, 2], Bruno Meloni[2, 3, 4], Jennifer Rodger[1, 2]

1. School of Biological Sciences, University of Western Australia, Crawley, WA, Australia

2. Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia

3. Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Crawley, WA, Australia

4. Department of Neurosurgery, Sir Charles Gairdner Hospital, QEII Medical Centre, Nedlands, WA, Australia

Critical period is a time of heightened plasticity during development when the brain becomes sensitive to environmental cues which can influence structure and function. As this period is important in stabilizing neuronal projections, any negative environmental influence during this time can lead to abnormal brain organization which has been linked to several neurodevelopmental disorders. Therefore, enhancing, or prolonging plasticity during critical neurodevelopmental periods can be of therapeutic importance.

We investigated the impact of low-intensity repetitive transcranial magnetic stimulation (LI-rTMS) on markers of critical period plasticity. We delivered LI-rTMS to the primary visual cortex (V1) in young ephrin-A2/A5$^{+/-}$ mice (sham: n=6, treatment: n=5), which have disorganised visual system projections, and in wildtype mice (sham: n=7, treatment: n=7), daily for two-weeks starting at postnatal day 28 (peak of critical period in V1). Following the treatment mice were euthanised, perfused and brains were cryosectioned. Sections containing V1 were immunostained for parvalbumin (PV)-expressing interneurons and their surrounding perineuronal nets (PNN) which are important determinants of the critical period.

Although following LI-rTMS there was no significant change in the density of PV-expressing interneurons or PNNs, we observed changes in the intensity of the nets surrounding PV-expressing interneurons. Following TMS, there were more low intensity nets compared to high intensity nets in the ephrin-A2/A5$^{+/-}$ mice, suggesting that TMS might delay or prevent the maturation of these nets. As PNNs play an important role in closing the critical period, our findings suggest TMS might prolong critical period plasticity when abnormal brain wiring is present.

id #21849

Positive Contrast Magnetic Resonance Imaging of Iron Oxide Nanoparticles in Patient-Derived Orthotopic Glioblastoma

Phillip W Janowicz[1], Caterina Brighi[1], Zdenka Kuncic[2], David EJ Waddington[1]

1. Image X Institute, The University of Sydney, Sydney, New South Wales, Australia

2. School of Physics, The University of Sydney, Sydney, New South Wales, Australia

Glioblastoma is the most common and aggressive brain cancer in adults, and spreads in an infiltrative manner into healthy brain tissue. We have evaluated superparamagnetic iron oxide nanoparticles (SPIONs) for the magnetic resonance imaging of glioblastoma infiltrate. Antibody-coated and untargeted 'stealth' SPIONs were imaged in orthotopic patient-derived xenografts (RN1) which maintain the closed blood brain barrier seen in patients, as well as in U87MG cell-line xenografts which have a relatively permeable blood barrier. The appropriate nanoparticle dose range, pharmacokinetic properties and susceptibility gradient maps for glioblastoma angiogenesis distinction, were evaluated. Moreover, susceptibility gradient mapping allowed the T2 (dark) nanoparticle contrast to be viewed as a sensitive positive (bright) contrast. Interestingly, the untargeted stealth SPIONs exhibited longer blood circulation times compared to the human IgG1 targeted SPIONs, by avoiding rapid reticuloendothelial blood clearance. These GMP-grade stealth SPIONs could potentially be a clinical advancement over the conventionally used gadolinium-based contrast agents by avoiding toxicity, especially in patients susceptible to nephrogenic systemic fibrosis. The nanoparticles and imaging methods used in this study could also potentially be applied to other brain malignancies such as metastases, and peripheral tumours.

id #21852

Neuronal Sez6 proteins: major BACE1 substrates modulating microglial engulfment

Benjamin Wu[1], Louis Liu[1], Kathleen Teng[1], Anja Schomann[1], Peer-Hendrik Kuhn[2, 3], Martina Pigoni[2, 3], Stephan Muller[2, 3], Amelia Nash[1], Kathryn Munro[1], Stefan Lichtenthaler[2, 3, 4], Jenny Gunnersen[1]

1. The University of Melbourne, Parkville, VIC, Australia

2. Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Munich, Germany

3. Neuroproteomics, Technische Universität München, Munich, Germany

4. Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

Microglia-mediated synaptic pruning is an essential process in normal postnatal brain development. In certain regions of the brain, microglia have been shown to refine neuronal synaptic connections through the complement system. Based on structural similarities to complement regulatory proteins, as well as inhibitory effects on the complement system demonstrated in vitro, neuronal Seizure-related 6 (Sez6) family proteins are proposed to regulate microglial engulfment of synapses. Furthermore, Sez6 family proteins are prominent BACE1 substrates and therapeutic BACE inhibition (BACEi) is predicted to interfere with neuron-microglia signalling. In this study, the effects of the lack of Sez6 proteins on synaptosome and microglial properties were investigated. Quantitative proteomics of synaptosomes prepared from brains of mice lacking the three members of the Sez6 family (Sez6 triple knockout, or TKO), revealed significant decreases in signature microglial proteins including complement receptor 3 (CR3). To determine the functional effect of the lack of Sez6 family proteins on microglial pruning, live microglial engulfment assays were conducted. Microglia were found to preferentially engulf synaptosomes of the same genotype as their own when given a choice, although wild-type (WT) microglia engulfed significantly more synaptosomes than Sez6 TKO microglia, and WT synaptosomes were engulfed more than TKO synaptosomes by microglia of either genotype. Preliminary results from chemotaxis assays indicated that synaptosomes containing Sez6 proteins enhance the directional motility of WT, but not Sez6 TKO microglia. Taken together, these in vitro experimental results confirm that Sez6 proteins regulate microglial motility and synaptic engulfment.

id #21598

DASH and Cognitive Health in Older Adults: Findings from the WA Memory Study.

Carolina Blagojevic Castro[1, 3, 2], Samantha Gardener[4, 5, 6], Farzana Jahan[1], Juliana Chen[7, 8], Belinda Brown[1, 6], Ruey-Leng Loo[1, 9], Ralph Martins[1, 7, 4, 5], Hamid Sohrabi[1, 7, 4, 5, 2]

1. Murdoch University, Perth, WA, Australia

2. Centre for Healthy Ageing, Health Futures Institute, Murdoch University, Perth, WA

3. Alzheimer's Research Australia, Perth, WA

4. Edith Cowan University, Perth, WA

5. Alzheimer's Research Australia, Perth, WA

6. Lifestyle Approaches Towards Cognitive Health Research Group, Perth WA

7. Macquarie University, Sydney, NSW, Australia

8. University of Sydney, Sydney NSW

9. Australian National Phenome Center, and Center for Computational and Systems Medicine, Health Futures Institute, Perth WA

Objectives: Previous research has demonstrated that adherence to the Dietary Approaches to Stop Hypertension (DASH) diet is associated with improved cognitive function and a reduced risk of cognitive decline and Alzheimer's disease (AD). Our study aimed to assess the association between the DASH diet and cognitive decline.

Methods: Cognitively unimpaired participants (n=196) from the Western Australia Memory Study (>60 years; 35% male) completed a food frequency questionnaire (baseline only) from which a DASH diet score was generated, and cognitive assessment at baseline and up to five additional timepoints at 18-month intervals. A linear mixed effect model with random intercept was fitted to both cohorts including potential confounders, stratified by apolipoprotein E (*APOE*) genotype.

Key findings: In *APOE* ε4 allele non-carriers, the DASH diet score was positively associated with a change in the verbal fluency measure Controlled Oral Word Association Test (COWAT) letter L (β=0.215; FDR adjusted *p*-value=0.033).

Conclusion: Greater baseline adherence to the DASH diet was associated with a reduced decline in verbal fluency in those at lower genetic risk of AD. Potentially, the benefits of the DASH diet are not sufficient to overcome the cognitive decline as a sign of increased risk of AD amongst carriers of the *APOE* ε4 allele. The study findings implicate the importance of a healthy diet in the context of brain health, prefacing randomized controlled trials investigating healthy dietary patterns and cognitive outcomes.

id #20831

Multi-scale 3D mapping of myelinated afferent projections and terminals innervating the lower urinary tract in male and female rats

John-Paul Fuller-Jackson[1], Maria Daglas[1], Luke D Bowden[1], Jonathan Chan[1], Peregrine B Osborne[1], Janet R Keast[1]

1. Department of Anatomy and Physiology, University of Melbourne, Parkville, VIC, Australia

Myelinated Aδ-fiber visceral afferents of the urinary bladder transduce and convey mechanosensory signals to the spinal cord via the pelvic nerve and are essential for micturition. We have recently identified a new approach to selectively label this afferent class in rats using a novel adeno-associated virus with tropism for peripheral neurons, AAV-PHP.S (PMID: 38102980). Our aims were to define the structural properties of myelinated bladder afferents within the pelvic nerve and bladder wall; and to characterize the structure of myelinated afferents in the urethra, where sensory function is less well understood. Studies were performed in organ whole mounts from male and female Sprague-Dawley rats. All interventions were performed under anaesthesia. Myelinated afferents

innervating the bladder were identified in the pelvic nerve by combining AAV-PHP.S (intravenous), microinjection of cholera toxin subunit B into the bladder and neurofascin immunofluorescence (node visualisation). This revealed heterogeneity in axon diameter (0.5-1.5 µm) and internode length (115-891 µm). Terminal structures of myelinated afferents varied in shape between bladder dome, bladder neck and urethra. In the urethra the terminal fields varied across region and sex. The intramural component of myelinated afferents was devoid of myelin in the bladder but not the urethra. This study provides the first detailed structural characterization of myelinated bladder and urethra afferents, revealing several major differences between organs and regions. Our data will inform physiology and modeling of sacral visceral afferent function and optimization of neuromodulation protocols targeted to lower urinary tract conditions.

id #21861

Mechanisms underlying diet-induced analgesia in rodent models of chronic pain

Wendy Imlach[1], Kelly O'Sullivan[1], Shen Chen[1]

1. Department of Physiology, Monash University, Clayton, Melbourne, VIC, Australia

Chronic pain is a major global health burden that results in hypersensitivity to sensory input allowing normally innocuous stimuli to become painful. Many chronic pain states are linked to neural circuit dysfunction, which can be traced back to the spinal level in the majority of cases. In this study we investigated the mechanism underlying analgesic effects of a high-amylose diet in rodent models of fibromyalgia and arthritis. We have found that diet-induced changes to the gut microbiota reduce hyperexcitability in spinal nociceptive circuits and acts to relieve inflammation induced sensitivity. This was driven by a reduction in the activation and infiltration of inflammatory cells in the spinal dorsal horn and a reduction in the expression of neurotrophic factors in the peripheral sensory neurons. Cytokine expression was changed in these regions leading to a reduction of pronociceptive cytokines and inflammation. Electrophysiological studies showed that the acetate diet restored the excitatory-inhibitory balance in neuronal activity in sensory circuits and reduced central sensitization. These findings show that modification of gut microbiota may provide a safe and effective therapeutic option for the treatment of chronic inflammatory pain conditions.

id #21864

Developmental dysfunction in a preclinical model for *Kcnq2* developmental and epileptic encephalopathy

Miaomiao Mao[1], Nikola Jancovski[1], Yafit Kushner[1], Lucas Teasdale[1], Phan Truong[1], Kun Zhou[1], Samuel Reid[1], Linghan Jia[1], Ye Htet Aung[1], Melody Li[1], Christopher Reid[1], Sean Byars[1], Ingrid Scheffer[1], Steven Petrou[1], Snezana Maljevic[1]

1. Florey Institute of Neuroscience and Mental Health, Parkville, VICTORIA, Australia

Developmental and epileptic encephalopathies (DEE) are rare but severe neurodevelopmental disorders characterised by early-onset seizures often combined with developmental delay, behavioural and cognitive deficits. Treatment for DEEs is currently limited to seizure control and provides no benefit on the patients' developmental and cognitive outcomes. Genetic variants are the most common cause of DEE with *KCNQ2* being one of the most frequently identified disease-causing genes. *KCNQ2* encodes a voltage-gated potassium channel widely expressed in the central nervous system and is critically involved in the regulation of neuronal excitability. Here we engineer and characterise a new *Kcnq2* DEE mouse model (K557E) based on the K556E variant discovered in a female patient with a milder form of DEE. While no significant biophysical changes were observed when the mutant channel was expressed in a heterologous system, the heterozygous knock-in mice showed a reduced survival rate and increased susceptibility to induced seizures. Electrophysiology recordings in brain slices revealed a hyperexcitable phenotype for cortical layer 2/3 pyramidal neurons with retigabine (K_V7 channel opener) able to rescue both the increased sensitivity to chemically- induced seizures *in vivo* and neuronal excitability *ex vivo*. Interestingly, whole brain RNA sequencing revealed numerous differentially expressed genes and biological pathways pointing at dysregulation of early developmental processes. Our study reports on a novel *Kcnq2* DEE mouse model recapitulating aspects of the disease phenotype with the electrophysiological and transcriptomic analysis providing insights into *KCNQ2* DEE mechanisms that can be leveraged for the future therapy development.

id #21610

As Time Ticks, Does Your Brain Pick Sides? Exploring Age-Related Shifts in Hemispheric Asymmetry

Eileen Luders[1], Nicolas Cherbuin[2], Christian Gaser[3], Florian Kurth[1]

1. University of Auckland, Auckland, New Zealand

2. Australian National University, Canberra, Australia

3. University of Jena, Jena, Germany

Hemispheric brain asymmetries exist early on in life but may change over time. However, there is no consensus on whether asymmetries become weaker or stronger and which brain regions are affected. Here we set out to further explore age-related changes in brain asymmetry, with particular focus on local gray matter asymmetry. For this purpose, we selected 2,324 participants (1,151 women / 1,173 men), ranging between 47 and 80 years of age, from the UK Biobank. Each participant was scanned twice; the time between baseline and follow-up scans ranged between 1 and 7 years (mean 2.4 years). Analyses were conducted using voxel-based morphometry and running the longitudinal pipeline as implemented in the CAT12 toolbox. After calculating an asymmetry index (AI) at each voxel, the resulting images were smoothed, and the smoothed voxel-wise AI values were converted to absolute AI values. The absolute AI values at baseline were then subtracted from the ones at follow-up. Analysing the resulting AI difference images, most brain areas showed no significant change in asymmetry over time. Notwithstanding, there were a few regions where asymmetry significantly increased (e.g., medial frontal and orbito-frontal cortex, posterior parietal cortex, insula, and cerebellum) or decreased (e.g., occipital cortex, inferior parietal cortex, and posterior temporal cortex). In conclusion, albeit both increases and decreases in asymmetry are evident, these changes are confined to only a few brain regions. Overall, brain asymmetry seems to

remain relatively stable, which might be attributable to the rather narrow time frame examined here.

id #21360

Intranasal antipsychotic administration: a viable route of administration to avoid debilitating peripherally-induced side-effects

Darryl W Eyles[1, 2], Suzy Alexander[2], Emma Hilsley, Harendra Parekh[3], Preeti Pandey[3], Masood Ali[3]

1. Queensland Brain Institute, University of Queensland, St Lucia, QLD, Australia

2. Queensland Centre for mental health research, Brisbane, Qld, Australia

3. PACE, University of Queensland, Brisbane, Qld

Clozapine is the most effective antipsychotic drug for Treatment Resistant Schizophrenia, yet it has severe debilitating metabolic and potentially lethal immune-related side effects. Most of these side effects are peripherally mediated. We are exploring whether administering clozapine via the intranasal (IN) route, which largely avoids the periphery, can still act as an antipsychotic while hopefully avoiding such side-effects.

Suppression of the Conditioned Avoidance Reaction (CAR) in experimental animals is a unique feature of all antipsychotic agents. The aim of this study was to determine if IN clozapine suspended in a patented Sol-Gel nano-emulsion could also suppress CAR.

Male Sprague-Dawley rats were trained to associate the presentation of a tone (Conditioned Stimulus, CS), with a mild footshock. Animals learn and on presentation of the CS move to the alternate chamber to avoid the foot shock. Antipsychotics all uniquely interfere with this learned behaviour largely via their actions at post-synaptic D2 receptors.

INCloz (0.7mg/kg) produced a similar profile of CAR suppression over time as 20mg/kg oral Cloz. These doses were selected based on their ability to suppress CAR without inducing unwanted cataleptic / tranquilizing effects which were observed at higher doses (INCloz 1.16mg/kg, oral Cloz 30mg/kg). Brain and blood collected 30 minutes post INCloz administration were analysed using HPLC and show clozapine levels 5.5 times higher in the brain vs the blood.

We show intranasal administration can produce an antipsychotic behavioural effect at a fraction (3.5%) of the dose used orally. This offers great promise for planned future clinical trials in patients.

id #21361

Progress towards viral gene therapy for Christianson syndrome

Collin Anderson[1]

1. University of Sydney, Camperdown, NSW, Australia

Introduction: Christianson syndrome (CS) is a rare x-linked neurodevelopmental and neurodegenerative disorder characterized by severe symptoms, including intellectual disability, cerebellar degeneration, ataxia, seizures, and autistic behaviour. CS is caused by loss-of-function mutations in the SLC9A6 gene, which encodes NHE6, a sodium/hydrogen exchanger that is highly expressed in endosomes. We sought to preclinically evaluate a viral gene therapeutic strategy aimed at replacing missing SLC9A6 in a rodent model of Christianson syndrome.

Methods: We developed AAV9-CAG-SLC9A6, making use of the AAV9 serotype, a ubiquitous promoter, and the human SLC9A6 gene. Using the Wistar Furth shaker rat, a spontaneous rat model of CS, we administered AAVs via intracerebroventricular injection and quantified effects on molecular, cellular, and motor dysfunction.

Results: AAV9-CAG-SLC9A6 yielded substantial improvements in cerebellar health markers and motor function at doses as low as 8E9 viral genome copies. We identified strong, significant relationships between NHE6 expression and motor function, NHE6 expression and CALB1 expression, and CALB1 expression and motor function. Interestingly, AAV gene replacement differentially modulated tremor and ataxia and substantially modified their temporal relationship.

Conclusions: Gene therapy is a promising, potential treatment strategy for Christianson syndrome. NHE6 loss of function leads to an overly acidified endosome, which may assist with viral transduction of target neurons, yielding strong expression and uptake at low doses. Underlying cerebellar health markers are highly predictive of motor coordination. Further, tremor and ataxia may arise from dissociable mechanisms in Christianson syndrome. Additional work is required, particularly in terms of non-motor symptoms and safety / toxicology.

id #21874

Repurposing GABA-Acting Drugs for Myelin Repair: Insights from the Cuprizone Demyelination Model of Multiple Sclerosis

Chanelle Hazim[1], Hooman Saei[1], Alessandro Castorina[1]

1. University of Technology, Sydney, Sydney, NSW, Australia

Multiple sclerosis (MS) is a neurological condition marked by ongoing demyelination and neuronal damage, impacting over 3 million individuals globally. Existing MS treatments offer symptomatic relief or necessitate long-term use of immunosuppressants, often with considerable side effects. This underscores the need for alternative therapies that promote myelin regeneration. Recent studies have revealed an unexpected remyelinating potential of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), leading to renewed interest in exploring existing drugs that modulate GABA activity.

This study investigates the remyelinating effects of two FDA-approved GABA-targeting drugs: Diazepam, a positive allosteric modulator of GABAA receptors, and Valproic Acid (VPA), an antiepileptic that increases GABA bioavailability. C57BL/6 mice were fed a 0.2% cuprizone diet for four weeks to induce demyelination, followed by standard diet to promote spontaneous remyelination. These mice were then divided into groups receiving daily intraperitoneal

injections of saline (control), VPA (200 mg/kg), or Diazepam (2 mg/kg) for a further four weeks. Locomotor skills were assessed using Open Field and Rotarod tests, while histological evaluations measured myelin recovery. Both VPA and Diazepam treatments improved locomotion and promoted myelin regeneration. Real-time qPCRs revealed that both GABA-targeting drugs upregulated the expression of several myelin genes (MOG, MBP, OLIG2 and PLP1). Luxol Fast Blue staining demonstrated increased remyelination in drug-treated mice, while immunostaining for oligodendrocyte markers OLIG2 and ASPA indicated enhanced oligodendrocyte regrowth. These results emphasize the significance of targeting GABA receptors in non-neuronal cells to promote myelin repair and suggest that existing GABA-acting drugs hold promise for repurposing as treatments for MS.

id #21619

Pericytes are prevalent at capillary loop structures in the Tasmanian Devil brain

Gary P Morris[1], Grace G Russell[2], Jake M Cashion[1], Ruth Pye[2], Andrew S Flies[2], Brad A Sutherland[1]

1. Tasmanian School of Medicine, University of Tasmania, Hobart, TASMANIA, Australia

2. Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia

The Tasmanian devil (*Sarcophilus harrisii*) is an endangered carnivorous marsupial confined to the island of Tasmania. Little is known about the structure and cellular organisation of the Tasmanian devil brain. We collected the brain of a 7-year-old Tasmanian devil that was euthanised for non-neurological reasons. The brain was hemi-dissected, and the right hemisphere was prepared for cryosectioning. It was serially sectioned and labelled with cresyl violet to create a coronal atlas. A challenge when studying the cellular architecture of marsupial tissue is a lack of antibodies to label different cell types. We therefore next assessed several commercially available antibodies to label cells of the neurovascular unit, a group of cells that together regulate cerebral blood flow (CBF). We successfully labelled astrocytes (GFAP), blood vessels (lectin), neurons (NeuN), pericytes (PDGFRβ) and microglia (lectin). Like all marsupials, we found that Tasmanian devil cerebral blood vessels display a unique paired structure, with pairs of arterioles and venules penetrating the brain and then branching in unison into a capillary network, before uniting at terminal capillary loop structures. Curiously, we observed PDGFRβ-positive cell soma within these capillary loops, which we hypothesise are pericytes, a contractile cell with roles in CBF regulation and blood-brain-barrier integrity. To the best of our knowledge this is the first description of this phenomenon. Collectively, our work enables a deeper understanding of Tasmanian devil brain cytoarchitecture which can be linked to behaviour, and we shed new light on the structure of marsupial capillary loop structures.

id #21878

Long-term, high-resolution telemetry monitoring of circadian rhythmicity of temperature and activity in socially housed mice.

Stephan Martin[1], Julio Alvarez[1], Troy Velie[1], Justin Van Hee[1], Kathryn Nichols[1], Kimberly White[1], Michael Hoover[1]

1. DSI | an affiliate of Harvard Bioscience Inc., Saint Paul, MN, United States

Studying the circadian rhythms governing physiological processes is vital to understanding their impact on health and diseases. However, technological restrictions can impose a practical hurdle in experimental settings employing socially housed animals. We developed a sophisticated telemetry platform, SoHo™, to monitor body temperature and locomotor activity in group-housed small animals. This study aims to validate the SoHo™ telemetry system for monitoring body temperature and locomotor activity in group-housed mice.

CD1 male mice (6 weeks old; N=5) were intraperitoneally instrumented with the SoHo™ telemetry transmitters. The animals were group-housed under a standardized 12-hour dark/light cycle. Synchronized body temperature and locomotor activity were continuously collected for 12 days. Actograms and hourly temperature box plots show the circadian regulation of locomotor activity and body temperature with a positive correlation (r = 0.85). Locomotor activity (in counts per minute; cpm) and body temperature were significantly increased during dark periods compared to light periods (75.7 ± 10.2 cpm vs. 48.1 ± 4.7 cpm; 37.6 ± 0.4 oC vs. 36.4 ± 0.4 oC). The magnitude of locomotor activity and body temperature fluctuations across the circadian cycle were 94.4 ± 37.9 cpm and 3.5 ± 0.7 oC, respectively. The temporal relationship between peaks of locomotor activity and peaks of body temperature yielded a phase angle of 12.9 ± 7.7 minutes.

The data demonstrates that SoHo™ telemetry is a powerful platform for evaluating temperature and activity variations in socially housed mice. Further experiments, such as photoperiod modifications and pharmacological intervention, are needed to address circadian rhythm modulation.

id #21367

Characterising α-synuclein in epithelial cells of the gut

Anastazja M Gorecki[3, 1, 2], Chidozie C Anyaegbu[1, 4], Varsha Singh[5], Kathy A Fuller[6], Subhash Kulkarni[5], Ryan S Anderton[3]

1. Curtin Health Innovation Research Institute, Curtin University, Nedlands, Western Australia, Australia

2. School of Biological Sciences, University of Western Australia, Crawley, Western Australia, Australia

3. University of Notre Dame, Fremantle, WESTERN AUSTRALIA, Australia

4. Perron Institute for Neurological and Translational Science, Nedlands, Western Australia, Australia

5. School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA

6. Translational Cancer Pathology Laboratory, School of Biomedical Sciences , University of Western Australia, Crawley

Pro-inflammatory environmental factors and disruptions to the gut microbiome are recognised contributors to Parkinson's disease, and exogenous α-synuclein fibrils spread from the gut to brain via the vagus nerve in animal models. However, the natural cause of enteric α-synuclein pathology is unknown, but

emerging research implicates enteroendocrine cells in the gut epithelium. We investigated epithelial α-synuclein through confocal microscopy and western blotting of human intestinal epithelial monolayers (Aim 1), and imaging flow cytometry (IFC) of murine enteroendocrine STC-1 cells (Aim 2). Aim 1 demonstrated α-synuclein staining in enteroendocrine cells and goblet cells, the latter being a novel result. Consequently, stimulation of the apical monolayer with faecal proteins revealed a non-significant trend for increased insoluble α-synuclein, suggesting modulation by gut luminal contents. To investigate the targeted effect of a microbial metabolite, STC-1 cells were exposed to lipopolysaccharide (8 and 32 ug/ml for 24 hours), with α-synuclein localisation and phosphorylation assessed through IFC. This enabled the robust quantification of morphological and fluorescent data from large populations of cells, using objective data-processing algorithms to analyse protein expression and localisation at the single-cell level. Interestingly, 8 ug/ml LPS significantly decreased levels and altered intracellular distribution of α-synuclein in a TLR4-dependent manner, and did not cause α-synuclein aggregation or increase phosphorylated α-synuclein, such as that seen in PD. Overall, these findings demonstrate the complex interplay between gut metabolites and α-synuclein in the gut epithelium, providing insights into potential mechanisms contributing to PD.

id #21880

An innovative approach for conducting 3D electrophysiological recordings within intact organoids.

Kerri Kukovetz[1], Peter Jones[2], Tom Stumpp[2], Sara Mirsadeghi[3], Michael Mierzejewski[2], Angelika Stumpf[2], Haein Chang[2], Udo Kraushaar[2], Ali Hosseini[4], Michele Giugliano[4], Jenny Hsieh[3]

1. Multi Channel Systems GmbH, Reutlingen, BADEN-WüRTTEMBERG, Germany

2. NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, BW, Germany

3. Department of Neuroscience, Developmental and Regenerative Biology, The University of Texas at San Antonio, San Antonio, TX, USA, San Antonio, Texas, United States

4. Neuroscience Area, International School for Advanced Sciences, Trieste, Friuli-Venezia Giulia, Italy

Organoid technology is a pivotal tool for exploring human physiology and diseases. Despite its potential, current readout capabilities constrain organoid electrophysiological research. Classical microelectrode arrays (MEA) fall short in capturing data from intact organoids, which may flatten in the 2D-MEA surface, jeopardizing physiological responses and data validity. To overcome this, we pioneered a mesh MEA, reducing morphological deformations, fostering 3D growth, and facilitating electrical activity recording within intact organoids over an extended period. Electrophysiological recordings of human brain organoids were performed in an MEA-2100 head stage from MultiChannel Systems, accommodating classical MEA and mesh MEA chips. Extracellular neural activity, sampled at 25 kHz and filtered at 400 Hz for spike detection, accurately reflected action potential events on the membrane. Neuronal migration around the mesh was monitored using light microscopy. The mesh MEA integrates 60 titanium nitride electrodes (30 µm diameter) at the nodes of a 2D polymer mesh with a pitch of 200 µm and filament width of ~20 µm and thickness of ~10 µm. The mesh scaffold is suspended 2 mm from the bottom of the well. From preliminary measurements, spike time analysis revealed heightened activity after seven days on the mesh MEA (mean firing rate 34 Hz) compared to acute recordings on a classical MEA (5 Hz). Microscopy images illustrated neuronal migration, dendritic growth, and axon development around the mesh structure and electrodes. These findings suggest that the mesh MEA holds great promise for comprehensive, long-term organoid electrophysiological studies, providing deeper insights into human functions and disorders.

id #21372

The role of MAST3 in neurodevelopment and disease

Carolina Duro[1], David Keays[1]

1. LMU Munich, Planegg-Martinsried, MUNCHEN, Germany

The development of the human brain occurs early in human life and requires the coordination of key cellular events, including the generation, migration and differentiation of neurons. Genetic mutations that perturb these processes cause neurodevelopmental disorders, such as microcephaly, epilepsy, or autism spectrum disorders. Recent genetic studies have implicated MAST3 (microtubule-associated serine/threonine kinase 3) in a range of neurological conditions; however, the underlying molecular and cellular mechanisms are unknown. In this PhD project, I aim to remedy this deficit. To this end I have three goals. To: (1) Generate novel MAST3 mouse models that recapitulate disease causing mutations; (2) Undertake behavioral and anatomical characterization of these mouse lines; and (3) Generate MAST3-disease related phenotypes *in vitro* using stem cell models and cerebral organoids, and further characterize them morphologically and cellullary. Collectively, my doctorate will shed light on how MAST3 contributes to neurodevelopment and will provide insight into the pathophysiological mechanisms associated with MAST3 mutations.

id #21885

An Innovative Therapy for Alzheimer's Disease: Reducing DYRK1A Expression using Antisense Oligonucleotides

Aidan J Murphy[2, 1], Kelly M Martinovich[2, 1], Steve D Wilton[2, 1], May T Aung-Htut[2, 1]

1. Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, WA, Australia

2. Molecular Therapies Laboratory, Perron Institute for Neurological and Translational Science, Perth, WA, Australia

Approximately 10.9% of people over 65 live with Alzheimer's Disease (AD). Individuals with Down Syndrome (DS) are disproportionally affected, with 40-80% developing AD by their 60s. Mouse models and *in vitro* studies have shown that DYRK1A overexpression is implicated in AD pathology. Reducing *DYRK1A* expression in AD animal models has shown to improve cognitive function and slow AD onset. Current AD treatments are limited, and DYRK1A inhibitors tested

so far show non-specific effects despite promising disease modification. Hence, we aim to reduce the activity of DYRK1A using a novel approach where antisense oligomers (ASOs) are designed to target the *DYRK1A* transcript. We designed 18 ASOs, resulting in an altered *DYRK1A* mRNA transcript. The efficiency of these ASOs was assessed using RT-PCR. The three most efficient ASOs were electroporated into DS patient cells and assessed for protein knockdown. This was followed by RNA sequencing and explicit analysis of global changes in both gene expression and splicing patterns to confirm the specificity. At 5μM concentrations, two of our three candidate ASOs achieved significant protein knockdown compared to a positive control. ASO1 and ASO2 showed reductions of 88% ±5% SEM (p = 0.014) and 84% ±5% SEM (p = 0.013), respectively. The analyses on RNA sequencing validated the specificity of the ASO without affecting other genes ($p = 3.80\times10^{-17}$). This research introduces a novel therapeutic approach for people at risk of AD including people with DS. We have shown that ASOs can effectively downregulate DYRK1A protein, a potential therapeutic target for AD.

id #21886

Anti-Sez6 antibodies modulate Sez6 surface levels and protein turnover

Mohammadhassan Tajik[1], Benjamin Wu[1], Igli Subashi[1], Richard Zhang[1], Kathryn M Munro[1], Martina Jones[2], Jenny Gunnersen[1]

1. The university of melbourne, Parkville, VIC, Australia

2. University of Queensland, Brisbane, QLD, Australia

Neuropathic pain is a persistent and debilitating condition, which is challenging to manage. Current first-line treatments have central nervous system side-effects which could be avoided if treatments targeting the peripheral nervous system (PNS), particularly sensory neurons in the dorsal root ganglia (DRG), were found to be effective. Seizure-related protein-6 (Sez6) is a neuronal protein essential for excitatory synapse development and maintenance and our previous studies have indicated Sez6 involvement in neuropathic pain. Sez6 is expressed in sensory neurons of the DRG, with high levels in peptidergic C-fibres which sense noxious heat. Moreover, conditional deletion of Sez6 in peripheral neurons, including DRG neurons, in Advillin-Cre^{ERT2}xSez6$^{fl/fl}$ mice, significantly ameliorated heat hyperalgesia after a chronic constriction injury of the sciatic nerve. These data indicate that blocking Sez6 function in the peripheral nervous system is a promising means of inhibiting chronic pain hypersensitivity while avoiding adverse central nervous system side-effects.

With the aim of mimicking the effects of Sez6 cKO in the peripheral nervous system, we isolated anti-Sez6 monoclonal antibodies (mAbs) through biopanning a naïve human antibody single-chain variable fragment [scFv] phage display library. Testing of the reformatted mAbs *in vitro*, in Cos 7 cells exogenously expressing transmembrane Sez6 (Sez6 tII) on their surface, has revealed that several of these mAbs enhance Sez6 endocytosis and decrease levels of internalised Sez6 over time. In conclusion, these mAbs are effective at modifying the cellular distribution of Sez6 and may interfere with Sez6 function.

id #21377

Increased blood-brain barrier permeability following paediatric cancer treatments

Maya Davies[1, 2], Courtney Cross[1, 2], Long Yu[3], Paul Trim[4], Marten Snel[4], Zarina Greenberg[5], Cedric Bardy[5, 3], Hannah Rose Wardill[1, 2]

1. Precision Medicine Theme, South Australian Medical Research Institute, Adelaide, South Australia, Australia

2. Faculty of Health and Medical Science, University of Adelaide, Adelaide, South Australia, Australia

3. College of Medicine and Public Health, Flinders University, Adelaide, South Australia, Australia

4. Proteomics, Metabolomics and MS-Imaging Facility, South Australian Health, and Medical Research Institute, Adelaide, South Australia, Australia

5. Lifelong Health Theme, South Australian Medical Research Institute, Adelaide, South Australia, Australia

Introduction: 1 in 3 childhood cancer survivors develop severe and persistent cognitive impairment as a direct result of their treatments, impacting academic, vocational, and independent functioning. Unfortunately, there are no effective therapies reflecting a limited understanding of how these systemically administered treatments impact the central nervous system. Hence, we aimed to characterise the impact of anti-cancer treatments on the blood-brain barrier.

Methods: Brain microvasculature endothelial cells (BMECs) derived from human induced pluripotent stem cells were cultured on Transwell inserts and treated with 1.28mM PM (active metabolite of chemotherapeutic cyclophosphamide) for 48-hours. BMECs were fixed and stained with ZO-1 and caspase-3 for immunofluorescent analysis of tight-junction structure and apoptosis, respectively. In parallel, plasma samples from N=47 children undergoing haematopoietic stem cell transplant (HSCT) for haematological malignancies was analysed for markers of blood-brain barrier damage (S100ß), inflammation (interleukin-6), and neurological function (BDNF), pre- and post-transplant.

Results: PM induced tight-junction malformation staining without inducing significant cell death. In addition, S100ß was significantly elevated in plasma after HSCT compared to baseline levels (78.75mM vs 5.70mM; P<0.0001) suggesting increased blood-brain barrier permeability. Increased plasma interleukin-6 (P=0.02) and decreased BDNF (P=0.0008) were also identified post-transplant.

Significance: These data suggest paediatric cancer treatments impair the integrity of the blood-brain barrier, providing a novel mechanism by which systemically administered anti-cancer treatments can access the brain to impair cognitive function. They also highlight the utility of S100ß in diagnosing blood-brain barrier disruption in the context of childhood cancer. Future work will look to test barrier strengthening compounds *in vitro*.

id #21891

AUS-mTBI Extend: An extended and innovative model to predict recovery for people with mild traumatic brain injury.

Jemma Keeves[2, 1, 3], Sarah Hellewell[1, 3], Rachel Singer[1], Daniel Fatovich[4, 5], Fatima Nasrallah[6], Stuart McDonald[7], Elizabeth Armstrong[8], Karen Caeyenberghs[9], Karen Barlow[10, 11], Biswadev Mitra[2, 12], Trish Ratajczak[13], Michael Makdissi[14, 15, 16, 17], Elena Chebkasova[18, 19, 20], John Gilroy[21],

Patrick Kwan[2, 22, 7], Nick Rushworth[23], George Charalambous[24], Kirsten Vallmuur[25, 26], Natasha Lannin[12, 7], Belinda Gabbe[2], Jennie Ponsford[27, 28], Vicki Anderson[29, 30], Melinda Fitzgerald[1, 3]

1. Curtin University, Perth, WA, Australia
2. Pre-Hospital, Emergency and Trauma, School of Public Health and Preventative Medicine , Monash University, Melbourne, Victoria, Australia
3. Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia
4. Emergency Medicine, Royal Perth Hospital, Perth, WA, Australia
5. Centre for Clinical Research in Emergency Medicine, Harry Perkins Institute of Medical Research, Nedlands, WA, Australia
6. Queensland Brain Institute, The University of Queensland, Brisbane, QLD, Australia
7. Department of Neurosciences, Central Clinical School, Monash University, Melbourne, VIC, Australia
8. School of Medical and Health Sciences, Edith Cowan University, Perth, WA, Australia
9. Cognitive Neuroscience Unit, School of Psychology, Deakin University, Melbourne, VIC, Australia
10. Queensland Children's Hospital, Brisbane, QLD, Australia
11. Centre for Children's Health Research, University of Queensland, Brisbane, QLD, Australia
12. Alfred Health, Melbourne, VIC, Australia
13. Murdoch University, Perth, WA, Australia
14. Olympic Park Sports Medicine Centre, Melbourne, VIC, Australia
15. Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, Australia
16. Latrobe University, Melbourne, VIC , Australia
17. Australian Football League, Melbourne, VIC, Australia
18. Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia
19. Centre for Adolescent Health, Murdoch Children's Research Institute, Parkville, VIC, Australia
20. National Drug Research Institute, Curtin University, Perth, WA, Australia
21. Aboriginal and Torres Strait Islander Research, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia
22. Department of Neurology, Alfred Health, Melbourne, VIC, Australia
23. Brain Injury Australia, Sydney, NSW, Australia
24. Curve Tomorrow, Melbourne, VIC, Australia
25. Centre for Healthcare Transformation, Australian Centre for Health Services Innovation, Queensland University of Technology, Brisbane, QLD, Australia
26. Jamieson Trauma Institute, Brisbane, QLD, Australia
27. Turner Institute for Brain and Mental Health, School of Psychological Sciences, Monash University, Melbourne, VIC, Australia
28. Monash Epworth Rehabilitation Research Centre, Epworth Healthcare, Melbourne, VIC, Australia
29. Clinical Sciences Research, Murdoch Children's Research Institute, Melbourne, VIC, Australia
30. Psychology Service, The Royal Children's Hospital, Melbourne, VIC, Australia

Understanding mild traumatic brain injury (mTBI) is a significant challenge in Australia and worldwide. At least one in five Australians will experience a mTBI over their lifetime, with approximately 180,000 cases reported each year. AUS-mTBI Extend aims to recruit 200 participants via three metropolitan Emergency Departments (Melbourne; Brisbane; Perth). Demographic, injury circumstance, health status, mTBI symptomology and care management data will be collected at the time of injury, with follow-up for 12-months or until symptom resolution. Participants will provide blood and saliva samples within 12 hours of injury and undergo a novel MRI scan protocol to assess blood brain barrier integrity, functional networks and white matter microstructure; vestibular/ocular motor screening; cognitive assessment; and balance assessment within 4 days of injury. Notably, the innovative MRI protocol has the potential to set a world standard for imaging after mTBI. Follow-up assessment will be completed by telephone at 2 weeks, 1, 3, 6 and 12 -months after injury to assess post-concussion symptoms, using the Rivermead Post-Concussion Questionnaire (adults), or the Post-Concussion Symptom Inventory (<18years), and quality of life using the Quality of Life after Brain Injury Overall Scale. By collecting data on lifestyle, injury circumstances and pre-existing health factors, and combining this with participant symptoms, we will build a detailed picture of concussion recovery and better direct care, particularly for those identified to be at risk of a delayed recovery.

id #21894

A Novel, Minimally Invasive Mode of Delivery for Red and Near-Infrared-Light Therapy of the Spinal Cord

Isabella Drew[1, 2], Zara van Zijl[1, 2], Mikayla Compton[1, 2], Daniel Flanagan[3], Ahmad Shah Idil [4], Vincent Wallace[5], Alan Harvey[1, 2], Stuart Hodgetts[1, 2]

1. Perron Institute for Neurological and Translational Science, Perth, WA, Australia
2. School of Human Sciences, The University of Western Australia, Perth, WA, Australia
3. Engineering, The University of Western Australia, Perth, WA, Australia
4. Medical Physics & Biomedical Engineering, University College London, London, WC1E 6BT, United Kingdom
5. Physics, The University of Western Australia, Perth, WA, Australia

Currently, attempts to treat experimental spinal cord injury (SCI) using light therapy involve the use of diffusion tip catheters, optic fibre cables or handheld devices. However, efficacy can be affected by dislodgment and/or infection, both leading to variable dosage absorbed at the injury site. These approaches also require restraint or anaesthesia during every treatment session which can lead to animal stress and may impact functional recovery. To address these issues, we have developed a novel, wirelessly powered, hermetically sealed LED implant (6 x12 mm) positioned directly above the lesion in our rat thoracic (T10) SCI model. This allows animals to move freely during treatment, which promotes exercise induced benefits, and has the capability of being upscaled to a mobile device for human application. In our pilot study, under anaesthesia the device was sutured in place over the thoracic spinal cord after a laminectomy. The implants did not impair locomotion (no change in BBB scores or ladder-walk accuracy). After two months, rats were perfused and implants were found still to

be positioned in line with the vertebral column. The morphology of the underlying spinal cords (Toluidine blue-stained sections) appeared normal. IBA-1 immunostaining revealed low-level reactive changes in the phenotype of microglia but there was no evidence of altered astrocytic phenotype (GFAP immunoreactivity). In short, our novel hermetically sealed implants did not significantly impact locomotion, did not result in dislodgment, infection, or elicit a significant inflammatory response, and therefore form the basis of a first line, minimally invasive therapy for SCI repair.

id #21897

The effect of contralateral cortex injection of AAV1-CRMP2 on C6/7 hemi-contusion spinal cord injury repair in Fischer rats

Zara van Zijl[1], Isabella Drew[1], Mikayla Compton[1], Steven Petratos[2], Alan Harvey[3], Stuart Hodgetts[1]

1. School of Human Sciences, The University of Western Australia / Perron Institute for Neurological and Translational Science, Perth, Western Australia, Australia

2. Department of Neuroscience, Monash University, Melbourne, Victoria, Australia

3. School of Human Sciences, The University of Western Australia, Perth, Western Australia, Australia

Collapsin response mediator protein 2 (CRMP2) is a microtubule-associated phosphoprotein in the mammalian CNS. It has multifunctional properties and is known to influence neuronal cytoskeleton integrity, a function that is inhibited following phosphorylation after injury. Importantly, preventing phosphorylation is reported to stabilise axons and promote neuronal survival after trauma. In this pilot study, CRMP2's neuroprotective capacity was investigated following a moderate cervical C6/7 hemi-lateral contusion spinal cord injury (SCI) in anaesthetised adult female F344 rats. Corticospinal neurons were transduced immediately after SCI by injecting bi-cistronic adeno-associated viral vectors (AAV1) expressing a mutated phospho-resistant form of CRMP2 ($mCRMP2^{GFP}$) (*n*=4) or $AAV1^{mCherry}$ in control rats (*n*=4) into contralateral forelimb sensorimotor cortex. Functional deficits and improvements were analysed for 8 weeks with behavioural tests. Morphology of corticospinal tract (CST) axons was compared between treatment groups by injecting biotinylated dextran amine into the same previously transduced region of cortex. Rats were perfused two weeks thereafter (D56 after SCI). Despite demonstrating significant functional deficits associated with CST impairment there were no significant differences between treatment and control groups. Qualitatively, we observed fewer degenerative CST axons, increased microglia infiltration and smaller cysts ($p>0.05$) near the injury in mCRMP2 treated rats. However, there was considerable variability, presumably from inconsistent damage sustained by the CST during the injury. In summary, transduction of mCRMP2 with AAV1 into the sensorimotor cortex of the rat brain can successfully target corticospinal neurons and their axons, providing a potential avenue to further explore the neuroprotective effects of mCRMP2 after SCI.

id #21898

Amacrine cells in the ganglion cell layer of human and macaque monkey retina

Paul R Martin[1], Ulrike Grunert[1], Alyssa Kristina Baldicano[1]

1. Faculty of Medicine and Health, University of Sydney, Save Sight Insitute, Sydney, NSW, Australia

Amacrine cells provide inhibitory synaptic input to bipolar, ganglion and other amacrine cells in the retina. Here, we investigated the spatial distribution and morphology of displaced amacrine cells (dACs) located in the ganglion cell layer (GCL). Postmortem human donor retinas and retinas from macaque monkeys (*Macaca fascicularis*) were triple labelled with antibodies to label amacrine cells (AP2-alpha, a generic amacrine cell marker, choline acetyltransferase, ChAT, a marker for starburst amacrine cells, substance P, secretagogin) and ganglion cells (RBPMD) and with DAPI (nuclei marker). Intracellular injections of labelled cells with the lipophilic dye DiI was used to reveal their morphology. In peripheral retina of both macaque and human, dACs constituted ~60% of all cells in the GCL. AP2-alpha labeled only 50% of the dACs in macaque and 70% of dACs in human retina indicating that AP2-alpha does not label all amacrine cells. In both species, substance P expressing cells constituted 2% of dACs and ~5-6% expressed secretagogin. ChAT expressing cells made up ~14% of dACs in macaque and ~21% of dACs in human. Intracellular injections revealed that substance P and secretagogin are expressed by a wide-field amacrine cell type named thorny type 2, which is characterized by thorny dendrites stratifying in the middle of the inner plexiform layer. In conclusion, dACs make up most cells in the GCL in peripheral retina, thorny type 2 cells make up 2%, and starburst amacrine cells make up between 15-20% of the dACs. Thus, a large proportion of dACS has yet to be identified.

id #21899

The Infectious Contribution to Alzheimer's disease -- assessing the role of Chlamydia pneumoniae infection

Amy McEwen[2, 1], Andrea Standke[2, 1], Edith Miller[2, 1], Ali Delbaz[2, 1], James St John[2, 1]

1. Griffith University, Southport, QLD, Australia

2. Clem Jones Centre for Neurobiology and Stem Cell Research, Southport, QLD, Australia

Chlamydia pneumoniae is an intracellular pathogen that infects the respiratory tract and has been linked to infecting the central nervous system. *C. pneumoniae* has been shown to be present in people with Alzheimer's disease, with prior work within our lab showing that this bacteria utilises nerves in the olfactory system and their associated glial cells to get direct entry into our brain. The presence of *C. pneumoniae* has been associated with amyloid beta accumulation, a hallmark feature of the disease. To assess how *C. pneumoniae* contributes to Alzheimer's disease progression, in vivo murine models were used. Assessing how intranasal inoculation of *C. pneumoniae* in childhood (Postnatal Day 14, P14), adulthood (P84) and re-infection (P14 & P84) was used to establish how the bacterium is linked to key pathways in Alzheimer's disease progression. RNA-Seq results of the olfactory mucosa showed how inoculation of the nasal cavity with *Chlamydia pneumoniae* led to the dysregulation of genes associated with Alzheimer's disease. Mice that were subject to a repeat

exposure had an increased susceptibility of gene expression linked to neurodegenerative diseases. Proteomic analysis of the olfactory bulb also showed a response consistent with disease progression. These results indicate that repeat infections of *Chlamydia pneumoniae* may contribute to an increased risk in Alzheimer's disease. Establishing pre-clinical testing, such as the Smell Clinic at Griffith University, will allow for individuals prior to any signs of neurodegeneration to be assessed for risks. Further research into the nose-brain axis may lead to the establishment of therapies.

1. Chacko, A., Delbaz, A., Walkden, H. et al. Chlamydia pneumoniae can infect the central nervous system via the olfactory and trigeminal nerves and contributes to Alzheimer's disease risk. Sci Rep 12, 2759 (2022). https://doi.org/10.1038/s41598-022-06749-9

id #21646

Insights into the role of the PAC1 receptor: Attenuation of ER stress in myelin degeneration and repair.

Minduli Withana[1], Alessandro Castorina[1]

1. Laboratory of Cellular and Molecular Neuroscience (LCMN), School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia

Multiple Sclerosis (MS) is a debilitating central nervous system (CNS) disorder that leads to significant motor and cognitive impairments. A hallmark of MS pathology is endoplasmic reticulum (ER) stress, typically counteracted by the Unfolded Protein Response (UPR). The PAC1 receptor, abundantly expressed in oligodendrocytes and neurons, has emerged as a promising therapeutic target due to its potential to mitigate ER stress.

This study investigates the impact of PAC1 deficiency on myelin density, neuronal health, and ER stress during *demyelination* and *remyelination* in the cuprizone mouse model of MS.

Real-time qPCR demonstrated a marked downregulation of myelin markers (MOG, MBP, Olig2) in the motor cortex and white matter of PAC1-/- mice relative to PAC1+/+ controls during demyelination ($p<0.0001$), corroborated by immunofluorescence analysis of myelin markers and diminished myelin density in the corpus callosum, as determined by Luxol Fast Blue staining. This downregulation persisted at both 1 and 3 weeks post-remyelination when compared to PAC1+/+. Additionally, UPR markers (ERN1, ATF4, ATF6, DDIT3) were significantly lower in PAC1-/- mice, reflecting a compromised ability to manage ER stress. Behavioral assessments (Open Field, Rotarod) revealed significant motor deficits in PAC1-/- mice. Maxadilan (PAC1-specific agonist) ameliorated cuprizone-induced motor coordination deficits compared to saline controls in PAC1+/+ mice ($p<0.05$).

This study identifies a distinct CNS phenotype in PAC1-/- mice during demyelination and after spontaneous remyelination, with impaired myelin health and behavioral deficits likely linked to unresolved ER stress. These findings underscore the PACAP/PAC1 axis as a potential therapeutic target for modulating ER stress in MS.

id #21902

Novel Methods to Achieve the Highest Resolution *in-vivo* Child Brain MRI — A Pilot Study

Steve Kassem[1, 2], Brooklyn Wright[2], Mark Schira[3]

1. NeuRA, Randwick, NSW, Australia

2. Medicine, University of NSW, Sydney, NSW, AU

3. School of Psychology, University of Wollongong, Wollongong, Australia

Introduction

Neurological development is a complex and dynamic process in which brain structure, function, and connectivity evolves throughout the first three decades of life. Despite recent quality and resolution advancements in MRI, there is no high-quality paediatric data.

Methods

This pilot study employs short (~8 min) structural acquisition protocols at 0.65mm isotropic. Four T1-w scans were collected for two female participants, aged 9 and 12. Multiple shorter scans facilitate breaks between scanning and thus navigate the issue of participant compliance. Then, using template generation protocols, single participant images are averaged where T1-w and T2-w 'templates' are generated for each participant. Our methods are publicly available at: https://osf.io/ckh5t/

Results

Applying template generation methods to single participant datasets reduces head-motion artefacts, reveals structural detail and facilitates up-sampling to a voxel size of 0.5mm isotropic in the final data. Figure 1 compares outputs from our method to the Haskins child atlas, illustrating the marked improvement in structure visibility and an 8-fold increase in resolution we achieve.

Discussion

To our best knowledge this represents the highest resolution pediatric MRI dataset by a significant margin. It permits the comprehensive investigation of all ~1000 brain structures, including challenging structures such as the hippocampal subfields which can only be sufficiently visualised at high resolution. Our methods, tailored to the child brain, are key to advancing paediatric clinical interventions and facilitating the investigation of child brain anatomy directly, instead of deduction from adult anatomy.

id #21393

The Enzyme-Responsive Delivery System for Reprogramming Reactive Glia into Neural Progenitors and Neurons after Brain

Injury

Zahra Eivazi Zadeh[2, 1], Richard Williams[3], Kiara Bruggeman[4], Clare Parish[5], David Nisbet[2, 1]

1. The Graeme Clark Institute, the University of Melbourne, Melborne, Please select..., Australia

2. Department of Biomedical Engineering, the University of Melbourne, Melborne, Victoria, Australia

3. iMPACT, School of Medicine, Deakin University, Melborne, Victoria, Australia

4. Laboratory of Advanced Biomaterials Research, School of Electrical and Energy Engineering, Australian National University, Canberra, ACT, Australia

5. The Florey Institute of Neuroscience and Mental Health, the University of Melbourne, Melborne, Victoria, Australia

Brain injuries are considered one of the most challenging cases because of the limited ability of neural cells to regenerate. Reprogramming reactive astrocytes, surrounding and within a lesion cavity, into functional neurons through Adeno-associated viruses (AAV) encoding SOX2 or NeuroD1 can provide an alternative therapeutic approach for the regeneration of the injured or diseased brain. However, delivering such AAV, especially into the brain, suffers from challenges like spreading AAV into the non-target tissues, and their neutralisation by the host immune responses.

In this research project, we have developed an injectable hydrogel made of self-assembling peptides (SAPs) that can hold the viral vectors within its nanostructure to keep them in the target tissue and protect them from the host immune responses. Moreover, this hydrogel can be activated by injection of a bio-orthogonal enzyme to release the viral vector. With this technique, we can control the time, location and condition of the viral vectors released in the target tissue.

The optimised sequences of SAPs were synthesised using routine solid-phase peptide synthesis. To characterise the prepared SAPs nanostructures, the samples were tested through Fourier Transform Infrared Spectroscopy (FT-IR), Circular Dichroism (CD), Transmission Electron Microscopy (TEM) and Zeta potential assay. Their functionality in response to the enzyme was also tested. The enzyme will ensure precise gene delivery timing by cleaving only the delivery systems holding the viral vector. The biocompatibility of this system was also tested in contact with the primary astrocytes.

1. https://doi.org/10.1021/acsnano.3c11337?urlappend=%3Fref%3DPDF&jav=VoR&rel=cite-as

id #21905

Excessive repetitive behavior, enhanced spatial learning, altered dendritic features, and aberrant protein expression in *Fbxo25* knockout mice

Li-Jen Lee[1, 2, 3], Szu-Yu Lin[1], Chi-Hou Ng[1], Ho-Ching Chang[1], Susan Shur-Fen Guo[2, 3, 4]

1. Graduate Institute of Anatomy and Cell Biology, National Taiwan University College of Medicine, Taipei, Taiwan

2. Institute of Brain and Mind Sciences, National Taiwan University College of Medicine, Taipei, Taiwan

3. Neurobiology and Cognitive Science Center, National Taiwan University, Taipei, Taiwan

4. Department of Psychiatry, National Taiwan University Hospital, Taipei, Taiwan

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by social communication impairments, restricted interests, and repetitive behaviors. A microdeletion in the 8p23.2-pter region has been identified in a Taiwanese ASD boy. The gene encodes F-box only protein 25 (FBXO25), a component of SCF (Skp1-Cullins-F-box) E3 ubiquitin ligase complexes, is located in this region. To evaluate the impact of *FBXO25* removal, we established *Fbxo25* knockout mice and characterized their phenotype. Both heterozygous (Het) and homozygous (Homo) *Fbxo25* knockout mice are fertile and develop normally. Compared with wild-type (WT) littermates, *Fbxo25* knockout mice exhibited comparable behaviors in the aspects of locomotion, emotion, social interaction, and aggression; notably, Homo *Fbxo25* displayed excessive repetitive behavior and enhanced spatial learning. Given that FBXO25 is highly expressed in the hippocampus, we found reduced dendritic complexity in the granule cells of the dentate gyrus of both Het and Homo *Fbxo25* knockout mice and increased dendritic spines in Het *Fbxo25* knockout mice. Additionally, altered hippocampal protein expressions were noticed in knockout mice. Comparing the hippocampal protein and mRNA profiles, we identified increased GluN2A, mGluR5, ERK, and ARC proteins with unchanged mRNA expression in the Homo *Fbxo25* knockout mice, suggesting the substrates of FBXO25-mediated protein degradation that impact the synaptic transmission and are relevant to the pathogenesis of ASD.

id #21650

Investigating CNS white matter defects in a murine model of Aicardi-Goutières syndrome

Christabella A Winata[1], Markus Hofer[2], Alessandro Castorina[1]

1. School of Life Science , Laboratory of Cellular and Molecular Neuroscience (University of Technology Sydney), Sydney, NSW, Australia

2. School of Life and Environmental Sciences and Charles Perkins Centre, University of Sydney , Sydney, NSW, Australia

Aicardi-Goutières syndrome (AGS) is a rare genetic disorder that affects the brain and immune system of paediatric patients. Clinical features of AGS include central nervous system (CNS) white matter abnormalities, developmental delays, progressive motor and cognitive decline, and elevated interferon-alpha (IFN-α) levels in cerebrospinal fluid, a hallmark feature of AGS. IFN-α is linked to disruptions in calcium homeostasis and chronic neuroinflammation, contributing to myelin and oligodendrocyte damage. To investigate white matter damage (WMD) and calcifications observed in AGS patients, we utilised transgenic (GIFN) mice with astrocyte-specific over-expression of IFN-α driven by GFAP protein as a preclinical model.

Brain tissue sections from two age groups of GIFN mice (2 and 7 months) were stained with Luxol Fast Blue (LFB), Hematoxylin & Eosin (H&E), Alizarin Red S (ARS), and immunofluorescence for IBA1 and ASPA. Age-related alterations were observed which correlates with disease severity. LFB revealed demyelination or areas of discolouration in the corpus callosum and cerebellar white matter of GIFN mice, alongside with dysregulation of myelin genes (MBP,

PLP1, MOG, OLIG2 and SOX10) as determined by RT-qPCR analysis. H&E identified subtle micro-aneurysms specifically in the cerebellum. ARS highlighted sparse cerebellar calcifications, more pronounced in older GIFN mice, and immunofluorescence staining indicated neuroinflammation.

Our findings establish GIFN mice as a valid preclinical model of AGS, demonstrating myelin defects and micro-angiopathy similar to those observed in AGS patients. This model provides insights into AGS pathophysiology and potential therapeutic strategies targeting IFN-α-mediated neuroinflammation and WMD.

id #21906

Boosting Brainwaves: How Low-Intensity rTMS Modulates Synaptic Plasticity During Skilled Learning in Mice

Ross C Langley[1], William Bennett[1], Alison J Canty[1]

1. Wicking Dementia Research and Education Centre, University of Tasmania, Hobart, TAS, Australia

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique that uses magnetic waves to stimulate neuronal elements. Administering low intensity (LI)-rTMS immediately before learning has been shown to enhance reaching accuracy in a rodent skilled reaching task (Tang et al., 2018). While the effects of rTMS can be long-lasting, the mechanism underlying these effects remains unclear.

Our study investigates how delivering LI-rTMS prior to a skilled reaching task affects structural synaptic plasticity in the mouse motor cortex. We employed longitudinal *in vivo* 2-photon microscopy to observe changes in dendritic spines of pyramidal neurons in Thy1-gfp-M mice. Mice were divided into two groups and received LI-rTMS (n=4) or sham LI-rTMS (n=5) immediately before a 10-day skilled reaching task. Daily imaging was performed through a cranial window after the task, with spines recorded as stable, new, or lost.

All mice improved in performance over the 10-day period. In the learning only (sham LI-rTMS) group, we observed a decrease in synaptic gains during the first three days of the task, with no changes in spine losses, stability, survival, or density. In contrast, mice that received LI-rTMS showed destabilization of newly formed dendritic spines, leading to lower spine survival rates compared to spines formed before the task. Additionally, LI-rTMS caused a small but significant increase in spine gains on day three, raising spine density. These alterations were transient returning to baseline following task cessation.

These findings suggest that LI-rTMS, when used as a primer for a learning task, can modulate synaptic plasticity.

id #21395

Structural studies of neutral amino acid transporters of the SLC6 family coupled with discovery of new inhibitors

Jedrzej Kukulowicz[1], Angelika Broer[2], Ziwei Hu[3], Aditya Yadav[2], Ting Zhang[3], Lu Dai[3], Renhong Yan[3], Stefan Broer[2], Marek Bajda[1]

1. Department of Physicochemical Drug Analysis, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland

2. Research School of Biology, Australian National University, Canberra, ACT, Australia

3. Department of Biochemistry, Key University Laboratory of Metabolism and Health of Guangdong, School of Medicine, Institute for Biological Electron Microscopy, Southern University of Science and Technology, Shenzhen, Guangdong, China

Sodium-dependent membrane transporters of the SLC6 family mediate the transport of amino acids and monoamines. Their neural representatives, such as B^0AT2 (SLC6A15), NTT4 (SLC6A17), and SIT1 (SLC6A20), transport neutral amino acids and thereby maintain glutaminergic homeostasis by supplying neurons and glial cells with precursors for the glutamine-glutamate-GABA cycle. Their dysfunction has been associated with anxiety, mental retardation, or NMDA receptor hypofunction [1]. The structures of these transporters are poorly understood and remain an area of research. In doing so, we carried out computational investigations followed by cryo-EM and kinetic studies. As a result, we have collected data that advance our understanding of the structure, membrane anchoring, mechanism of substrate transport and inhibition. Fine feedback, with the discovery of first-in-class potent inhibitors of B^0AT2 and SIT1, has validated our computational methods. Our findings will guide further structural studies toward a mechanistic understanding of transporter function. Furthermore, the discovery of new inhibitors will also be an aid in the design of another. Such a compounds will be useful for investigating the therapeutic relevance of their targets as well as for deciphering the physiology of the glutaminergic system.

1. Kukułowicz J, Pietrzak-Lichwa K, Klimończyk K, Idlin N, Bajda M. The SLC6A15-SLC6A20 Neutral Amino Acid Transporter Subfamily: Functions, Diseases, and Their Therapeutic Relevance. Pharmacol Rev. 2023;76(1):142-193. 2023 Dec 15. doi:10.1124/pharmrev.123.000886

id #21651

Biobehavioural assessment of methamphetamine use disorder: an interplay between genes, cognition, and duration of drug use

Alexandre Guerin[1], Briana Spolding[2], Kiymet Bozaoglu[1], Courtney Swinton[2], Zoe Liu[2], Bruna Parry[2], Trang Truong[2], Brian Dean[3], Andrew Lawrence[3], Yvonne Bonomo[4], Eric Nestler[5], Peter Hamilton[5], Michael Berk[2], Susan Rossell[6], Ken Walder[2], Jee Hyun Kim[2]

1. The University of Melbourne, Parkville

2. Medicine, IMPACT, Deakin University, Geelong

3. Florey Department of Neuroscience and Mental Health, University of Melbourne, Parkville

4. St Vincent's Hospital, Fitzroy

5. Icahn School of Medicine at Mt Sinai, New york

6. Swinburne University of Technology, Hawthorn

Background: A range of risk factors are associated with the development of methamphetamine use disorder, including genetics. Assessing sequence variations and expression of candidate genes can help understand the disorder and inform potential treatments. This study is an investigation of single nucleotide polymorphisms and gene expression in four candidate genes: *SLC18A1, SLC18A2, BDNF,* and *FAAH,* between controls and people with methamphetamine use disorder.

Method: Fifty-nine participants (29 people with methamphetamine use disorder and 30 controls) completed a clinical interview, cognitive tasks, and provided a blood sample. *SLC18A1, SLC18A2, BDNF*, and *FAAH* single nucleotide polymorphisms were genotyped, and gene expression was assessed with real-time quantitative PCR.

Results: *SLC18A1* Pro4Thr was associated with methamphetamine use disorder (OR = 6.22; p = 0.007). *SLC18A2* variants, rs363227 and rs363387, were negatively associated with severity of methamphetamine dependence (p = 0.003) and positively associated with inhibitory control performance (p = 0.006), respectively. *BDNF* Val66Met was associated with severity of dependence (p = 0.008). SLC18A2 and FAAH blood mRNA levels were lower in people who use methamphetamine relative to controls (p = 0.021 and 0.010, respectively).

Conclusions: *SLC18A1,* which codes for the vesicular monoamine transporter 1 (VMAT1), is identified for the first time to play a potential role in conferring a risk for methamphetamine use disorder. Lower levels of blood SLC18A2 and FAAH mRNA in people with methamphetamine use disorder suggest reduced monoamine reuptake, recycling, or release and higher anandamide levels in this clinical group, which may be potential therapeutic targets.

id #21907

Oxygenating Hydrogels: A Therapeutic Approach for Brain Injury

Yi Wang[1, 2], David R Nisbet[1, 2]

1. Department of Biomedical Engineering, The University of Melbourne, Melbourne, VIC, Australia

2. The Graeme Clark Institute, The University of Melbourne, Melbourne, VIC, Australia

Background: Approximately 700,000 Australians suffer from a brain injury, along with the time and financial burden on our healthcare system. However, there is no long-lasting approved treatments for brain injury. Engineered biomaterials for delivery and support of cells hold significant promise for the replacement and repair of damaged or diseased brain tissues. Currently, there are critical issues of their use post-transplantation; namely, hypoxia, poor cell survival and cell differentiation.

Methods: In this work, we have engineered oxygenating hydrogels by protein-engineering techniques and solid-phase peptide synthesis. We have characterised those oxygenating hydrogels and explored oxygen release kinetics, followed by the assessment of the functional integration *in vivo* after 28 days.

Results: The oxygenating hydrogels can perform nanofibrous scaffolds with mechanical support, as well as being the functional state for oxygen binding and releasing. The oxygenating hydrogels have no effect on host inflammatory response while supporting cell survival and graft innervation. Most importantly, the oxygenating hydrogel can promote the differentiation of primary stem cells into neurons, which are considered essential for brain injury.

Discussions and Conclusions: We have successfully engineered oxygenating hydrogels that are capable of concomitantly delivering stem cells and oxygen within the brain to support engraftment. These oxygenating hydrogels can promote extensive innervation, ensuring functional synaptic connectivity while increasing of neuronal differentiation, demonstrating modulating cell fate specification. These oxygenating hydrogels are the promising approach for the treatment of neural injuries and diseases affecting the central and peripheral nervous systems.

id #21652

A systems-based strategy for personalised schizophrenia treatment using patient-derived olfactory neuronal stem cells

Jamila Iqbal[1], Daniel Russell[1], Caio Damski[1], Oak Hatzimanolis[1], Alex M Sykes[1], Alexandre S Cristino[1]

1. Institute for Biomedicine and Glycomics, Griffith University, Brisbane, Queensland, Australia

Schizophrenia is a complex polygenic disorder with hundreds of genetic variants contributing to disease risk, most of which are in regulatory non-coding regions of the genome. These variants impact gene networks and biological pathways that control fundamental cellular processes and subcellular organisation beyond synapses and neurotransmitters. Our goal is to develop a systems-based approach using patient-derived olfactory neuronal stem cells (ONS cells) to identify optimal therapeutic interventions for schizophrenia. We generated multi-omics data from 57 samples of ONS cells (30 schizophrenia patients and 27 healthy controls). The data includes high-content cell imaging, whole genome sequencing, transcriptome profiling (long, small and circular RNAs), and proteome analysis. Our approach integrates genome-wide association studies (GWAS), Connectivity Map drug-induced expression signatures (CMap), ONS cell gene expression profiles, bioinformatics predictions of binding motif sequences, and correlation analyses. This comprehensive integration aims to identify disease-associated gene networks and cell traits, enabling the selection of FDA-approved drugs that can potentially reverse the dysfunctional cell traits and gene signatures in individual patients. Our results reveal that schizophrenia patient-derived ONS cells have significant differences in cell traits related to subcellular organisation and cell motion, including cell area and shape, mitochondria and endoplasmic reticulum morphology, and cell motility (travel distance and speed). We identify gene networks associated with cell adhesion and migration that correlate with genes identified in GWAS and CMap gene-drug signatures. Our systems-based drug discovery pipeline provides new insights into the mechanisms of FDA-approved drugs and assists clinicians in selecting optimal treatments based on individual stem cell responses.

id #21908

RNA editing of the GluA2 Q/R site regulates dendritic spines in hippocampal neurons

Luke T Milham[1], Bryce Vissel[1]

1. St Vincents Hospital Sydney, Sydney, NSW, Australia

Objective: With a growing base of literature finding widespread hypo-editing in neurodegenerative diseases characterised by synapse loss, we aimed to investigate the role of ADAR2-mediated RNA editing, particularly at the GluA2 Q/R site, in regulating dendritic spine density in hippocampal neurons.

Methods: We used siRNA knockdown (KD) and viral overexpression of ADAR2 and and force-edited GluA2(R) in primary hippocampal neurons. RNA editing efficiency was assessed using RT-qPCR, while dendritic spine density was analysed through fluorescent microscopy and 3D reconstruction.

Results: ADAR2 KD significantly reduced editing at both GluA2 and GluK1 Q/R sites and led to a substantial loss of dendritic spines, with spine numbers correlating strongly with ADAR2 expression. Overexpression of ADAR2 increased GluK1 Q/R site editing and restored GluA2 Q/R editing in ADAR2 KD neurons. Importantly, ADAR2 overexpression increased dendritic spine numbers compared to controls, even in ADAR2 KD cells. Furthermore, GluA2(R) overexpression specifically restored GluA2 Q/R editing following ADAR2 KD without affecting GluK1 editing. Notably, GluA2(R) overexpression increased spine density and rescued spine loss in ADAR2 KD neurons.

Conclusion: Our findings establish a direct link between ADAR2-mediated GluA2 Q/R site editing and dendritic spine maintenance in hippocampal neurons. The rescue of spine density by both ADAR2 and GluA2(R) overexpression suggests that GluA2 Q/R site editing is not only necessary but sufficient for spine regulation. This mechanism provides a potential target for therapeutic interventions in neurodegenerative diseases characterised by RNA-editing deficits and synaptic loss, offering a novel approach to maintaining cognitive function through the preservation of dendritic spines.

id #21397

Hybrid Optogenetic and Electrical Retinal Prosthesis for Vision Restoration

Wei Tong[1], William Kwan[2], Emma Brunton[1], James Begeng[3], Toon Goris[1], Tatiana Kameneva[3], Paul Stoddart[3], Michael Ibbotson[1], Rachael Richardson[4]

1. University of Melbourne, Parkville, PLEASE SELECT, Australia

2. Monash University, Clayton

3. Swinburne University of Technology, Hawthorn, VIC, Australia

4. Bionic Institute, East Melbourne, VIC, Australia

Retinal prostheses aimed at stimulating retinal ganglion cells hold promise for restoring vision in patients with photoreceptor degeneration, such as retinitis pigmentosa and age-related macular degeneration. Current clinically approved retinal prostheses utilizing electrical stimulation have limited spatial resolution due to electrical spread, resulting in vision restoration not even up to the threshold of legal blindness. Optogenetic approaches offer greater spatial precision but are hindered by lower temporal fidelity and safety concerns due to the high intensity of light required for stimulation.

In this study, we present a novel hybrid approach that combines optogenetic and electrical stimulation of retinal ganglion cells. Through ex vivo retinal electrophysiology experiments, including patch clamping and calcium imaging, we demonstrated that hybrid stimulation could lower optogenetic activation thresholds and enhance high-frequency responses without compromising spatial resolution. Our findings suggest that integrating hybrid stimulation into future retinal prostheses could improve the safety, efficiency, and resolution of vision restoration.

id #21909

Impaired TYROBP/DAP12 signalling alters postnatal brain homeostasis

Alison K Carlisle[1], Riku Sato[1], Adam D Ewing [2], Jürgen Götz[1], Liviu-Gabriel Bodea[1, 3]

1. Clem Jones Centre for Ageing Dementia Research (CJCADR), Queensland Brain Institute (QBI), The University of Queensland, St Lucia, QLD, Australia

2. Mater Research Institute, The University of Queensland, Woolloongabba QLD, Australia

3. School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, St Lucia, QLD, Australia

A key regulator of microglial physiology is TYROBP (aka DAP12), an adaptor protein that has been extensively studied in the context of age-related neurodegenerative diseases. However, little is known about its role in brain postnatal development. To investigate this, we generated a novel mouse model expressing mutant TYROBP with a disrupted signalling motif. Proteomic analysis of total brain lysates revealed dysregulations in synapse maturation-related functions. In addition, we found mild age- and brain region-specific differences in microglia density and volumes in the mutant TYROBP brains. Co-expressing TREM2, a cognate receptor of TYROBP, together with either wild-type or mutant TYROBP in HeLa cells, we observed unaltered TREM2-TYROBP interactions and subcellular localisation. However, synaptosomal uptake was significantly reduced in cells co-expressing mutant TYROBP. As these results could imply altered synaptic pruning with potentially life-long consequences, we examined mutant mice behaviour at 6 months of age and identified subtle impairments in males but not females compared with wild-type control animals. Together, our data reveal a role for TYROBP in postnatal brain development, with mild consequences on the behaviour of adult male mice.

id #21399

Rapid degeneration and neurochemical plasticity of the lateral geniculate nucleus following lesions of the primary visual

cortex in marmoset monkeys

Gaoyuan Ma[1], Jonathan M Chan[1], Katrina H Worthy[1], Marcello GP Rosa[1], Nafiseh Atapour[1]

1. Neuroscience Program, Biomedicine Discovery Institute and Department of Physiology, Monash University, Clayton, VIC, Australia

Lesions of the primary visual cortex (V1) cause retrograde neuronal degeneration, volume loss and neurochemical changes in the lateral geniculate nucleus (LGN). We characterised the timeline of these processes in adult marmoset monkeys, after various recovery times following unilateral V1 lesions. Observations in NeuN-stained sections obtained from animals with short recovery times (2, 3 or 15 days) showed that the volume and neuronal density in the LGN ipsilateral to the lesions were similar to those in the contralateral hemispheres. However, neuronal density in the retrograde degeneration zone of LGN dropped rapidly thereafter, with approximately 50% of the population lost within a month post-lesion. This level of neuronal loss remained stable for over three years post-lesion. In comparison, shrinkage of the LGN volume progressed more gradually, not reaching a stable value until 6 months post lesion. We also determined the time course of the expression of the calcium-binding protein calbindin (CB) in magnocellular (M) and parvocellular (P) layer neurons, a form of neurochemical plasticity previously reported to be triggered by V1 lesions. We found that CB expression could be detected in surviving M and P neurons as early as two weeks after lesion, with the percentage of neurons showing this neurochemical phenotype gradually increasing over 6 months. Thus, neurochemical change precedes neuronal degeneration, suggesting it may be linked to a protective mechanism. This study highlights the limited time window for any possible interventions aimed at reducing secondary neuronal loss in the visual afferent pathways following damage to V1.

id #21655

Making sense of a noisy world: How Does sensory variability account for motor noise?

Nicholas SC Price[1], Hoi MK Yip[1], Maureen A Hagan[1], Shaun Cloherty[2]

1. Monash University, Clayton, VIC, Australia

2. RMIT, Melbourne, VIC, Australia

It is largely unknown how sensory information represented by a neuronal population is transformed to generate motor responses. Ocular following is a useful model system for studying these sensory-motor transformations as it is a eye movement reflexively triggered by motion of a visual stimulus within less than 100 ms. Previous electrophysiological studies have shown that neurons in the middle temporal area (MT) represent sensory information critical for generating ocular following responses. However, single-neuron studies were not able to assess how neuronal variability correlates with behavioural variability on the level of single trials. We used Neuropixels probes to simultaneously record neurons (n = 29-139) in area MT of marmosets viewing visual motion stimuli that evoked tracking eye movements. We explored how well the population activity correlated with the timing and velocity of eye movements. We used partial-least-square regression (PLSR) to predict horizontal and vertical eye velocities from the population spiking rates on a trial-by-trial basis. For each trial, we extracted the mean firing rates of each neuron and mean eye velocities in 20 ms time windows relative to motion onset. The model started to predict eye velocities well 60 ms after stimulus motion onset (r = 0.58), when the eyes just started moving, and stayed high (r>0.72) in subsequent time windows (80-160 ms). Eye velocities at 60-160 ms were also well predicted by neural activity at 20-40 ms (r = 0.52). In conclusion, single-trial motor variability can be predicted from variations in sensory activity in the MT population activity.

id #21911

Functional abilities are associated with Alzheimer disease-related fluid biomarkers: A systematic review.

Joanne Scotney[2, 1], Michael Slee[2], Belinda Brown[2, 1, 3, 4], Stephanie Rainey-Smith[2, 3, 4, 5], Paul Maruff[6, 7], Ralph Martins[1, 3, 4, 8], Michael Weinborn[9], Hamid Sohrabi[2, 1, 3, 4]

1. Centre for Healthy Ageing, Health Futures Institute, Murdoch University, Perth, WA, Australia

2. School of Psychology, Murdoch University, Perth, WA, Australia

3. School of Medical and Health Sciences, Sarich Neuroscience Research Institute Edith Cowan University, Perth, WA, Australia

4. Australian Alzheimer's Research Foundation, Perth, WA, Australia

5. School of Psychological Science, The University of Western Australia, Perth, WA, Australia

6. The Florey Institute, University of Melbourne, Melbourne, VIC, Australia

7. CogState Ltd, Melbourne, VIC, Australia

8. Faculty of Medicine, Human and Health Sciences, Macquarie University, Sydney, NSW, Australia

9. School of Psychological Science, University of Western Australia, Perth, WA, Australia

Background: Alzheimer's disease (AD), the most common form of dementia, is a progressive neurodegenerative disorder marked by memory loss, functional impairments in activities of daily living (ADLs) and cognitive decline. By 2050, an estimated 153 million people globally will have AD. **Objective:** This systematic review aims to synthesise the literature to determine if the performance of ADLs is associated with changes in AD-related biomarkers across the AD spectrum. **Methods:** A comprehensive electronic database search across CINAHL, Cochrane, PsycINFO and PubMed to retrieve suitable publications. Five studies met inclusion criteria, including, but not limited to, cognitively unimpaired, mild cognitive impairment (MCI) or AD participants and using standardised scales to measure ADLs. Data on associations between fluid biomarkers (amyloid-beta, total tau, phosphorylated tau) and ADLs were extracted. **Results:** Significant associations were reported between cerebrospinal fluid (CSF) levels of amyloid-beta and tau proteins and a decline in ADLs. Lower baseline CSF amyloid-beta levels and higher baseline tau levels were consistently linked to worsening ADL abilities in MCI and AD participants. The findings also suggest that a decline in ADLs may occur independently of cognitive decline, particularly in individuals with MCI. **Conclusion:** This systematic review highlights the potential of AD-related fluid biomarkers as early indicators of declining functional abilities, which may aid in implementing targeted interventions to maintain independence for those at risk of AD. Future research should focus on standardised biomarker cut-offs and longitudinal studies to further elucidate the complex interplay between AD biomarkers and change in daily living activities outcome measures.

id #21912

Role of Connexin 36 in the Development of Neuropathic Pain

Yufei Xu[1], Christine Lu[1], Kelly O'Sullivan[1], David Spanswick[2], Wendy Imlach[1]

1. Department of Physiology, Monash University, Clayton, VIC, Australia

2. Neurosolutions Ltd, Coventry, United Kingdom

In contrast to acute pain, pathological chronic pain has no biological purpose or set endpoint and is often not well relieved by common analgesics. Changes that drive the development of chronic pain are mediated by complex interactions between neurons and glial cells. These cells rapidly communicate with each other through gap junctions, including Connexin36 (Cx36). In this study we investigated the role of Cx36 in pain signaling using Cx36 knockout mice. We generated a number of neuropathic pain models in Cx36 KO mice wild-type littermate controls, including sciatic nerve injury, chemotherapy-induced neuropathy, reserpine-induced fibromyalgia, and streptozotocin-induced diabetic neuropathy. Sensitivity to touch, heat and cold were tested. Data showed significant decreases in mechanical allodynia using a von Frey assay in the Cx36 KO mice in the weeks following pain development. Spinal cords were harvested for immunohistochemical labeling, which showed a decrease in microglial activation in the KO group. Electrophysiological analysis using dual whole-cell patch clamp recording techniques in spinal cord slices showed gap-junction-mediated electrical synaptic transmission and electrotonic coupling between neurons in wild-type mice was absent in Cx36 KO mice, electrotonic coupling driving synchronous firing and burst firing patterns of activity. This data suggests that targeting the Cx36 hemichannels may be an effective therapeutic option for the treatment of neuropathic pain conditions.

id #21658

Exploratory research into the effects of exercise, rhythm, and music on Central Auditory Processing Disorder in children.

Robyn Choi[1, 2, 3], Bonnie Furzer[1], Christopher Brennan-Jones[4, 2, 3], Ben Jackson[1, 2], Greta Edwards[1], Hadeel Y Tarawneh[1], Wilhelmina Mulders[1]

1. Human Sciences, The University of Western Australia , Crawley, WA, Australia

2. Telethon Kids Institute, The University of Western Australia , Crawley, WA, Australia

3. School of Allied Health, Curtin University, Perth, WA, Australia

4. Medical School, The University of Western Australia , Crawley, WA, Australia

Central auditory processing disorder (CAPD) refers to difficulties in the perceptual processing of auditory information in the central auditory nervous system. CAPD affects 3 to 7% of children in the general population and 9 to 15% of children in Aboriginal populations. CAPD can lead to social, emotional, mental health and academic problems, that can persist into adulthood. There are currently limited effective treatment options for CAPD and associated mental, social and academic development. Of options that do exist, many are costly, complex and do not treat co-occurring challenges, resulting in CAPD being left untreated. This study aimed to investigate whether a novel treatment using auditory cued exercise therapy (ACET) could improve auditory processing abilities in children with CAPD. ACET uses a custom designed rhythmic movement (exercise) to music to promote specific brain circuitry development, which could improve the auditory processing ability of a child while also addressing other co-morbidities such as mental health. Movement with music has been shown to improve communication, emotional regulation and temporal processing needed to understand speech and environmental sounds. Auditory-motor training has been shown to improve phonological skills important for reading and cognitive functions such as attention and memory. Exercise more generally has been shown to positively influence the brain and physical and mental health. ACET as CAPD treatment can engage children in a positive environment with benefits for physical and mental health. Preliminary analyses indicate that children show improvements in binaural integration, auditory closure and auditory word memory after attending an 8-week ACET program.

id #21914

Visualisation of cuprizone-induced copper depletion in mouse brain

Brittney Lins[1, 2, 3], Gaewyn Ellison[1, 4], Meg Willans[1, 4], David Paterson[5], Daryl Howard[5], Melinda Fitzgerald[1, 2], Mark Hackett[1, 4]

1. Curtin Health Innovation Research Institute, Curtin University, Bentley, WA, Australia

2. Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia

3. Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Crawley, WA, Australia

4. School of Molecular and Life Sciences, Curtin University, Bentley, WA, Australia

5. ANSTO-Australian Synchrotron, Monash University, Clayton, VIC, Australia

Cuprizone toxicity is a commonly used model of demyelination, however its modes of action are not known. Cuprizone is a copper chelator, so copper deficiency has been suggested to underlie the demyelination. Proof of cuprizone-induced brain-Cu deficiency, however, has remained elusive. Our aim was to directly visualise Cu distribution at micron spatial resolution with nM detection limits to determine if brain-copper levels are disturbed in the cuprizone model. C57BL/6 mice (male, 8-weeks age) were fed 0.2% w/w cuprizone-containing diet or control diet for 3 or 6 weeks (n=7/group) before brain tissue collection. Tissue was rapidly frozen in liquid nitrogen-cooled isopentane without perfusion or fixation. Cryosections (coronal plane, +1 mm bregma, 10-µm) were air-dried onto X-ray transparent substrate for elemental mapping with X-ray fluorescence microscopy (XFM) at ANSTO-Australian Synchrotron. XFM revealed brain-Cu content at 2 µm spatial resolution and 0.1 ng/cm^2 detection limits. 2-way ANOVA of myelin density (luxol fast blue) revealed pronounced cuprizone-induced demyelination ($P<0.0001$) in lateral corpus callosum (3-wk $p<0.0001$; 6-wk $p<0.0001$), medial corpus callosum (3-wk $p<0.0001$; 6-wk $p<0.0001$), lateral striatum white matter (3-wk $p<0.0001$; 6-wk $p<0.0001$), and medial striatum white matter (6-wk $p=0.0035$). One-way ANOVA revealed significant localised

reduction in copper in lateral cortex (6-wk p=0.0073), and striatum (3-wk p=0.0405; 6-wk p=0.0008), but not medial regions. This is the first-time brain-Cu has been directly visualised in the cuprizone demyelination model and provides evidence that cuprizone toxicity results in localized brain-Cu deficiency. These findings may lead to new insights into glial biology and demyelinating diseases.

id #21917

Incremental Value of Baseline Cognition, *APOE* ε4, and Amyloid-beta for Predicting Progression to Mild Cognitive Impairment in Cognitively Normal Older Adults

Bhargav Tallapragada[2, 1], Brook Galna[3, 1, 4], Rosita Shishegar[5, 6], Shaun J. Markovic[7], Belinda M. Brown[1, 8, 9], Simon M. Laws[8, 10], Jurgen Fripp[5], James D. Doecke[11], Colin L. Masters[12], Paul Maruff[13], Hamid R. Sohrabi[2, 1, 9]

1. Centre for Healthy Ageing, Health Futures Institute, Murdoch University, Perth, Western Australia, Australia

2. School of Psychology , Murdoch University, Perth, Western Australia, Australia

3. School of Allied Health (Exercise Science), Murdoch University, Perth, Western Australia, Australia

4. Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth, Western Australia, Australia

5. The Australian e-Health Research Centre, CSIRO, Melbourne, Victoria, Australia

6. School of Psychological Sciences and Turner Institute for Brain and Mental Health, Monash University, Melbourne, Victoria, Australia

7. Discipline of Psychology, Counselling and Criminology, Edith Cowan University, Joondalup, Western Australia, Australia

8. Centre for Precision Health, Edith Cowan University, Joondalup, Western Australia, Australia

9. Alzheimer's Research Australia, Sarich Neuroscience Research Institute, Nedlands, Western Australia, Australia

10. Curtin Medical School, Curtin University, Bentley, Western Australia, Australia

11. The Australian e-Health Research Centre, CSIRO, Brisbane, Western Australia, Australia

12. Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Melbourne, Western Australia, Australia

13. Cogstate, Cogstate Ltd, Melbourne, Victoria, Australia

Objective: Amyloid-beta (Aβ) deposition predicts cognitive decline in preclinical Alzheimer's disease; however, measuring Aβ levels is not always possible. In lieu of Aβ quantification, apolipoprotein (*APOE*) ε4 allele carriage and cognitive test scores, which are both associated with Aβ deposition, may be useful in the prediction of cognitive impairment. We aimed to evaluate the incremental value of cognition, *APOE* ε4, and baseline Aβ status to predict progression to mild cognitive impairment (MCI).

Methods: Baseline data of 1,964 cognitively unimpaired (CU) participants were obtained from the ADOPIC consortium. Cognitive composite scores were created for the domains of memory, executive function, and attention, derived from principal components analysis. We trained four random forest classifiers on 1,000 bootstrapped samples to predict progression to MCI at 8.35 years from baseline. The first uses age, sex, and education as predictors; the second adds the three cognitive composites; the third and fourth add *APOE* ε4 and baseline Aβ status, respectively. Performance was measured using ROC-AUC scores.

Results: At 8.35 years follow-up, 255 participants had progressed to MCI, while 1,709 remained CU (Table 1). Demographics as predictors resulted in a ROC-AUC of 0.57. Adding cognition, *APOE* ε4, and baseline Aβ status improved ROC-AUC scores to 0.68, 0.70, and 0.70 respectively (Figure 1). Although *APOE* ε4 carriage improved the model prediction, adding baseline Aβ status did not improve it further.

Conclusion: In the absence of biomarkers, neuropsychological assessment and demographic characteristics can provide an acceptable and accurate prediction of the future risk of MCI over 8.35 years.

id #21406

Paternal SARS-CoV-2 Infection Increases Anxiety in Offspring and Changes Sperm Small Noncoding RNA Profiles

Elizabeth A Kleeman[1], Carolina Gubert[1], Sonali N Reisinger[1], Kathryn C Davidson[2], Merle Dayton[2], Liana Mackiewicz[2], Bethany A Masson[1], Pranav Adithya[1], Alexandra Garnham[2], Shanshan Li[1], Huan Liao[1], Shae McLaughlin[1], Michelle Y Wheeler[1], Pamudika Kiridena[1], Marcel Doerflinger[2], Marc Pellegrini[2], Anthony J Hannan[1, 3]

1. The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Melbourne, Victoria, Australia

2. The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

3. Department of Anatomy and Physiology, The University of Melbourne, Melbourne, Victoria, Australia

Publish consent withheld

id #21918

Altered post stress coping and reward seeking behaviours in chronically stressed mice

Laura Stanton[1], Nick Burton[1], Fay Waldrip[1], Jakob Slack-Smith[1], Jacqueline Iredale[1], Erin Campbell[1], Lizzie Manning[1], Christopher Dayas[1]

1. University of Newcastle, Callaghan, NSW, Australia

Increasing evidence links chronic stress to the development of neuropsychiatric conditions such as depression. Maladaptive stress-coping behaviours (e.g. avoidance) and dysfunctional reward seeking behaviours (e.g. anhedonia) are often observed in depression. We aimed to investigate these stress- and reward-related behaviours in a rodent model of depression, to facilitate studies of underlying neural mechanisms.

The Unpredictable Chronic Mild Stress (UCMS) model was used, where mice are exposed to 2-4 mild stressors daily for 5 weeks to induce a depressive-like phenotype. Behaviour responses following acute stress and palatable reward consumption were measured in separate sessions.

Post-stress, UCMS exposed mice showed a significant increase in digging ($p<0.0001$) and a significant decrease in rearing ($p<0.0001$), jumps ($p<0.001$), and escapes ($p<0.001$). This suggests the UCMS mice exhibited more passive stress coping than controls. In the palatable food test, UCMS mice displayed a significantly higher number of eating bouts ($p<0.05$) and increased eating duration ($p<0.05$) compared to controls. This disturbed eating behaviour possibly suggests that chronic stress induces anxiety-like behaviours even when eating a palatable reward.

Establishing the use of acute footshock induced behaviours following UCMS to show changes in stress coping, in addition to assessing reward-related behaviours during palatable food exposure, provides a framework for future studies. Future studies will combine the use of UCMS and these behaviour assessments with approaches such as fibre photometry to investigate the specific circuitry underlying stress-induced depression and how its affected by novel treatments.

id #21663

Mitigation of Oxidative Stress-Induced Damage in SH-SY5Y Cells by Two Aqueous Phytoextracts

Okobi E Ekpo[1], Megan L Petersen[2], Sylvester I Omoruyi[3], Gail Hughes[4]

1. Biological Sciences, Khalifa University, Abu Dhabi, United Arab Emirates

2. Division of Clinical Anatomy and Biology Anthropology, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

3. School of Anatomical Sciences, University of the Witwatersrand, Johannesburg, South Africa

4. Department of Medical Biosciences, University of the Western Cape, Cape Town, South Africa

Exposure to hydrogen peroxide (H_2O_2) is a well-established method for inducing oxidative stress (OS) in cellular models, mainly through the generation of reactive oxygen species (ROS). There is evidence in the literature of a strong association between the production of free radicals and neurodegenerative diseases. Plant-derived natural products have been considered important sources of biologically active compounds, most of which possess strong antioxidant, anti-inflammatory, anti-cancer, anti-bacterial, and anti-viral properties. There are limited studies in the literature on the neuroprotective potentials of phytoextracts from two botanicals with well-known health benefits, *Sutherlandia frutescens (SF)* and *Carpobrotus edulis (CE)*, hence this study investigated the possibility of the aqueous extracts (SF_e and CE_e) of both these botanicals to attenuate possible hydrogen peroxide (H_2O_2)-induced physiochemical and bioenergetic damage in SH-SY5Y cells. The cells were pre-treated for 24 hours with the maximum non-toxic concentrations (MNTC) of SF_e and CE_e respectively, after which 250 µM of H_2O_2 was added, and the cells incubated for 3 hours followed by assays to evaluate cell viability, caspase-9 activity, nitric oxide activity, intracellular calcium, and ROS levels, superoxide dismutase and catalase activity, as well as mitochondrial membrane permeability. The results showed that the cellular dysfunction caused by H_2O_2 in the SH-SY5Y cells was significantly attenuated by pre-treatment with both SF_e and CE_e. The observed benefits of these treatments could be attributed to the synergistic effects of the phytocompounds in the phytoextracts. Further *in vitro* and *in vivo* investigations of these compounds are recommended.

id #21919

Role and therapeutic targeting of *NFIX* in the neurodevelopmental Malan syndrome

Isabella Trew[1, 2], Craig McIntosh[3], Steve Wilton[1, 2], Jessica Cale[1, 2], May Aung-Htut[1, 2]

1. Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Murdoch, WA, Australia

2. Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia

3. n-Lorem Foundation, San Diego, CA, USA

Malan syndrome is a rare neurodevelopmental disorder characterised by macrocephaly, developmental delay, intellectual disability and seizures. Symptoms typically emerge in childhood and there are no treatments currently available. Causative *de novo* mutations have been identified in the Nuclear factor I X-type (*NFIX)* gene, particularly exons 1-3, that confer haploinsufficiency. *NFIX* encodes a transcription factor critically associated with proper neuromuscular development; although, exact mechanisms are not fully elucidated. We utilised molecular techniques including RT-PCR, qPCR, Sanger sequencing, Western blotting and RNA-sequencing to characterise the mutations in 5 patient-derived fibroblast lines and analysed differences in their transcriptomic profiles compared to unaffected individuals. Results identified several altered biological processes, metabolic pathways and downstream targets. Additionally, we identified complex splicing mutations beyond those originally genotyped. Our findings provide significant insight into the extensive roles of *NFIX* and potential avenues for therapeutic development. In fact, this data is currently informing the refinement of *NFIX*-targeting antisense oligonucleotides. We utilised the neuroblastoma cell line, SH-SY5Y, to screen 44 antisense oligonucleotides and evaluated their efficacy at both RNA and protein level by qPCR and Western blotting. Furthermore, we are developing patient-derived neuronal stem cell models of Malan syndrome, the first of knowledge. These disease-relevant models will be used to validate our top therapeutic candidates and serve as a platform to explore mechanisms of Malan syndrome throughout neural development, an area yet to be fully understood. Overall, this project provides significant insight into the mechanisms behind the causative gene of Malan syndrome, *NFIX*, and

approaches for its therapeutic targeting.

id #21921

Circuits underlying hyperexcitability and epilepsy in the piriform cortex

Muqin Zhang[1]

1. The Eccles Institute of Neuroscience, The Australian National University, Canberra, ACT, Australia

Disinhibition – characterized by a reduction in network inhibition via interactions between inhibitory interneurons that increases the excitability of principal neurons – has been proposed as an important factor in numerous neurological diseases, including epilepsy. Electrical connections via gap junctions between interneurons can affect the synchronisation of neuronal firing. Thus, gap junctions are also likely to play a role in epilepsy.

HCN channels are widely expressed in the central nervous system, and mounting evidence indicates their functional involvement in human epilepsies (Hung et al., 2021, Bleakley et al., 2021, Shah, 2014). Of the four known isoforms, the HCN1 subtype appears to have the strongest association with epilepsy, with several de novo variants reported in patients with developmental and epileptic encephalopathy (Hung et al., 2021, Bleakley et al., 2021, Bonzanni et al., 2018).

The piriform cortex, a three-layered primary olfactory cortex, has long been recognised as a highly epileptogenic site. However, the connections between inhibitory interneurons in the piriform cortex are still unclear. Hence, this project aimed to: 1) investigate the chemical and electrical synaptic connections between specific subtypes of inhibitory interneurons in the piriform cortex using dual whole-cell recordings, and 2) explore the functional properties of these interneurons in the piriform cortex, utilizing a transgenic mouse model with mutant HCN1 channels.

id #21922

AUS-mTBI: designing and implementing novel health informatics approaches to improve outcomes for people with mild TBI across Australia

Jemma Keeves[1, 2, 3], **Rachel Singer**[2], **Sarah Hellewell**[2, 3], **Natasha Lannin**[4, 5], **Karen Caeyenberghs**[6], **Elena Chebkasova**[7, 8, 9, 10], **Michael Makdissi**[11, 12, 13], **Gary Mitchell**[14, 15, 16, 17], **Karen Barlow**[18, 19], **Nick Rushworth**[20], **John Gilroy**[21], **Elizabeth Armstrong**[22], **Trish Ratajczak**[23], **Kirsten Vallmuur**[24, 15], **Patrick Kwan**[1, 25, 5], **George Charalambous**[26], **Jennie Ponsford**[27, 28], **Belinda Gabbe**[1], **Vicki Anderson**[29, 30], **Melinda Fitzgerald**[2, 3]

1. Pre-Hospital, Emergency and Trauma, School of Public Health and Preventative Medicine , Monash University, Melbourne, Victoria, Australia
2. Curtin University, Perth, WA, Australia
3. Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia
4. Alfred Health, Melbourne, VIC, Australia
5. Department of Neurosciences, Central Clinical School, Monash University, Melbourne, VIC, Australia
6. Cognitive Neuroscience Unit, School of Psychology, Deakin University, Melbourne, VIC, Australia
7. Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia
8. Centre for Adolescent Health, Murdoch Children's Research Institute, Parkville, VIC, Australia
9. National Drug Research Institute, Curtin University, Perth, WA, Australia
10. School of Population and Global Health, University of Western Australia, Perth, WA, Australia
11. Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, Australia
12. Latrobe University, Melbourne, VIC , Australia
13. Australian Football League, Melbourne, VIC, Australia
14. Emergency and Trauma Unit, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia
15. Jamieson Trauma Institute, Brisbane, QLD, Australia
16. Royal Brisbane Clinical Unit, University of Queensland, Br, QLD, Australia
17. Queenland Rubgy Union, Brisbane, QLD
18. Queensland Children's Hospital, Brisbane, QLD, Australia
19. Centre for Children's Health Research, University of Queensland, Brisbane, QLD, Australia
20. Brain Injury Australia, Sydney, NSW, Australia
21. Aboriginal and Torres Strait Islander Research, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia
22. School of Medical and Health Sciences, Edith Cowan University, Perth, WA, Australia
23. Murdoch University, Perth, WA, Australia
24. Centre for Healthcare Transformation, Australian Centre for Health Services Innovation, Queensland University of Technology, Brisbane, QLD, Australia
25. Department of Neurology, Alfred Health, Melbourne, VIC, Australia
26. Curve Tomorrow, Melbourne, VIC, Australia
27. Turner Institute for Brain and Mental Health, School of Psychological Sciences, Monash University, Melbourne, VIC, Australia
28. Monash Epworth Rehabilitation Research Centre, Epworth Healthcare, Melbourne, VIC, Australia
29. Clinical Sciences Research, Murdoch Children's Research Institute, Melbourne, VIC, Australia
30. Psychology Service, The Royal Children's Hospital, Melbourne, VIC, Australia

In Australia, there are approximately 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. The AUS-mTBI national consortium has established a multi-faceted online platform that uses web-based app technology to gather data to aid reliable prediction of outcome and improved management of mTBI. AUS-mTBI aims to recruit 5000 people who have experienced mTBI in the community. Self-reported demographic, injury circumstance, health status, mTBI symptomatology and care management data will be collected for all participants. The presence of persistent post-concussion symptoms will be assessed fortnightly for 3 months after injury and then monthly until symptom resolution using the Rivermead Post-Concussion Questionnaire (adults), or the Post-Concussion Symptom Inventory (< 18 years). Through the online platforms, participants will also be provided with an evidence-based recovery program, personalised to their own circumstances, and be able to track their symptom changes over time. AUS-mTBI is the first study of its kind in the world to integrate best practice, cutting edge research and patient recovery experiences for people with mTBI. The data will be used to inform our understanding of predictors of recovery after mTBI and use machine learning models to predict those at risk of poor outcome and enhance care pathways to reduce the public health burden of mTBI.

id #21923

Characterizing the role of movement in ventromedial striatal dopamine signals related to reward

Eugenia Z Poh[1, 2], Gino Hulshof[1, 2], Lucie Mazé[1, 2], Ingo Willuhn[1, 2]

1. Neuromodulation and Behavior, Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Sciences, Amsterdam, Netherlands

2. Department of Psychiatry, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

Situation-appropriate action initiation and suppression are essential behavioural functions. In particular, striatal dopamine signalling is critical for motor control and encoding reward-related information. Using a go/no-go task reflecting action initiation and suppression, a previous study elegantly showed the necessity of movement for the expression of reward-related dopamine release in the ventromedial striatum (VMS). However, action requirements for 'go' and 'no-go' trials can vary within and between animal and human studies, and self-initiation versus cued-initiation has been shown to alter dopamine activity. Here, we studied VMS dopamine release during variations of the go/no-go task. Rats were implanted with microelectrodes to measure real-time dopamine dynamics using fast-scan cyclic voltammetry and were instructed by auditory cues to initiate (‘go’), suppress (‘no-go’), or express no overt (‘free’) action to earn food rewards. In freely-reward trials, rats could immediately walk to the food magazine, a condition more akin to human 'no-go' trials. During self-initiated trials, we observed an increase in dopamine release contingent on action initiation but not when the trial was cued. Increased dopamine release was largest during freely-rewarded trials as compared to the other trial types, peaking prior to magazine entry. Unexpectedly, rats exhibited different behavioural strategies for no-go trials, complemented by differences in dopamine release dynamics. Our results suggest that striatal dopamine signalling is not only altered by the action requirements to obtain a reward but the ability to self-initiate its pursuit.

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id #21669

Role of Butyrate in the expression of Interleukin 4,6,8 in Alzheimer’s disease

R.M. Uththara Senarath[1, 2], Lotta Oikari[3], Prashant Bharadwaj[1, 2], Vijay Jayasena[4], Juliana Cristina da Silva Chaves[3], Ralph Martins[1, 2, 5, 6], Binosha fernando[1, 2]

1. School of Medical and Health Sciences, Edith Cowan University, Joondalup, WA, Australia

2. Australian Alzheimer’s Research Foundation, Ralph and Patricia Sarich Neuroscience Research Institute, Nedlands, WA, Australia

3. Cellular and Molecular Neurodegeneration, , QIMR Berghofer Medical Research Institute, Brisbaine, QLD, Australia

4. Nutrition and Food Science, School of Science, Western Sydney University, Penrith , NSW, Australia

5. Lions Alzheimer’s Foundation, Nedlands, WA, Australia

6. Department of Biomedical Sciences, Macquarie University, Sydney, NSW, Australia

BackgroundIn Alzheimer's Disease (AD), astrocytes become reactive, which can both protect and harm neurons, contributing to the complex pathology of the disease; research often focuses on how astrocyte dysfunction and neuroinflammation contribute to neuronal damage and cognitive impairment, aiming to find potential therapeutic targets. Butyrate, a short-chain fatty acid produced by gut microbiota, has emerged as a potential therapeutic agent due to its anti-inflammatory properties. This study explores the impact of butyrate on the expression of interleukins IL-4, IL-6, and IL-8 in AD-affected astrocytes and neurons.

MethodologySynthetic Aβ (20μM) and butyrate (100μM) were administered to cultures of astrocytes, neurons and spontaneous cultures derived from iPSCs obtained from a healthy human. Inflammatory marker IL-4,6,8 of these cells was measured using Quantitative PCR and quantified using Legend Plex Human Inflammatory Panel. Statistical analysis was performed using GraphPad Prism version 10.1.2, and one-way ANOVA.

ResultsWhen Aβ-induced cells were treated with butyrate, they exhibited higher expression of IL-8, with a significant increase observed in astrocytes ($P<0.05$). In contrast, IL-6 expression was low in these cells, with a significant difference noted in neurons and spontaneous cultures. However, IL-4 did not show a significant increase in expression, though there was a tendency for higher expression across all cell types.

ConclusionsThe role of butyrate in modulating the expression of IL-4, IL-6, and IL-8 in AD astrocytes underscores its potential as a therapeutic agent. By selectively influencing cytokine expression, butyrate may contribute to altering the neuroinflammatory milieu in AD. Further research is needed to elucidate this.

id #21927

Using the live rat stress paradigm to investigate approach-avoidance behaviour under threat

Jacqueline A Iredale[1, 2], Fay J Waldrip[1, 2], Jakob A Slack-Smith[1, 2], Zane A Andrews[3], Erin J Campbell[1, 2], Christopher V Dayas[1, 2], Lizzie E Manning[1, 2]

1. University of Newcastle, Callaghan, NSW, Australia

2. Brain Neuromodulation Research Program, Hunter Medical Research Institute, New Lambton, NSW, Australia

3. Monash Biomedicine Discovery Institute and Department of Physiology, Monash University, Clayton, VIC, Australia

Hunger is a key physiological behaviour and approach-avoidance feeding behaviour is known to be modified by threat. Animals undertake riskier behaviour to obtain food when it is scarce. To examine food approach-avoidance behaviour under threat, we utilised the live rat stress paradigm. C57BL/6 mice had free access, or no access, to food for >16hrs prior to testing and were placed in an arena divided into mouse (40x40cm) and rat (20x40cm) areas, separated by a transparent, perforated wall. The mouse side of the arena included a 'hide' space, diagonally to which was an automated pellet dispensing device (FED), dispensing sucrose pellets on a free-feeding schedule, located adjacent to the rat wall. Results indicated fasted animals spent significantly more time near the FED and subsequently near the live rat compared to non-fasted animals (41.19±5.70 seconds vs. 18.37±1.94 seconds; $p<0.01$) and consumed significantly more pellets (15.96±1.87 pellets vs. 3.08±0.79 pellets; $p<0.001$). Comparatively, fasted animals exposed to the live rat spent significantly less time near the FED than those exposed to the control 3D-printed rat (41.19±5.70 seconds vs. 69.74±4.65 seconds; $p<0.0001$). Future studies will aim to examine the brain pathways involved in food approach behaviour under threat. We will utilise *in vivo* calcium imaging, paired with the live rat stress paradigm, and investigate connections between the hypothalamic regions underlying threat dependant changes in feeding behaviour, and the effect of hunger on these. This future research may identify the role of hypothalamic circuits in modulating reward-seeking in a risky environment.

id #21160

Polyphenols from *Sorghum bicolor* Suppress Tauopathies: Evidence from *In Silico* and In *Vivo* Studies

Rasheed A Abdulraheem[1, 2], Prashant Bharadwaj[1, 2], Ralph N Martins[1, 2, 3], Zhaoyu Li[4], Ranil Coorey[5], Stuart Johnson[6], Binosha fernando[1, 2]

1. Edith Cowan University, Perth, WA, Australia

2. Alzheimer's Research Australia, Perth, WA, Australia

3. Faculty of Medicine, Human and Health Sciences, Macquarie University, Sydney, New South Wales, Australia

4. Queensland Brain Institute, University of Queensland, Brisbane, Queensland, Australia

5. School of Molecular and Life Sciences, Curtin University, Perth, WA, Australia

6. Ingredients by Design Pty Ltd, Perth, WA, Australia

Polyphenols, abundant in plant sources, have antioxidant and anti-inflammatory properties, which may benefit neurological health. *Sorghum bicolor*, a cereal grain rich in polyphenols, presents promising therapeutic candidate for tauopathies. Studies have suggested that polyphenols inhibit tau protein aggregation, potentially mitigating the progression of neurodegeneration.

Objective: In this context, our study investigates the inhibitory effects of polyphenol extracts from *Sorghum bicolor* on tauopathies using computational molecular docking simulations and a validated *Caenorhabditis elegans* model of tau aggregation. By integrating *in silico* predictions with *in vivo* validation, we elucidate the therapeutic potential of *Sorghum bicolor* polyphenols in combating tau-related neurodegenerative diseases.

Method: Polyphenols identified from *Sorghum bicolor* were docked against tau protein (503L PHF in Alzheimer's brain) using MCULE, RCSB Protein Data Bank and Discovery Studio Analyser. We evaluated the effects of these polyphenol extracts on tau-induced toxicity in *Caenorhabditis elegans* through body movements, chemosensory, and lifespan assays.

Results: The polyphenols exhibited strong binding affinities with specific amino acid residues (Valine, Glutamic Acid, and Isoleucine) of the tau protein, as identified by consensus scoring methods and binding pockets from docking poses. These interactions involved hydrogen bonds that potentially inhibit the formation of PHF. At a concentration of 500 µg/mL, the polyphenol extracts improved the healthspan and extended lifespan of the tau model of *Caenorhabditis elegans* by two days compared to controls.

Conclusions: Our findings suggest that *Sorghum bicolor* polyphenols hold promise as a complementary therapy for tau-associated neurodegenerative diseases, particularly Alzheimer's disease. However, further investigation is warranted to elucidate the underlying mechanisms.

id #21928

Diphtheria toxin mediated microglia depletion in CD11b-DTR mice

Jessica JI Irving[1, 2], Peggy PR Rentsch[1, 2], Bryce BV Vissel[1, 2]

1. University of New South Wales, Sydney

2. St Vincent's Centre for Applied Medical Research, Sydney

The CD11b-diphtheria toxin receptor (DTR) mouse has been widely used to study the role of microglia and macrophages in disease. By taking advantage of the insensitivity of murine cells to diphtheria toxin (DT), transgenic expression of the DTR renders CD11b-expressing cells DT sensitive, leading to toxin-induced apoptosis. Despite use of these mice across the literature, we found DT treatment caused high mortality and behavioural deficits in naive CD11b-DTR and wild-type mice, highlighting the need for DTR mouse line characterisation and consideration of potential side-effects, before use in disease models.

In this study, CD11b-DTR and C57BL/6 mice were implanted with a cannula into the lateral ventricles and allowed to recover for two-weeks. Mice were then infused with differing doses of DT for up to 10 days. Throughout the treatment period, mice underwent the open field test, rotarod and Y-maze to assess motor function and memory. Blood testing and histological analysis are being conducted, and CD11b depletion efficiency in the transgenic mice will be quantified in the midbrain, striatum and hippocampus.

We found CD11b-DTR mortality sensitive to all doses of DT, and observed dose-dependent survival in wild-type mice. Unexpectedly, wild-type DT treated mice exhibited severe loss of motor function and mild impairment of memory, and DT severely affected CD11b-DTR mice performance across all doses in the Y-maze and rotarod. Taken together, our results suggest possible shortcomings of the DT-mediated ablation system as wild-type mice are non-specifically effected by DT, reiterating the importance of employing appropriate controls in studies using transgenic DTR mice.

id #21419

Design and Development of Choline Acetyltransferase Fluorescence Imaging Probes

Christine Law[1], Keng Yoon Yeong[1]

1. Monash University Malaysia, Subang Jaya, SELANGOR, Malaysia

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which commonly affects the elderly. Despite the arduous effort in developing an effecting treatment for AD, there is still no cure for this disease. Treatment at early stages of AD has been postulated to deliver better pathological benefits as neuropathological changes start taking place about two decades prior to the appearance of clinical symptoms. Bioimaging has been proposed to be a better alternative to the conventional behavior-based clinical diagnosis. The attractive advantages of fluorescence imaging, such as its non-radioactive nature, have made fluorescent probes an indispensable tool for bioimaging. This study aims to develop novel choline acetyltransferase (ChAT) ligands which can be used as fluorescence imaging probes for early-AD detection. ChAT is the enzyme that catalyzes the biosynthesis of acetylcholine neurotransmitter, and acetylcholine deficiency manifests as cognitive and non-cognitive dysfunctions. Hence, its activity can be used to document the health of cholinergic neurons. Following *in silico* structure-based screening of a known chemical database, 20 compounds were developed and structurally characterized. **N1-DMA** has been identified as the lead compound, demonstrating high binding affinity towards ChAT (K_i = 1.89 μM), selectivity over anti-targets; acetylcholinesterase and butyrylcholinesterase (>14-fold), intrinsic fluorescence (ϕ = 59%), no significant neurotoxicity towards SH-SY5Y cells (at 50 μM), predicted blood-brain barrier permeation and cellular binding to target protein ChAT. As one of the most potent ChAT ligands reported to date, these favorable characteristics of **N1-DMA** highlight its potential to be developed into a fluorescence imaging probe for early-AD detection.

id #21931

Dimension of visual information interacts with working memory in monkeys and humans

Daniel J Fehring[1], Alexander Pascoe[1], Zakia Z Haque[1], Ranshi Samandra[1], Seiichirou Yukoo[2], Marcello Rosa[1], Keiji Tanaka[2], Tetsuo Yamamori[2], Farshad A Mansouri[1]

1. Department of Physiology, Monash University, Melbourne, VIC, Australia

2. RIKEN Centre for Brain Science, Tokyo, Japan

Humans demonstrate behavioural advantages (biases) towards particular dimensions (colour or shape of visual objects), but such biases are significantly altered in neuropsychological disorders. Recent studies have shown that lesions in the prefrontal cortex do not abolish dimensional biases, and therefore suggest that such biases might not depend on top-down prefrontal-mediated attention and instead emerge as bottom-up processing advantages. We hypothesised that if dimensional biases merely emerge from an enhancement of object features, the presence of visual objects would be necessary for the manifestation of dimensional biases. In a specifically-designed working memory task, in which macaque monkeys and humans performed matching based on the object memory rather than the actual object, we found significant dimensional biases in both species, which appeared as a shorter response time and higher accuracy in the preferred dimension (colour and shape dimension in humans and monkeys, respectively). Moreover, the mnemonic demands of the task influenced the magnitude of dimensional bias. Our findings in two primate species indicate that the dichotomy of top-down and bottom-up processing does not fully explain the emergence of dimensional biases. Instead, dimensional biases may emerge when processed information regarding visual object features interact with mnemonic and executive functions to guide goal-directed behaviour.

id #21933

A spatially resolved single-nucleus multi-omics approach to understand how stress contributes to psychiatric disorders

Tamim Ahsan[1, 2, 3, 4], Dominic Kaul[1, 2, 3], Amber Curry[1, 2, 3], Katrina Edmond[1, 2, 3], Nathalie Gerstner[5, 4, 6], Anna Fröhlich[5, 4, 6], Michael Ziller[7, 8], Elisabeth Binder[4, 9], Lezanne Ooi[3], Janine Knauer-Arloth[4, 6], Natalie Matosin[1, 2, 3]

1. Charles Perkins Centre, University of Sydney, Sydney, New South Wales, Australia

2. School of Medical Sciences, University of Sydney, Sydney, New South Wales, Australia

3. School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, New South Wales, Australia

4. Department Genes and Environment, Max Planck Institute of Psychiatry, München, Germany

5. International Max Planck Research School for Translational Psychiatry, München, Germany

6. Institute of Computational Biology, Helmholtz Zentrum München, Neuherberg, Germany

7. Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, München, Germany

8. Department of Psychiatry, University of Münster, Münster, Germany

9. Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, USA

Stress is a major risk factor for psychiatric disorders. Understanding how stress raises risks of psychopathology is essential for identifying new treatments. Here, we examined human postmortem orbitofrontal cortex (BA11) samples to unravel stress-induced cell-type-specific transcriptomic and epigenomic changes in psychiatric disorders and their spatial distribution. We examined 86 individuals (n = 32 controls vs n=54 cases with mixed major psychiatric disorders: schizophrenia, major depression and bipolar disorder) with or without history of profound psychological stress exposure (n=23 with severe stress and n=31 without). After filtering out low-quality nuclei from single-nucleus RNA sequencing data, about ~800,000 nuclei from 15 different cell types were retained (4 excitatory neuronal subtypes, 5 inhibitory neuronal subtypes, 2 astrocyte subtypes, oligodendrocytes, oligodendrocyte precursor cells, microglia and endothelial cells). Compositional analysis revealed that the proportions of inhibitory neurons and astrocytes were significantly lower in individuals exposed to stress vs those without stress, while the proportion of oligodendrocytes was higher (Log2FoldChange of -0.51, -1.02 and 0.33, respectively). Although we identified 37 and 0 differentially expressed genes (DEGs) respectively in oligodendrocytes and reelin-expressing inhibitory neurons when all cases were compared against the controls, the numbers rose to 160 and 265, respectively, when comparisons were made among the cases based on their history of stress exposure. These DEGs showed significant enrichment of metabolic processes and phenotypes involved in psychopathology. The results highlight striking cell-type-specific impacts of psychological stress in the orbitofrontal cortex of individuals with psychiatric disorders, and experiments and analyses are in progress to validate these findings.

id #21934

Generation of induced pluripotent stem cells (iPSCs) from Monotremes

Florian Walter[1], Elly Walters[2, 3], Fillip Pajpach[2], Frank Grützner[2], Jose Polo[2, 3], David Keays[1]

1. Ludwig-Maximilians-Universität München, Munich, BAVARIA, Germany

2. The University of Adelaide, Adelaide

3. Monash University, Melbourne

Monotremes are remarkable animals, a mosaic of anatomical and physiological features that reflect reptilian, avian, and mammalian traits. Given their inaccessibility, little is known of the molecular and cellular mechanisms that underlie their neurodevelopment. Strikingly the Platypus has a lissencephalic neocortex, whereas its closest relative the Echidna has a large gyrified brain. We hypothesize that this is due to a difference in the number, type, or output of neuronal progenitors. As primary tissue is not available to test this hypothesis, we will exploit advances in induced pluripotent stem cell (iPSC) generation and cerebral organoid technology. Specifically, the aims are to: (1) Generate induced pluripotent stem cells derived from the platypus and echidna; (2) Model monotreme brain development using cerebral organoids; and (3) Characterize these organoids with a focus on the proliferative ventricular zone. This characterization will employ single-cell sequencing as well as traditional histological analysis. By leveraging these state-of-the-art methods, we will shed light on conserved mechanisms underpinning gyration in humans, as well as their divergence in monotremes.

id #21423

Chronic traumatic encephalopathy in Australia: six years of the Australian Sports Brain Bank

Andrew J Affleck[1, 2], Catherine M Suter[1, 2], Monica A Clarke[1, 2], Alan J Pearce[3], Michael E Buckland[1, 2]

1. Brain and Mind Centre, University of Sydney, Camperdown, NSW, Australia

2. Department of Neuropathology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

3. School of Health Science, Swinburne University of Technology, Hawthorn, VIC, Australia

Chronic traumatic encephalopathy (CTE) is a degenerative brain disease characterised by the abnormal accumulation of hyperphosphorylated tau around blood vessels in the sulcal depths of the cerebral cortex. CTE is almost exclusively seen in individuals with a history of repetitive head injury (RHI) and is associated with a range of cognitive, behavioural and psychological symptoms experienced during life.

The Australian Sports Brain Bank (ASBB) was established in 2018 to identify the prevalence of CTE in at risk Australians and to support research to understand CTE further. In 2021, we reported some outcomes of the first three years of the ASBB. Here, a further three years on, we provide an update.

Brain donations have increased from twenty-one brains donated in 2021 to ninety-one to date. The prevalence of CTE has reduced from 57% (12 CTE cases out of 21) in 2021 to 50% (45 CTE cases out of 91) currently. RHI remains the greatest risk factor for CTE. In 2022 we found the first case of CTE in a female ex-professional athlete and serving defence force member. CTE is still being detected in younger individuals with death occurring on average 10 years earlier in those with CTE. Though suicide is a complex issue, a notable similarity since 2021 is the high proportion of individuals with CTE who died due to suicide (42%) compared to those without CTE (17%).

This latest update on CTE reiterates the need for and importance of brain donation to underpin current and future CTE research.

id #21935

Morphological and functional alterations of Müller cells during early Diabetic Retinopathy

Mayu Aburaya[1], Erica Fletcher[1], Andrew Jobling[1], Una Greferath[1]

1. University of Melbourne, Melbourne, VICTORIA, Australia

Diabetic retinopathy (DR) is one of the most feared complications of diabetes and a leading cause of visual impairment. One of the common causes of vision loss with DR is diabetic macular edema (DME), characterised by swelling within the central macula. Müller cells, specialised macroglia within the retina, regulate ions and subsequent water movement via the inwardly rectifying potassium channel 4.1 (Kir4.1) and aquaporin 4 (AQP4). Here, we evaluated the role of Müller cells in the development of swelling in a mouse model of type I diabetes. Müller cell-specific nestin-cre transgenic mice were rendered diabetic after injection of streptozotocin (STZ group, n = 6, 50 mg/kg, blood glucose: >15mmol/L) or citrate buffer (control group, n = 6). Following 12 weeks of diabetes, retinas were imaged, and Müller cell volume was quantified using Imaris. Changes in the gene expression of *Kcnj10* (Kir4.1) and *Aqp4* were quantified using qPCR. The effect of $BaCl_2$ (100 µmol/l), VU0134992 (2 µmol/l) and inflammatory mediators on Müller cell volume was also quantified using *ex vivo* retinal explants. We observed a significantly increased soma volume in the retinae of 12-week STZ mice compared to the control groups, however, gene expressions of *Kcnj10* and *Aqp4* were unchanged. An increase in Müller cell soma volume was observed in *ex vivo* retinas incubated with $BaCl_2$, VU0134992, and inflammatory molecules, including LPS and TNF-α. These results suggest that diabetes is associated with increased swelling of Müller cells and that inflammatory mediators may be important regulators of this effect.

id #21424

Evaluating mineralocorticoid receptor modulators and gene variants on microglial function

Amy O'Keeffe[1], Marnie Petrucci[1], Ayeisha Milligan Armstrong[1], David Groth[1], Giuseppe Verdile[1, 2]

1. Curtin Medical School and Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia, Australia

2. School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia

Chronic stress has been associated with promoting neurodegenerative diseases, such as Alzheimer's disease (AD), through dysregulating the Hypothalamic-Pituitary-Adrenal (HPA) axis and increasing cortisol levels. The cellular action of cortisol is mediated by the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), with MR being involved in setting the HPA-axis activation threshold. Studies have revealed the importance of the MR in neuronal cells. However, little is known about its role in microglia which are thought to become maladaptive in AD. Thus, the project aims to 1) assess the impact of modulating MR activity on microglia function and 2) to evaluate effect of MR haplotypes that are known to alter MR activation. Human microglia cell-line (HMC3) were treated with MR agonist, Fludrocortisone, or MR antagonist, Spironolactone and cell survival, gene expression and inflammatory responses were assessed. These molecules modulated microglial MR activity and led to differential expression of several genes, including some related to stress responses, neuroinflammation, and AD. To confirm a major role for the MR in microglia function, current work is assessing the effects of combinations of MR and GR modulators and establishing HMC3 models of MR haplotypes prior to undertaking morphology, phagocytosis, migration, and cytokine production analyses. Overall, the findings from this study will provide novel insights into the role of the MR in microglial function and have implications in targeting this receptor in neurodegenerative diseases.

id #22192

Animals in Research Committee

Nicholas Price[1]

1. Monash University, Australia

ANS Animals in Research Committee

id #21938

Anterograde trafficking of the BACE1 substrate Seizure-related Protein 6 in neurons

Alina Krumme[1], Kye Kudo[1, 2], Victoria Li[1], Shepard HE[1], Jenny Gunnersen[1], Lou Fourriere-Chea[1], Kathryn Munro[1]

1. The University of Melbourne, Parkville, VIC, Australia

2. Queensland Brain Institute, Brisbane, QLD, Australia

Seizure-related Protein 6 (Sez6) is involved in neuronal development and synaptic function. Sez6 is cleaved near its transmembrane region by the protease BACE1, producing a soluble protein (sSez6). This shed sSez6 is detected in cerebrospinal fluid (CSF) and sSez6 CSF levels are altered in multiple neurological diseases and disorders. We aim to understand the cellular processes that affect sSez6 shedding from neurons, in addition to the functional consequences of this change. In this study, immunocytochemical analysis revealed that endogenous Sez6 is localised in the Golgi, early, late and recycling endosomes and at the cell surface of cultured rat hippocampal neurons. We used the Retention Using Selective Hooks (RUSH) system to synchronise and follow the anterograde trafficking of Sez6 in real time. HeLa cells and hippocampal neurons were transfected with the Sez6 RUSH plasmid. Newly synthesized Sez6-RUSH is retained in the endoplasmic reticulum and is released by the addition of biotin. Using spinning-disk and super-resolution microscopy to image live and fixed cells, Sez6 trafficking was examined for up to 120 minutes after release. Newly synthesized SBP-GFP-Sez6 was first concentrated in the Golgi apparatus before being distributed in vesicles throughout the soma and neuronal dendrites. SBP-GFP-Sez6 cell surface staining tended to increase from 45 to 120 minutes post release. Further work will examine the effect of altered BACE1 activity on Sez6 localisation and trafficking in neuronal subcellar compartments. These results provide insight into the mechanisms of sSez6 production and increase our understanding of molecular factors contributing to altered Sez6 levels in disease.

id #21940

Closed-loop stimulation in periods with less epileptiform activity drives improved epilepsy outcomes

Daria Anderson[1], Chantel M Charlebois[2], Elliot H Smith[3], Angela Y Peters[4], Blake J Newman[4], Amir Arain[4], Christopher R Butson[5], John D Rolston[6]

1. The University of Sydney, Darlington, NSW, Australia

2. Biomedical Engineering, University of Utah, Salt Lake City, UT, United States

3. Neurosurgery, University of Utah, Salt Lake City, UT, United States

4. Neurology, University of Utah, Salt Lake City, UT, United States

5. Neurology, University of Florida, Gainsville, FL, USA

6. Neurosurgery, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Objective:

Responsive neurostimulation (RNS) for epilepsy applies therapeutic stimulation in response to detected epileptic activity using intracranial electrodes. Prior studies have found that people with successful seizure reduction using RNS devices undergo functional network changes[1]. We hypothesise that the timing of stimulation, specifically stimulation during non-epileptic brain states, is critical in driving long-term changes to restore healthy brain networks.

Methods:

Simulation episodes occurring in low-risk epochs was characterized in a cohort of 40 people with epilepsy treated with the RNS System at the University of Utah, USA. People were categorized into three groups based on their seizure reduction values: super responders (>90% reduction, n=10), intermediate responders (≥ 50% reduction and ≤ 90% reduction), n=19, and poor responders (<50% reduction, n=11). Low-risk periods were defined as days which had fewer long epileptic episode counts than the weekly average from the prior week.

Results:

Super-responders spent more time in and experienced more frequent stimulation during low-risk seizure periods (Pearson's correlation: p=1.88e-3 and p=1.59e-3). Additionally, circadian rhythmicity was an indicator of improved seizure reduction (Pearson's correlation: p=0.045 and 1.55e-3), and stimulation of super-responders was less phase- locked to long epileptic episodes (two-sample Kuiper test; p<0.01). Baseline seizure frequency, epilepsy duration, and age was not a statistical predictor of outcome.

Conclusions:

Individuals with greater seizure reduction experienced more stimulation episodes during low-risk periods and spent more time in low-risk periods from stimulation onset. Targeting low-risk brain states may improve seizure reduction though neuromodulation via network restructuring driven by neuromodulation-induced plasticity.

1. 1. Khambhati, A.N., Shafi, A., Rao, V.R. and Chang, E.F., 2021. Long-term brain network reorganization predicts responsive neurostimulation outcomes for focal epilepsy. Science Translational Medicine, 13(608), p.eabf6588.

id #21941

3D assessment of iron & autophagy dysfunction in human post-mortem tissue of synucleinopathies.

Matthew Boag[1, 2], Ali Delbaz[2], Linlin Ma[3], Dean Pountney[1]

1. Griffith University, Southport, QLD, Australia

2. Clem Jones Centre of Neurobiology and Stem Cell Research, Griffith University, Gold Coast, Queensland, Australia

3. Griffith Institute of Drug Discovery, Griffith University, Nathan, Queensland, Australia

Autophagy is a lysosomal-dependant pathway mediating the degradation of aggregated proteins (aggrephagy) and dysfunctional mitochondria (mitophagy). Autophagy is the only method that releases iron from the intracellular storage protein ferritin (ferritinophagy). As iron accumulation accompanies aggregated α-synuclein in disease (synucleinopathies), we aimed to assess if iron overload is consequent to diminished autophagic flux. To investigate ferritinophagy impairment, we used human post-mortem brain tissue from 6 patients with pathologically confirmed Lewy body dementia (DLB) and multiple system atrophy (MSA), assessing the hippocampus, frontal, and temporal lobe. Using a spinning disk confocal microscope, we assessed autophagy function by immunostaining for ferritin and autophagy markers (*i.e.,* LC3B). Images were acquired as Z-stacks and assessed within a 3D context. Indeed, we confirmed that ferritin accumulates within glial cells of synucleinopathies, most significantly within MSA. We uniquely found significant intraneuronal ferritin accumulation within DLB tissue compared to healthy controls. We further identified that ferritin in DLB tissue colocalises with LC3B, supporting that observed ferritin is accumulating within autophagosomes and cannot be cleared. Moreover, ferritin significantly colocalised with α-synuclein, possibly indicating that protein aggregates directly hinder the autophagosome-lysosome fusion, a phenotype we have also observed in cell culture models of α-synuclein aggregation. Thus, we have illustrated that autophagy impairment is a true consequence of α-synuclein aggregation, particularly within patient pathologies. Therapies targeting the restoration of autophagy will likely provide significant neuroprotective benefits in clinical settings.

1. Boag, Matthew & Roberts, Angus & Uversky, Vladimir & Ma, Linlin & Richardson, Des & Pountney, Dean. (2022). Ferritinophagy and α-Synuclein: Pharmacological Targeting of Autophagy to Restore Iron Regulation in Parkinson's Disease. International Journal of Molecular Sciences. 23. 2378. 10.3390/ijms23042378.

id #21175

Metabolomic profiling of salvia predicts risk of cognitive impairment in people with breast cancer

Courtney Cross[1, 2], Joanne Bowen[1], Maya Davies[1, 2], Janet Coller[1], Wayne Leifert[1, 3], Maxime Francois[3], Rohit Joshi[1, 4], Beverly Fosh[5], Bogda Koczwara[6], Hannah Rose Wardill[1, 2]

1. The University of Adelaide, Adelaide, SA, Australia

2. South Australian Health and Medical Research Institute (SAHMRI), Adelaide, SA, Australia

3. Molecular Diagnostic Solutions Group, CSIRO Health & Biosecurity, Nutrition and Health Program, Adelaide, SA, Australia

4. Northern Cancer Centre, Lyell McEwin Hospital, Adelaide, SA, Australia

5. Adelaide Plastic Surgery, Adelaide, SA, Australia

6. Flinders Medical Centre, Flinders University, Adelaise, SA, Australia

INTRODUCTION: Chemotherapy causes profound and often chronic cognitive impairment (CICI), consistently identified as a priority concern by cancer survivors. CICI is highly unpredictable even in homogeneous populations. This means, i) patients cannot be given accurate predictions about their risk of developing CICI, and ii) symptoms are managed reactively. Here, we tested a novel saliva-based profiling technique to predict patients at risk of CICI, to provide clarity to patients and care providers on individual treatment response and risks.

METHODS: Untargeted and targeted metabolomic analyses using GCMS were performed in N=65 pre-chemotherapy saliva samples of newly diagnosed patients with cancer. Metabolomic data was assessed using multivariate analysis software SIMCA and MetaboAnalyst to compare the saliva-metabolome of patient cohorts aggregated on diagnosis and, in patients with breast cancer, their development of CICI (defined by the Functional Assessment of Cancer Therapy – Cognitive Function (FACT-Cog)). Individual metabolites predictive of cognitive impairment were also identified by measuring the % area under the ROC curve.

RESULTS: Metabolomic signatures in pre-chemotherapy saliva accurately discriminated patients based on cancer type (R2Y=0.82) and, in people with breast cancer, those who did and did not develop cognitive impairment (ROC=0.86). Biomarker analysis identified two potential metabolites that independently predicted the risk of developing cognitive impairment: D-maltose (ROC=0.84) and salicylic acid (ROC=0.84).

CONCLUSIONS: “Saliva-omic” profiling presents as a minimally invasive and inexpensive tool to predict a patient’s risk of CICI. Translation of this knowledge would provide much needed clarity for patients and care providers, as well as precise and targeted supportive care.

id #22200

The Sporadic ALS Australia Systems Genomics Consortium: SALSA-SGC

Anjali K Henders[1], Laura Ziser[1], Fleur Garton[1], Allan McRae[1], Shyuan Ngo[1], Matthew Kiernan[2], Nigel Laing[3], Susan Mathers[4], Merrilee Needham[5], Dominic Rowe[6], David Schultz[7], Steve Vucic[8], Pamela McCombe[9], Robert Henderson[10], Ian P Blair[6], Naomi R Wray[11], SALSA-SG Consortium[12]

1. The University of Queensland, Brisbane, QLD, Australia

2. Neuroscience Research Australia; and Institute of Neurological Sciences, UNSW and the South Eastern Sydney Local Health District, Sydney, NSW, Australia

3. University of Western Australia, Harry Perkins Institute for Medical Research, Perth, WA, Australia

4. Calvary Health Care Bethlehem, Parkdale, VIC, Australia

5. Fiona Stanley Hospital, Perth, WA, Australia

6. Macquarie University, Sydney, NSW, Australia

7. Department of Neurology, Flinders Medical Centre, Adelaide, SA, Australia

8. Brain & Mind Centre, University of Sydney, Institute of Clinical Neurosciences, Royal Prince Alfred Hospital, Sydney, NSW, Australia

9. Centre for Clinical Research, The University of Queensland, Brisbane, QLD, Australia

10. Neurology, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia

11. Department of Psychiatry and Big Data Institute, University of Oxford, Oxford, UK

12. Sporadic ALS Australia, Systems Genomics Consortium, Members in Australia/Netherlands/UK

The **Sporadic ALS Australia – Systems Genomics Consortium (SALSA)** was established in 2015 to provide dedicated infrastructure to support longitudinal collection of clinical data and biological samples from individuals with ALS in Australia. The aim to use collaborative system genomics approaches will accelerate understanding of risk factors and pathways underlying the aetiology of the disease. Additional data via linkage to the National MindAUS registry has also been established.

At census (March 2024) the cohort consisted of **1261 ALS cases and 140 controls** recruited across seven clinical sites (5 states) in Australia between 2015 and 2023. Consistent with international registry data, within cases, 64.7% are male and 35.3% are female, with 1 in 10 reporting having a first degree relative with an ALS/FTD diagnosis. Approximately 55% have a primary diagnosis of Classic ALS with onset of symptoms most common in lumbar (35%) and cervical (31%) regions. Routine genetic screening has identified individuals carrying known pathogenic variants in *C9orf72* (7.3%) and *SOD1* (1.4%) and *TARDBP43* (<1%).

Post pandemic we see recruitments rates increasing, and with new clinics to be added, we expect rapid growth. An online data explorer website has been made publicly available to enable data mine clinical and genetic phenotypes in real-time (http://salsasgc.org/explore/).

The SALSA-SGC is providing centralised support to ALS/MND research in Australia. It includes support for genetic screening for clinical trials, and novel genomic research strategies being applied to the samples. We expect additional resources to be added to facilitate translational, precision-based therapy.

id #21433

The moderating effect of dietary patterns on the longitudinal relationship between depression, anxiety, and cognitive decline

Hilal Al Shamsi[1], Samantha L. Gardener[1, 2, 3], Stephanie R. Rainey-Smith[1, 2, 3, 4, 5], Hamid R. Sohrabi[1, 2, 6, 4], Kevin Taddei[1, 2], Colin L. Masters[7], Christopher Rowe[7, 8], Ralph N. Martins[1, 2, 6, 4], Binosha fernando[1, 2]

1. Centre of Excellence for Alzheimer's Disease Research and Care, School of Medical and Health Sciences, Edith Cowan University, Joondalup, WA, Australia

2. Alzheimer's Research Australia, Ralph and Patricia Sarich Neuroscience Research Institute, Nedlands, WA, Australia

3. Lifestyle Approaches Towards Cognitive Health Research Group, Murdoch University, Murdoch, WA, Australia

4. Centre for Healthy Ageing, Health Futures Institute, Murdoch University, Murdoch, WA, Australia

5. School of Psychological Science, University of Western Australia, Perth, WA, Australia

6. Department of Biomedical Sciences, Macquarie University, Sydney, New South Wales, Australia

7. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia

8. Department of Molecular Imaging, Austin Health, Heidelberg, Victoria, Australia

Background: The link between depression and an increased risk of Alzheimer's disease highlights the need to explore how dietary patterns may moderate the relationship between mood and cognitive decline.

Methods: This study included 407 cognitively unimpaired participants (>60 years, 41% male) from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study. Participants completed a food frequency questionnaire (baseline only), along with cognitive and mood assessments at baseline and four additional time points. Scores were generated for six cognitive domains and three dietary patterns: Mediterranean (MeDi), Dietary Approaches to Stop Hypertension (DASH), and Western diet. Moderation and simple slope analyses examined interactions between baseline dietary patterns, mood changes, and cognitive decline

Results: After adjusting for false discovery rate, increasing depression was associated with greater decline in the AIBL PACC score among males with mean and below mean MeDI adherence (β=-0.006, SE=0.004, p=0.011 and β=-0.006, SE=0.003, p=0.025, respectively). This was also observed in males with mean and above mean Western diet adherence (β=-0.006, SE=0.004, p=0.019 and β=-0.006, SE=0.003, p=0.033, respectively).

Increasing depression was associated with greater decline in language in Apolipoprotein E (*APOE)* ε4 carriers with mean MeDi adherence (β=-0.006, SE=0.003, p=0.048). Additionally, increasing anxiety was associated with greater decline in the AIBL PACC score in *APOE* ε4 non-carriers with lower than mean DASH diet adherence (β=-0.003, SE=0.014, p=0.045).

Conclusion: This study highlights healthier dietary intake may moderate the relationship between changes in mood and cognitive decline. These findings suggest the importance of gender- and genotype-specific approaches in mood, cognition, and diet research.

id #21434

Tadpoles, seizures, and leaky brains: an intriguing model of severe epilepsy

Sulagna Banerjee[1, 2], Paul Szyszka[1, 2], Caroline Beck[1, 2, 3]

1. University of Otago, Dunedin, OTAGO, New Zealand

2. Brain Health Research Centre, University of Otago, Dunedin, New Zealand

3. Genetics Otago Research Centre, University of Otago, Dunedin, New Zealand

Developmental epileptic encephalopathies (DEEs) are severe genetic disorders in infants and children, marked by persistent, drug-resistant seizures and profound physical and cognitive impairments. Despite the identification of numerous DEE-associated genes, functional studies in animal or cell models are lacking for the majority of DEE which hinders the understanding of the common pathways involved in DEE development. To bridge this gap, this study developed a high-throughput DEE model using CRISPR/Cas9-mediated loss-of-function in *Xenopus laevis* tadpoles, aiming to elucidate the role of gene mutations in spontaneous seizure occurrence and abnormal brain signalling.

Focusing on DEE75, caused by de novo mutations in the *NEUROD2* gene, it was observed that CRISPR/Cas9-edited *NeuroD2.S* tadpoles exhibited heightened activity, faster swimming, and increased seizure frequency compared to controls. Live brain calcium imaging revealed prolonged, intense signals indicative of neuronal hyperactivity, and the crispants also showed a compromised blood-brain barrier (BBB) [1].

Additionally, recent research highlighted neuroinflammation (NI) as a contributing factor in seizure severity, suggesting a promising therapeutic target. The study utilized repurposed anti-inflammatory drugs (AIDs) to target NI in this model. The findings indicate that pathogenic variants lead to abnormal neuronal signalling and chronic seizures, resulting in sustained NI. Specifically, the TGF-β antagonist Losartan demonstrated a protective effect, reducing seizure events and neuronal hyperactivity, and temporarily improving BBB permeability.

These results support a model where diminished NeuroD2 activity during brain development contributed to DEE75 pathogenesis. Moreover, they suggest that targeting NI with AIDs like Losartan present a novel therapeutic strategy for managing intractable seizures in DEEs.

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id #21946

Chemistry Matters: Targeted Therapies for Neurological Disorders.

Jessica M Cale[1, 2], Kristin A Ham[1, 2], Anuradha Sooda[1, 2], Isabella Trew[1, 2], Steve D Wilton[1, 2], May T Aung-Htut[1, 2]

1. Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia

2. Centre for Molecular Medicine and Innovative Therapeutics, Health Futures Institute, Murdoch University, Murdoch, WA, Australia

In recent years, several synthetic nucleic acid sequences that modulate gene expression known as antisense oligonucleotides (ASOs) have been approved to treat various neurological and neuromuscular disorders, including spinal muscular atrophy, Batten disease and Duchenne muscular dystrophy. These therapies primarily work by sterically blocking complementary sequences on RNA molecules, thereby selectively altering gene expression. Decades of research have been dedicated to chemically modifying ASOs to enhance their stability, specificity, and affinity while reducing toxicity from hybridisation-dependent and independent off-target effects.

While there is a wealth of knowledge on the off-target effects of nucleic acids that recruit RNase H for their activities, few reports discuss the off-target effects of steric-blocking ASOs. This study investigated sequence-independent off-target splicing defects caused by ASOs with a combination of ribose and backbone modifications designed as steric blockers. Various ASO chemistries were delivered into cultured cells, and RT-PCR, Sanger sequencing, RNA sequencing, and immunolabeling were used to observe off-target effects. The results indicated chemistry-dependent impacts on gene expression, including cryptic splicing, exon skipping, up- and down-regulation, and isoform-switching. Notably, these off-target defects were absent when the same sequences were synthesised as phosphorodiamidate morpholino oligomers. This highlights that chemistry matters when developing therapeutic ASOs.

As research into tailored splice-modulating therapies continues to expand, our findings provide valuable data that will aid in identifying safe and effective therapeutic candidates.

id #22202

THE NEURAL CELL ADHESION MOLECULE 2 (NCAM2) PROMOTES SYNAPTOGENESIS THROUGH INCREASING INSULIN AND INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR SIGNALING

Nishat Zaman[1], Vladimir Sytnyk[1]

1. School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales, Australia

Publish consent withheld

id #21179

Familiarity and time influence how the brain integrates sensory and emotional information.

Alina B Thomas[1], Francesca S Wong[1], Fred Westbrook[1], Nathan M Holmes[1]

1. University of New South Wales (UNSW), Sydney, NSW, Australia

The current study examined how sensory and emotional information is integrated in the mammalian brain. To do so, it used a sensory preconditioning protocol in which rats integrate a tone-light association (sensory information) formed in stage 1 with a light-shock association (emotional information) formed in stage 2 to generate fear responses (freezing) when tested with the tone alone in stage 3. This integration could occur through chaining of the tone-light and light-shock associations during testing with the tone (e.g., tone-->light-->shock); or through formation of a mediated tone-shock association during conditioning of the light. Following previous work, we distinguished between these alternatives by blocking NMDA receptors (using micro-infusions of the antagonist, D-AP5) in the perirhinal cortex (PRh) during stage 2. We found that: 1) after just a few tone-light pairings in stage 1, the D-AP5 infusion disrupted freezing to the tone (but not the light), consistent with integration through mediated learning; 2) after many tone-light pairings in stage 1, the D-AP5 infusion no longer disrupted freezing to the tone, consistent with integration through chaining at test; but 3) in the latter case, the effect of the D-AP5 infusion was restored (i.e., freezing to tone was disrupted) when the interval between stages 1 and 2 increased from 24 hours (standard) to 15 days, suggesting a return to integration via mediated learning. These results show that familiarity and time influence how the brain integrates sensory and emotional information. They are discussed with respect to theories of information processing in the medial temporal lobe.

id #21435

Multi-scale imaging of the hypogastric nerves in the adult human

Kayleigh Scotcher[1], John-Paul Fuller-Jackson[1], Peregrine Osborne[1], Janet Keast[1], Martin Bertrand[2]

1. Department of Anatomy and Physiology, University of Melbourne, Melbourne, Australia

2. Faculté de Médecine, Montpellier-Nîmes, France

The bilateral hypogastric nerves (HGn) are a major trajectory of sympathetic (pre- and postganglionic) and visceral afferent axons that supply the pelvic organs. These nerves link the superior hypogastric plexus (sympathetic; superficial to the distal abdominal aorta) with the inferior hypogastric plexus (mixed sympathetic-parasympathetic; in the pelvic cavity). The cellular components and microscopic organization of the HGn have been poorly defined in humans. Our aim was to define the anatomical organisation of the HGn and its vasculature from the macroscopic to microscopic levels. This was performed by applying immunohistochemistry with multi-scale imaging, including large-volume light sheet microscopy of cleared tissues (modified iDISCO method) and high-resolution confocal microscopy of cryosections. Our studies have visualised distinct vascular trajectories and axon fascicles contributing to the HGn. We also identified several large aggregates of tyrosine hydroxylase (TH) positive neurons (n=7714) embedded within the HGn. Additional neural markers visualised in cryosections of the HGn were identified in subclasses of ganglion neurons, synaptic boutons (potential preganglionic terminals) and axon tracts. These include neuropeptide Y, neuronal nitric oxide synthase, somatostatin, and vasoactive intestinal peptide. Putative afferent fibres (calcitonin gene-related peptide and substance P) traversed the HGn and occasionally encircled individual ganglion neurons, raising the possibility of direct sensory-motor communication at these sites. Multiple small intensely fluorescent (SIF) cell nodules were also observed close to neural tracts along the length of the HGn. This study has provided

new insights into the structure, diverse neural elements and vascular supply within the adult human HGn.

id #22203

Resistance training and cognition in adults with and without cognitive decline: an umbrella review

Kieran J Marston[1, 2], Kelsey R Sewell[1], Brook Galna[1, 2], Hamid R Sohrabi[1, 5, 3, 4], Belinda M Brown[1, 3, 4, 6]

1. Centre for Healthy Ageing, Murdoch University, Murdoch, Western Australia, Australia

2. PHysical Activity, Sport and Exercise (PHASE) Research Group, Murdoch University, Murdoch, Western Australia, Australia

3. Centre of Excellence for Alzheimer's Disease Research and Care, Edith Cowan University, Joondalup, Western Australia, Australia

4. Alzheimer's Research Australia, Sarich Neurosciences Research Institute, Nedlands, Western Australia, Australia

5. Department of Biomedical Sciences, Macquarie University, North Ryde, New South Wales, Australia

6. Centre for Precision Health, Edith Cowan University, Joondalup, Western Australia, Australia

Title: Resistance training and cognition in adults with and without cognitive decline: an umbrella review

Objectives: The purpose of this umbrella review is to summarise the evidence from previous meta-analyses to provide an overall indication of the efficacy that resistance training has on cognitive function outcomes in older adults.

Methods: A systematic search of the literature was conducted. Eligible studies recruited adults ≥50y with or without cognitive decline and administered solely resistance training interventions of at least four weeks in duration. Reporting quality was assessed with AMSTAR 2. Overall effects of standardised mean change scores were calculated using the robust variance estimation method.

Findings: Reporting quality for the included meta-analyses was deemed moderate (n = 5), low (n = 8) or critically low (n = 12). We included 33 primary studies (from 25 meta-analyses). Studies had a median total sample of 40 per study (range: 16 to 210 participants). We observed an overall effect in favour of resistance training compared to control arms (d = 0.32, CI 95%: 0.16 to 0.47, $p < 0.001$), with large heterogeneity ($I^2 = 66.3\%$). Larger effects were seen for studies with passive control arms, and for global cognition compared to specific cognitive domains.

Conclusion: There is a low-to-moderate effect for resistance training to improve cognition in older adults. However, current meta-analyses have relied on small, poor quality studies. Large randomised controlled trials with active control arms are warranted.

id #21436

Impact of third hand exposure to e-cigarettes on cognition and brain health

Rochelle Alexia Yarak[1], Tharathip Hikasem[1], Catherine Gorrie[1], Hui Chen[1]

1. School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia

Introduction: Third-hand e-vaping refers to the residues left behind by vaping, which include nicotine and other chemicals. While e-vaping can adversely affect cognitive functions, research is limited on the effects of third-hand impacts, which formed the aim of this study.

Objective: To measure the changes in short-term memory and anxiety-like behaviours, as well as changes in cell numbers in the hippocampus.

Methods and Results: Balb/c mice (4 weeks, male) were exposed to freshly prepared towels daily for 4 weeks. Towels for the sham group were autoclaved only, while towels for the third-hand e-vaping groups were exposed to e-vapours with (18mg group) or without (0mg group) nicotine for 2 hours before being placed in the mouse cages. Mice exposed to the nicotine-free towels presented reduced short-term memory function during the Novel Objective Recognition Test, and those in the 18mg group spent the least time in the open arm of the elevated plus maze, suggesting increased anxiety. GFAP staining was increased in the cornu ammonis (CA)1, CA2, CA3, and dentate gyrus regions of the hippocampus ($p<0.05$) in the 18mg group, while hippocampal Iba1 staining was increased in the 0mg group.

Conclusions: Third hand exposure to nicotine and nicotine-free e-cigarettes leads to changes in cognitive function and numbers of astrocytes and microglia in the hippocampus.

id #21948

Seizure-responsive hydrogel programmed to modulate ion channels involved in epileptic seizure

Nafiseh Olov[1, 2], Samuel Cheeseman[1, 2], Dan Li[3], Richard J Williams[4, 5, 2], David R Nisbet[6, 1, 2]

1. Department of Biomedical Engineering, Faculty of Engineering and Information Technology, The University of Melbourne, Parkville, VIC 3010, Australia

2. The Graeme Clark Institute, The University of Melbourne, Parkville, VIC 3010, Australia

3. Department of Chemical Engineering, The University of Melbourne, Parkville, VIC 3010, Australia

4. IMPACT, School of Medicine, Deakin University , Waurn Ponds, VIC, Australia

5. Centre for Sustainable Bioproducts, Deakin University, Waurn Ponds, VIC 3217, Australia

6. Medical School, Faculty of Medicine, Dentistry and Health Science, The University of Melbourne, Parkville, VIC 3010, Australia

Epilepsy is a type of central nervous system-related disease commonly known for its seizures and affects approximately 3% to 3.5% of Australians at some point in their lives. Seizures occur when there is a sudden, unusual firing of electrical impulses in the brain, disrupting regular electrical activity. The most

common and primary treatment for epilepsy involves the use of antiepileptic drugs (AEDs) administered orally. However, due to the short life of the AEDs and the presence of the blood-brain barrier, which decreases the delivery efficacy of the drugs to the brain, AEDs need to be taken daily, often multiple times a day, even with extended-release preparations. This creates a burden for the user and undesirable side effects due to the required high doses.

One promising approach is local drug delivery to the brain in response to seizures. To this end, we have developed a conductive and adhesive hydrogel with a novel formulation based on polyacrylamide. This hydrogel is programmed to release a small amount of FOS-phenytoin in the absence of seizures, modulating Na+ ion channels to prevent their occurrence. However, if a seizure does occur, the hydrogel increases the dosage to suppress it. The stiffness of the hydrogel has been engineered to be compatible with the soft tissue of the brain. It adheres to the brain's surface to deliver the drug directly to the epileptic focus area at the appropriate time, thereby minimizing the side effects associated with indirect delivery.

id #21437

Modulating Brain Network Connectivity with Transcranial Alternating Current Stimulation

Brooke Lebihan[1], Hakuei Fujiyama[1]

1. Murdoch University, SCARBOROUGH, WA, Australia

Objective

Concurrent application of transcranial alternating current stimulation (tACS) over distant cortical regions appeared to modulate functional connectivity between these regions; however, the precise mechanisms by which functional connectivity is modulated between cortical regions remain unclear. Here, we investigated how dual-site tACS (ds-tACS) applied over bilateral motor cortices (M1s) modulates the functional connectivity between M1s using transcranial magnetic stimulation (TMS) and electroencephalogram (EEG).

Methods

Using a cross-over sham-controlled triple-blind within-subject design, 35 (24 female, age 18-40 yrs) healthy younger participants received tACS (1.0mA, 20Hz) over bilateral M1s for 20 min. Before and after tACS, functional connectivity between M1s was assessed using imaginary coherence (ImCoh) measure via resting-state EEG and interhemispheric inhibition (IHI) via dual-site TMS protocol.

Findings

The preliminary analyses indicated that ds-tACS modulated interhemispheric inhibition between M1s, whereas no such modulation was found in the sham session. While ImCoh showed no change after the simulation, spectral power analysis showed a significant decrease in beta (13-30 Hz) power was found in the tACS session.

Conclusion

Based on our preliminary results, we suggest that ds-tACS is capable of modulating IHI between the M1s. This change in IHI is likely associated with a decrease in beta power, indicating that the IHI changes following tACS are unlikely due to the induction of phase coupling in the oscillatory activity of neural populations. Since functional connectivity underlies many clinical symptoms in neurological and psychiatric disorders, these findings are invaluable in developing non-invasive therapeutic interventions that target specific neural networks to alleviate symptoms.

id #22205

Investigating what drives liquid-liquid phase separation in the ALS/MND-associated protein SFPQ

Emma G Knowling[1], Charlie S Bond[2], Archa H Fox[1]

1. School of Human Sciences, University of Western Australia, Crawley, WA, Australia

2. School of Molecular Sciences, University of Western Australia, Crawley, WA, Australia

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease characterised by the degeneration of motor neurons in the motor cortex and spinal cord. Many ALS-associated RNA-binding proteins contain Intrinsically Disordered Regions (IDRs) that facilitate an essential biological process called Liquid-Liquid Phase Separation (LLPS). LLPS refers to the reversible and controlled formation of liquid-like biomolecular condensates via the self-organisation of biomolecules. One RNA-binding protein implicated in ALS is Splicing Factor Proline- and Glutamine-rich (SFPQ). Previous studies have demonstrated that the SFPQ C-terminal IDR is essential for driving LLPS, where SFPQ co-localises to long non-coding RNA NEAT Paraspeckle Assembly Transcript 1 (NEAT1) to form paraspeckles. Paraspeckles are molecular hubs that facilitate many essential cellular processes commonly affected in ALS. Although the domain responsible for driving SFPQ LLPS has been identified, the specific features within the SFPQ C-terminal IDR that drive LLPS remain unknown. A series of substitution mutations that target various amino acids were designed, and the phase-separating ability of the mutant constructs was tested *in vivo* using cellular paraspeckle assays and *in vitro* using droplet assays with recombinant proteins. Substitutions of Arg and Tyr amino acids drastically decreased the phase-separating ability of SFPQ. In comparison, amino acids such as Pro and Gly altered the molecular dynamics of the protein droplets. This work contributes to understanding how amino acids with the SFPQ C-IDR facilitate LLPS. This work is particularly significant as phase separation underpins many biological functions, and aberrant phase separation is implicated in many neurodegenerative diseases and cancers.

id #21950

Tissue concentrations of a highly bioavailable phytosomal curcumin formulation and their correlation with glial inflammatory markers in the GFAP- IL6 mouse model of chronic neuroinflammation

Lillian J Jabur[1], Anushka Chatterjee[1], Natalie Mikhael[1], Ingrid Wagnon[1], Anandwardhan Hardikar[2], Mugdha Joglekar[2], Wilson Wong[2], Gerald Muench[1], Erika Gyengesi[1]

1. Neuropharmacology Group, School of Medicine, Western Sydney University, Campbelltown, NSW, Australia

2. Islet Biology Group, School of Medicine, Western Sydney University, Penrith, NSW, Australia

Aims: Chronic neuroinflammation is a hallmark of several neurodegenerative diseases, and uncovering the underlying mechanisms is crucial for developing effective therapies. Curcuminoids are well-known for their anti-inflammatory and neuroprotective properties. This study investigated the anti-inflammatory potential of a phytosomal curcumin (highly bioavailable formulation) in a mouse model of chronic neuroinflammation, overexpressing interleukin-6 under the control of the glial fibrillary acidic protein promoter (GFAP-IL6).

Method: WT and homozygous GFAP-IL6 mice, both sexes, were fed with phytosomal curcumin enriched diet (2000 ppm) or control chow from 1-month old. Brains were collected for quantitative analysis of glia numbers and morphology, using stereology and 3D single cell reconstructions, and for transcriptomic analysis via RT-qPCR in brain regions involved in motor control, learning and memory. Plasma curcuminoid levels were quantified with ultra-high pressure liquid chromatography-mass spectrometry (UPLC-MS) to assess bioavailability of curcumin formulation.

Results: Immunostaining of microglia in GFAP-IL6 mice revealed a significant decreased of glia activation in the hippocampus and cerebellum. Curcumin supplementation demonstrated an interference with neuroinflammatory pathways by downregulating the mRNA levels of pro-inflammatory markers *P2rx7* and *Nfkb1* in the hippocampus as compared to controls. The concentration of curcumin in plasma of GFAP-IL6 (2268.71ng/ml) mice was slightly higher than those of the WT (2088.04ng/ml).

Conclusion: The current study demonstrates a positive impact of phytosomal curcumin supplementation in homozygous GFAP-IL6 mice on neuroinflammatory markers and glial cells. This provides foundational evidence for the therapeutic potential of modified, highly bioavailable curcumin preparations as an anti-inflammatory agent against the detrimental effects of neuroinflammation.

id #22206

Impaired locus coeruleus function in Alzheimer's disease

Yasmin Potts[1], Stuart McDougall[1], Heather Daykin[2], Laura Jacobson-hoyer[1, 2], Daniel Hoyer[1, 2, 3], Maarten Van Den Bossche[4, 5]

1. Florey Institute of Neuroscience, Parkville, VIC, Australia

2. Department of Biochemistry and Pharmacology, University of Melbourne, Melbourne, VIC , Australia

3. Scripps Research Institute, La Jolla, California, USA

4. Geriatric Psychiatry , University Psychiatric Center KU Leuven, Leuven, Belgium

5. Neuropsychiatry, Department of Neurosciences, Leuven Brain Institute KU Leuven, Leuven, Belgium

The locus coeruleus (LC) is a predominant nucleus of the brainstem involved in the modulation of sleep, wakefulness and stress response. Tau pathology develops in the LC decades before Alzheimer's disease (AD) symptoms manifest in humans, and widespread LC degeneration occurs as the disease progresses. While LC degeneration has been characterised by total neuron death in AD, little research has explored the effect of tau pathology on the electrophysiological properties of LC neurons. The intrinsic electrical properties of LC neurons were investigated in the PS19 and rTg4510 tau transgenic murine models of AD. LC neuronal properties were not significantly different from wildtype littermates in both strains at 9 months of age, which represents a stage where AD symptoms persist and tau pathology is present in the LC. However, the resting potential of LC neurons was significantly depolarised in 9 mo PS19 transgenic mice, compared to 5 mo PS19 transgenic mice. Since the LC densely expresses orexin 1 receptors (OX1Rs), we then aimed to identify whether antagonising the OX1R, which is involved in modulation of stress and motivated behaviour, would alter cognitive symptoms in aged PS19 mice. Interestingly, the OX1R antagonist 1-SORA-51 exacerbated REM sleep deficits in male transgenic mice while, contrastingly, rescuing anxiety-like behaviours. These results suggest that tau pathology may act to 'accelerate' aging, contributing to hyperactivity of the LC and consequent sleep disruption in AD patients, and that modulation of the OX1R can produce divisive effects on sleep architecture while ameliorating anxiety symptoms.

id #21439

Cerebral blood flow alterations in brain regions associated with cardiovascular autonomic control following mild traumatic brain injury: an arterial spin labelling study

Jacinta Thorne[3, 1, 2], Sarah C Hellewell[1, 2], Gill Cowen[4, 5], Jun Chih[4], Michael Bynevelt[6], Anoek Van Houselt[7], Aleksandra Gozt[8], Andre Avila[5, 1, 2], Caerwen Beaton[5, 1, 2], Melissa Papini[5, 1, 2], Francesca Buhagiar[9], Amanda Jefferson [5], Elizabeth Thomas[4], Alexander Ring[3], Antonio Celenza [10], Glenn Arendts[10], Melissa Licari[11], Dan Xu[5], Stephen Honeybul[12], Suzanne Robinson[13], Carmela Pestell[9], Daniel Fatovich[14], Melinda Fitzgerald[1, 2]

1. Perron Institute for Neurological and Translational Science , Nedlands, WA, Australia

2. Curtin Health Innovation Research Institute, Curtin University , Bentley, WA, Australia

3. School of Allied Health , Curtin University , Bentley, WA, Australia

4. School of Population Health , Curtin University, Bentley, WA, Australia

5. Curtin Medical School , Curtin University , Bentley , WA, Australia

6. Neurological Intervention and Imaging Service, Sir Charles Gairdner Hospital , Perth, WA, Australia

7. Curtin University, Nedlands, WA, Australia
8. Connectivity Traumatic Brain Injury Australia, Nedlands, WA, Australia
9. School of Psychological Science , University of Western Australia, Nedlands, WA, Australia
10. Division of Emergency Medicine, University of Western Australia, Nedlands, WA, Australia
11. Telethon Kids Institute, Nedlands, WA, Australia
12. Department of Neurosurgery , Sir Charles Gairdner Hospital , Nedlands, WA, Australia
13. School of Health and Development , Deakin University , Melbourne, VIC, Australia
14. Emergency Medicine, Royal Perth Hospital , Perth, WA, Australia

Purpose: Following mild traumatic brain injury (mTBI) some individuals experience heart rate (HR) alterations and exercise intolerance(1,2). It is postulated these alterations are related to autonomic nervous system dysfunction and impaired cerebral blood flow (CBF)(3), yet the neurobiological mechanisms remain unclear.

Methods: Prospective observational study of 30 adults aged 18-65 years within ten days of mTBI, and 33 age- and sex-matched controls. HR and exercise intolerance were assessed using the Buffalo Concussion Bike Test(4), and symptoms using the Post-Concussion Symptom Scale(5). Pseudo-continuous arterial spin labelling was used to examine the relationship between CBF in brain regions recognised for their role in cardiovascular autonomic response (insular cortex, anterior cingulate cortex (ACC) and amygdala)(6,7), with resting HR and exercise intolerance.

Results: Resting HR (p=0.042) and HR change (p=0.004) were significantly lower for mTBI participants than controls. CBF was increased in the right insula (p=0.027) and right amygdala (p=0.025) in people who had recently sustained mTBI compared to controls. A positive association between resting HR and CBF in the left insula (r=0.378, p=0.033) and right ACC (r=0.423, p=0.016) was identified for controls, but not mTBI participants. Similarly, a positive association was observed between CBF in the left insula and right ACC and both symptom severity and autonomic-type symptoms in controls, but not following mTBI.

Conclusion: This study provides preliminary evidence that the natural regulatory relationship between CBF and resting HR may be disrupted in brain regions involved in cardiovascular autonomic control following mTBI.

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id #21951

Small Molecule Base Exchange Inhibitor of TIR:TIR Domain Formation and Activation in Human SARM1

Paige Harten[1]

1. Griffith University, Southport, QLD, Australia

Axon loss is a recurrent feature in many prevalent neurological disorders, such as peripheral neuropathies, traumatic brain injury, Parkinson's disease, and glaucoma. At the heart of the process leading to axon loss is SARM1, the principal executor of the pathway responsible for axonal degeneration. After axonal injury SARM1's TIR domain initiates the degradation of NAD+, resulting in a type of axonal demise known as Wallerian degeneration. SARM1 is characterized by an N-terminal armadillo repeat (ARM) domain, which is followed by a tandem set of sterile alpha motif (SAM) domains, and a C-terminal toll/interleukin receptor (TIR) domain. The TIR domain possesses intrinsic nicotinamide adenine dinucleotide (NAD) hydrolase (NADase) activity. With catalytic cleavage executed by a conserved glutamic acid (E642) cleaving NAD+ into ADP Ribose (ADPR), cyclic ADPR, and Nicotinamide mononucleotide (NMN), NMN is a forward-feeding byproduct. Base exchange reactions between NAD+ and small molecules/fragments inhibit NMN byproduct formation. Observed through fluorescence assays, multiple fragment structures reduce fluorescence intensity signifying E642 binding and inhibition of active SARM1. Compounds shown to inhibit assembly formation will be characterized in more detail for protein interaction using Saturation Transfer Difference (STD) NMR, surface plasmon/isothermal titration calorimetry, and X-ray Crystallography. This information will then be used with molecular modeling and structure analysis to generate more effective small-molecule inhibitors as potential leads for drugs.

id #22208

"*Rock on technology*": Perspectives on robot-assisted lower limb and gait neurorehabilitation from people with neurological conditions

Rachel Buckingham[1, 2], Saran Chamberlain[3], Amanda Timler[1, 4], Matthew Bagg[1, 5, 6], Nikki Bakhtazad[1], Taya Hamilton[5, 7], Trish Leonard[7, 8], Bianca Haagman[1, 9], Stuart Hodgetts[5, 10], Barby Singer[11], Ann-Maree Vallence[12], Jodie Marquez[13], Jessica Nolan[1, 14, 15]

1. School of Health Sciences, University of Notre Dame Australia, Fremantle,, Western Australia , Australia

2. Sir Charles Gardiner Osborne Park Health Care Group, Subiaco, WA, Australia
3. Lived experience expert of Stroke and Trauma , .
4. Institute for Health Research, , University of Notre Dame Australia, Fremantle, Western Australia
5. Perron Institute for Neurological and Translational Science, University of Western Australia Medical School, Nedlands, Western Australia , Australia
6. Centre for Pain IMPACT, Neuroscience Research Australia, Randwick, New South Wales,, Australia
7. Australian Institute of Neuro-rehabilitation, Osborne Park, Western Australia, Australia
8. State Rehabilitation Service, Fiona Stanley Hospital,, Murdoch, Western Australia
9. ActiveLINC Pty Ltd, Innaloo, Western Australia , Australia
10. School of Human Sciences, Anatomy, Physiology and Human Biology, University of Western Australia, Crawley, Western Australia
11. School of Medical & Health Sciences, Edith Cowan University , Joondalup, Western Australia
12. School Of Psychology, College of Health and Education, Murdoch University , Murdoch , Western Australia
13. School of Health Sciences, University of Newcastle , Newcastle, New South Whales, Australia
14. School of Allied Health, Curtin University, Bentley, Western Australia, Australia
15. Physiotherapy Department, Sir Charles Gairdner Osborne Park Health Care Group , Nedlands, Western Australia

Background

Robot-assisted rehabilitation (RAR) is a promising approach to improve function and quality of life in people living with neurological conditions. There are no studies of the perspectives of people with neurological conditions on RAR in Western Australia (WA). The aim is to explore the perspectives and lived experience of people living with neurological conditions on RAR in WA, whilst evaluating the need for RAR implementation in WA.

Methods

This co-designed, qualitative, descriptive study included people living with neurological conditions in WA. In-person focus groups (FGs) were held in Perth, WA, and facilitated by a person with lived experience of stroke. FGs were recorded, data transcribed, then thematically analysed using a reflexive approach.

Results

Twenty-four participants (54.2% female), of whom 13 had RAR experience. Participant median age was 50.5 (23-77). Eligible participants had experience of rehabilitation for a neurological condition, aged ≥18 years old, resided in WA and were able to participate in English. Three neurological conditions were represented among the participants [spinal cord injury n=11, stroke n=3, multiple sclerosis n=2, other n=8]. Three main themes were established in relation to RAR in WA: perceived benefits (physiological, psychosocial, therapy, ambulation, independence and pain), barriers (lack of awareness, access, cost, psychological challenges and device limitations), and future implementation in WA (improved access, design and purpose of rehabilitation robotic devices).

Conclusion

Participants identified perceived benefits, barriers and considerations for future implementation of RAR in WA, but this can only be achieved through addressing barriers of cost, design limitations, improved access and awareness.

id #21954

Stress-related changes to the spatial localisation of hippocampal cortisol receptors in psychiatric disorders

Amber R Curry[1, 2, 3], Dominic Kaul[1, 2, 3], Lezanne Ooi[3], Natalie Matosin[1, 2, 3]

1. Charles Perkins Centre, The University of Sydney, Sydney, New South Wales, Australia
2. School of Medical Sciences, The University of Sydney, Sydney, New South Wales, Australia
3. School of Chemistry and Molecular Biosciences and Molecular Horizons, University of Wollongong, Wollongong, New South Wales, Australia

Dysfunction of the stress response is a major risk factor for psychopathology, however, molecular evidence of the mechanism in humans is limited. Hippocampal cortisol receptors *NR3C1* and *NR3C2* and their modulators (e.g. *FKBP5*) are key coordinators of this stress response. Here, we explored how expression of *NR3C1/2* and *FKBP5* are affected by stress in the human brain with spatial transcriptomics.

Postmortem hippocampal tissues from individuals with schizophrenia or bipolar disorder were categorized by lifetime trauma exposure (childhood: n=3; adulthood: n=5; no stress: n=3; controls: n=3). Spatial transcriptomics (Visium, 10x Genomics) was performed to measure spatial gene expression. Hippocampal subregions were manually annotated using subregion specific markers, and the expression of cortisol receptors and FKBP5 was compared across groups for all hippocampal subregions and layers.

Psychiatric cases with childhood stress exposure exhibited significantly lower FKBP5 expression in the CA4 subregion compared to controls (-35%, P_{FDR} = 0.046) and no stress cases (-53.2%, P_{FDR} = 9.150e-15). FKBP5 was lower in the polymorphic layer of the dentate gyrus compared to controls (-19.7%, P_{FDR} = 0.016) and no stress cases (-29.5%, P_{FDR} = 0.0034). Adulthood stress cases displayed higher FKBP5 expression in the molecular layer of the dentate gyrus compared to controls (+4.54%, P_{FDR} = 0.01242) and no stress cases (+4.75%, P_{FDR} = 0.000573). Cortisol receptor expression was uniform throughout the hippocampus with no difference in expression between stress groups.

The lasting FKBP5 expression changes indicate a potential target for future therapeutic development for psychiatric disorders associated with childhood stress exposure.

id #22210

Assessing Firmicutes and Bacteroidetes Distributions in relation to Age, Gender, and APOE-ε4 allele Status in an Aging

Australian Cohort

Samantha Ramachandra[1], Hamid Sohrabi[2, 3], Vincent Ho[4], BGDNK De Silva[5], Ralph Martins[1, 6, 3], Binosha WMAD fernando[1, 3]

1. School of Medical and Health Sciences , Edith Cowan University, Perth, WA, Australia

2. Centre for Healthy Aging, Murdoch University, Perth, WA, Australia

3. Alzheimer's Research Australia, Perth, WA, Australia

4. Western Sydney University, Sydney, NSW

5. University of Sri Jayewardenepura, Colombo, Sri Lanka

6. Macquarie University, Sydney, NSW, Australia

Alzheimer's disease (AD) is a neurodegenerative condition marked by cognitive decline and impairment in daily functioning. Emerging research suggests that gut microbiota may contribute to disease progression via the gut-brain axis. This cross-sectional study investigated the gut microbiota composition among older adults in the Australian Imaging, Biomarkers, and Lifestyle (AIBL) and Western Australian Memory Study (WAMS) cohorts to understand microbial patterns associated with AD risk (age, sex, and APOE).

Participants included 123 individuals (55% female), 17% with apolipoprotein E (APOE-ε4 allele), the major genetic risk factor for AD. Faecal samples were analysed using shotgun metagenomic sequencing to characterise gut microbiota composition. Analysis (ANOVA and T-test) revealed that Bacteroidetes was the dominant phylum, followed by Firmicutes, consistent with age-related microbial composition trends in older populations.

The Firmicute/Bacteroidetes (F/B) ratio, a marker of gut health, appeared to decrease with age (0.956 for <65yrs, 0.935 for 65-75yrs, and 0.846 for >75yrs), though this trend was not statistically significant. Though it is insignificant, a higher (F/B) ratio was seen in male and non-carriers. The most abundant 10 phyla, classes, and order distribution showed no statistically significant difference against demographic variables except phylum Verrucomicrobia against gender ($p = 0.008$) and age categories ($p=0.05$). Alpha diversity, representing species richness within samples, showed no significant associations with age, sex, or APOE-ε4 status but showed a decreasing trend in advancing age and the presence of APOE-ε4.

These findings provide a basic understanding of gut microbiota in an aging Australian cohort, with Bacteroidetes and Firmicutes as the prevalent phyla.

id #21443

Assessing the effect of repetitive TMS on people with multiple sclerosis

Madison R Boda[1], Eva Moore[1], Ashleigh Smith[2], Mitchell Goldsworthy[3], Simranjit K Sidhu[1]

1. School of Biomedicine, The University of Adelaide, Adelaide, SA, Australia

2. Alliance for Research in Exercise, Nutrition and Activity, University of South Australia, Adelaide, SA, Australia

3. Behaviour-Brain-Body Research Centre, Justice and Society, University of South Australia, Adelaide, SA, Australia

80% of people with multiple sclerosis (PwMS) experience motor impairment associated with decreased corticospinal excitability from demyelination of the corticospinal tract. Restoration of corticospinal excitability by repetitive transcranial magnetic stimulation (TMS) techniques can enhance the performance of voluntary muscle contractions for motor rehabilitation. There is some evidence suggesting that repetitive i-wave periodicity TMS (iTMS) enhances corticospinal excitability in healthy individuals, but it is not known whether the same neuroplastic effects can be induced by iTMS in PwMS. Therefore, this study aimed to investigate if differences exist in iTMS-induced corticospinal excitability between PwMS and healthy individuals. Ten PwMS (47 ± 11 years) and ten healthy age-matched controls (51 ± 21 years) participated in the study. Corticospinal excitability was induced with a 15-minute iTMS intervention on the primary motor cortex (M1), and quantified with motor evoked potential (MEP) by applying single pulse TMS on M1. MEP was recorded before intervention at baseline, and after intervention at 5-minute and 30-minute post-iTMS timepoints. MEP was compared across time and groups to assess change in corticospinal excitability. There was no change in MEP from baseline to post-iTMS timepoints in either group (all P values ≥ 0.30). These preliminary findings do not support clinical application of iTMS for motor rehabilitation in PwMS. Additionally, MEP did not change in controls, which is inconsistent with existing literature. This inconsistency highlights the need for further research into factors affecting iTMS-induced plasticity.

id #21955

Freezing and Analgesic Learned Fear Behaviours in a Mouse Neuropathic Pain Model

Eddy ES Sokolaj[1]

1. University of Sydney, Sydney, NEW SOUTH WALES, Australia

Background

Chronic neuropathic pain is a debilitating condition that deeply affects quality of life and is a major socioeconomic burden. The anticipation of pain can be a major source of fear and anxiety for chronic pain sufferers, leading to or compounding stress and anxiety related disorders. However, the underlying mechanisms and relationship between pain and fear are poorly understood. We addressed this by using an animal model of neuropathic pain to examine fear-

like and analgesic responses in a fear-conditioning conditioning protocol.

Methods

Neuropathic pain was modelled by chronic constriction injury (CCI) of the sciatic in male C57BL/6 mice. These were compared to control surgery (sham) and no surgery (naïve) mice. At 14-days post-surgery, mice then underwent a fear conditioning protocol to assess fear learning behaviour by associating an innocuous audio tone with an electrical footshock. On the following days, mice underwent assessment of their fear-like freezing or analgesic responses (Hargreaves tester) to the audio tone alone in a different environmental context.

Findings

On the day following fear-conditioning, naïve mice expressed not only fear-like freezing behaviour, but also an analgesic response to the cue conditioned stimulus. Compare to control sham mice, CCI mice displayed disrupted analgesic and freezing responses to the cue conditioned stimulus.

Conclusion

As observed previously for fear-behaviour, the present study demonstrates that an analgesic response can be evoked by a non-contextual cue in a fear-learning paradigm in mice. This co-ordinated fear-analgesic response is disrupted in a chronic neuropathic pain model.

id #22211

Blood biomarkers for adult and childhood dementia

Prashant Bharadwaj[1, 2], Fraulien Arigo[1, 2], Maina Kava[3, 4], Ralph Martins[1, 2, 5]

1. Edith Cowan University, Nedlands, WA, Australia

2. Alzheimer's Research Australia, Nedlands, WA, Australia

3. Perth Children's Hospital, Perth, WA, Australia

4. UWA Medical School, University of Western Australia, Perth, WA, Australia

5. Macquarie University, Sydney, NSW, Australia

Objective: Childhood dementia (CD) is caused by more than 100 rare genetic disorders, and it is estimated that 143 children are living with dementia in Western Australia (WA). Monitoring of neurocognitive function is challenging in CD and a reliable blood test to assess neurodegeneration is a health care priority. Lysosomal storage disorders (LSDs) contribute to 20% of all CD and our main objective is to assemble a cohort of children affected by LSDs, determine changes in blood biomarkers and evaluate its association with neurodegeneration.

Methods: The participants for this study will include children with a confirmed, documented diagnosis of LSD and age-matched healthy controls. Participants for this study were recruited through Perth Children's Hospital (Ethics RGS5097). The research plan will focus on developing a combined signature of protein and gene biomarkers using SIMOA (SIngle MOlecule Array), Olink and RNA sequencing technologies. Diagnostic biomarkers for CD including oligosaccharides in urine and gangliosides in blood plasma will also be quantified.

Findings: Our LSD patient cohort currently comprises of mucopolysaccharidosis (MPS) type 1 (n= 1), MPS type II (n = 1), MPS type III (n =3), MPS type IV (n=2), Batten disease (n=2), Neurodegeneration with Brain Iron Accumulation (n=1) and Adrenoleukodystrophy (n = 3). Baseline collection of samples is complete, and analysis of protein, gene and diagnostic biomarkers is underway. In addition to improving prognosis, access to clinical trials and care pathways for WA children, this study will establish WA as a world leader and improve outcomes for children with dementia globally.

id #21444

Visualization and chemoproteomic-analysis of psychedelic drugs using click-chemistry compatible analogs.

Joseph Benetatos[1], Sara Zimmerman[1], Andrew Stewart[2], Giuseppina di Salvo [2], Lauren Arias[3], Andy Domkos[3], David Olson[3], Ian Maze[2], Loren Looger[1]

1. Neuroscience, University of California San Diego, La Jolla, CA, United States

2. Neuroscience, Icahn School of Medicine at Mount Sinai, New York, USA

3. Chemistry, University of California Davis, Davis, CA, United States

Psychedelic drugs produce profound states of altered consciousness and show promise for treating mental health disorders. These drugs promote synaptogenesis, increase neurite outgrowth, and increase spine size, processes which are thought to underlie their beneficial long-term effects. Psychedelics structurally resemble the neuromodulators serotonin and dopamine, and indeed the prevailing theory of psychedelic mechanism of action is agonism of 5-HT2A receptors. However, psychedelics are so potent, long-lasting, and varied in their effects on cognition and neuroplasticity that additional mechanisms seem likely. We hypothesized that in addition to binding to receptors, psychedelic drugs, like amine neuromodulators, are chemically reactive and can covalently react with specific glutamine residues of diverse proteins through enzymatic addition.

To test this hypothesis, we utilized click-chemistry compatible psychedelic analogs of both tryptamines and phenethylamines. These analogs allow the downstream incorporation of functional groups such as a fluorophore for microscopy or an affinity tag for purification and proteomics analysis. Visualization of the analogs in cultured human neurons revealed a rapid localization to the nucleus. Using larval zebrafish we are conducting a brain wide analysis of drug localization. Purification and proteomic analysis of covalent drug interactions in human neurons showed an enrichment of nuclear proteins, including Histone H3. A nuclear function of psychedelics was further supported by phospho-proteomics of human neurons treated with parent compounds. Further research is needed to determine the functional consequences of protein modification by psychedelic drugs. We predict that an epigenetic mechanism could underlie the beneficial long-term effects of a single administration of a psychedelic drug.

id #21956

Unlocking The Origin: Deciphering The Critical Role of Neural Progenitor Cells In Pediatric High Grade Glioma Progression

Kenny Chi Kin Ip[1], Grace Yan Hay YH Lee[1], Maria MT Tsoli[1], David DZ Ziegler[1]

1. Brain Tumour Group, Children's Cancer Institute; University of New South Wales, Randwick, NSW, Australia

Pediatric high-grade glioma (pHGG) is one of the deadliest cancers, with an extremely poor prognosis in children and no effective treatments available at diagnosis. Neural progenitor cells (NPCs), precursors to various neural lineages, have been proposed as the potential cell-of-origin for these aggressive brain tumours. NPCs are highly proliferative and can secrete factors that promote the growth and differentiation of neighboring cells. Strikingly, clinical studies have shown that gliomas in adult have a significantly higher recurrence rate when the tumor is located closer to the subventricular zone, a region which is enriched with NPCs. However, the role of NPCs in promoting pHGG tumor growth and invasion has not been explored.

In this project, we have demonstrated that NPC-conditioned medium significantly enhances cell proliferation and migration in patient-derived pHGG cells harboring various types of oncogenic mutations (e.g., H3.K27M and H3.G34R). Mechanistically, treatment of pHGG cells with NPC-conditioned medium significantly upregulates the expression of growth and proliferative markers (p-MTOR, p-FGF and FOS) alongside with neural stem cell markers (NESTIN and SOX2). These findings suggest that NPCs may contribute to tumor growth by activating the cancer stem cell population through secreted oncogenic factors, leading to systemic tumour growth and invasion. Taken together, we have shown for the first time NPCs play a direct oncogenic function in advancing pHGG tumour development.

id #22213

Persisting symptoms, quality of life and return to work following mTBI in an Australian community-based cohort: results from the Concussion Recovery Study (CREST)

Jacinta Thorne[3, 1, 2], Ola Gozt[4], Sarah Hellewell[1, 2], Jemma Keeves[1, 2], Gill Cowen[6, 5, 1, 2], Libby Thomas[7], Alexander Ring[3], Jun Chih[6], Caerwen Beaton[1, 2], Francesca Buhagiar[8], Amanda Jefferson [5], Melissa Papini[1, 2], Glenn Arendts[9, 10], Antonio Celenza[9, 11], Ben Smedley [12], Sjinene VanSchalkwyk[13], John Iliff[5, 14, 15, 16], Nicole Ghedina[17], Russell Young[18], Monica Marton [19], Suzanne Robinson[20], Melissa Licari[21], Dan Xu[5, 7, 22], Stephen Honeybul[23], Michael Bynevelt[24], Carmela Pestell[8], Daniel Fatovich[25, 26], Melinda Fitzgerald[1, 2]

1. Perron Institute for Neurological and Translational Science , Nedlands, WA, Australia

2. Curtin Health Innovation Research Institute, Curtin University , Bentley, WA, Australia

3. School of Allied Health , Curtin University , Bentley, WA, Australia

4. Connectivity Traumatic Brain Injury Australia, Nedlands, WA, Australia

5. Curtin Medical School , Curtin University , Bentley , WA, Australia

6. School of Population Health , Curtin University, Bentley, WA, Australia

7. School of Population Health , Centre for Clinical Research Excellence , Perth, WA, Australia

8. School of Psychological Science , University of Western Australia, Nedlands, WA, Australia

9. Division of Emergency Medicine, University of Western Australia, Nedlands, WA, Australia

10. Emergency Department, Fiona Stanley Hospital, Murdoch, WA, Australia

11. Emergency Department, Sir Charles Gairdner Hospital , Nedlands, WA, Australia

12. Emergency Department, Rockingham General Hospital, Rockingham, WA, Australia

13. Emergency Department, Joondalup Health Campus, Joondalup, WA, Australia

14. Emergency Department, St John of God Hospital, Murdoch, WA, Australia

15. School of Medicine, Notre Dame University , Fremantle, WA, Australia

16. Royal Flying Doctor Service, Western Operations, WA, Australia

17. Emergency Department , St John of God Hospital , Midland , WA, Australia

18. Emergency Department, Albany Regional Hospital, Albany, WA, Australia

19. Emergency Department, Geraldton Regional Hospital , Geraldton , WA, Australia

20. School of Health and Development , Deakin University , Melbourne, VIC, Australia

21. The Kids Research Institute Australia, Nedlands, WA, Australia

22. The First Affiliated Hospital , Sun Yat-Sen University , Guangzhou, China

23. Department of Neurosurgery , Sir Charles Gairdner Hospital , Nedlands, WA, Australia

24. Neurological Intervention and Imaging Service, Sir Charles Gairdner Hospital , Perth, WA, Australia

25. Emergency Medicine, Royal Perth Hospital , Perth, WA, Australia

26. Centre for Clinical Research in Emergency Medicine, Harry Perkins Institute of Medical Research , Nedlands, WA, Australia

Background: Some people experience persisting post-concussion symptoms (PPCS) for months or years after mild traumatic brain injury (mTBI). Our aim was to evaluate the presence of PPCS, impact on quality of life (QoL) and ability to return to work (RTW) at 3- and 12-months after mTBI in an Australian population.

Methods: Prospective, longitudinal cohort study of adults aged 18-65 years who sustained mTBI from a range of mechanisms. Demographic, pre- and peri-injury data were collected by telephone within seven days of injury. Primary outcome measures were PPCS (Post-Concussion Symptom Scale symptom severity score >6 for males; >7 for females), health-related QoL (QOLIBRI-OS); and RTW at 3- and 12-months post-mTBI.

Findings: 232 participants, median age 33y (IQR 24-50y), 101(43.5%) female, 74(31.9%) sports-related mTBI, were included in analysis. Of 164(70.7%) participants completing 3-month follow-up, 81(49.7%) had PPCS. At 3-months median QOLIBRI-OS score was 87.5% (IQR 67-96). Of those working pre-

injury, 131(95.6%) had RTW. Notably, 59(43.1%) of people who had RTW at 3-months had PPCS. At 12-months, 137(59.1%) people completed follow-up; 62(45.3%) were experiencing PPCS and median QOLIBRI-OS score was 88.0% (IQR 71-96). Of those working pre-injury, 109(94.8%) had RTW, with 38.3% working with a degree of PPCS.

Interpretation: Of those participants who completed follow-up, almost half reported experiencing persisting symptoms at 12 months after mTBI. Over a third of participants continue to work while symptomatic. Outcomes for those lost to follow-up are unknown, representing potential response bias. Further investigation into the social, financial and economic burden of PPCS is warranted.

id #21446

Using Brain Microglia Autofluorescence as an Index of Neuroinflammation

Mary Slayo[1], Blanca Del Rosal[2], Sarah J Spencer[1], Kylie M Quinn[1, 3]

1. School of Health and Biomedical Sciences, RMIT University, Melbourne, Victoria, Australia

2. School of Science, RMIT University, Melbourne, Victoria, Australia

3. Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

During aging-associated inflammation, microglia adopt chronic overactivity of their pro-inflammatory response that increases risk of neurodegenerative. Aging microglia also accumulate ROS-producing autofluorescent lipofuscin. Identifying aging-related changes in the spectral profile of this autofluorescent brain lipofuscin will help to develop autofluorescent-based biomarkers. Here we hypothesized that aging increases microglia autofluorescence and elicits a distinct spectral signature from young brain autofluorescence. To detect changes in the microglial autofluorescence signal with aging, we used young (3 months) and aged (21 months) female C57BL/6 mice. Brain microglia were immunolabelled for ionized calcium-binding adapter molecule 1 (Iba-1) with Alexa Fluor 647. We assessed microglial autofluorescence granules in the hippocampus. Emission spectra of granules from young and aged unstained brain tissue were analysed using a confocal microspectroscopy Using Bitplane Imaris software, we established that aging significantly increased the total volume of autofluorescence in hippocampal microglia, with significantly higher number of large (> 10 μm^3) granules in the aged. Spectral signatures show that both ages displayed a broad emission band with peaks between 510 nm and 675 nm, eight of which were common for both ages. When signatures were examined separately according to size, larger aggregates in aged brains were more intense in far-red peaks compared to smaller aggregates from both the aged and young, causing a spectral redshift. Our findings suggest that autofluorescent material aggregates with age in microglia and may be an autofluorescent biomarker of inflammaging. These findings highlight an opportunity for future spectral identification of aging-related autofluorescent signals of microglia during brain inflammaging.

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id #21958

Distinct Neural Activity Patterns across Fear-Related Learning Paradigms using Engram Tagging

Roger Marek[1], Matthew Kenna[1], Li Xu[1], Pankaj Sah[1]

1. Queensland Brain Institute, St. Lucia, QUEENSLAND, Australia

Fear learning, where a neural cue or environment (CS) is paired with a fearful event such as a shock (US), is a widely used model for investigating the mechanism of learning. Although learning typically involves plastic changes in individual neurons, the activated neural circuitry varies depending on the learning paradigm.

We here present learning-specific neural activation patterns using the engram tagging technique in prefrontal cortices and the hippocampus - regions implicated in converting learned information into long-term memories. Engram tagging allows for labelling of neurons that become active during specific phases of learning. We employed a technique called robust activity marking to label neurons active during the learning or retrieval of classical fear conditioning, trace conditioning, cued and contextual conditioning.

While many published studies have tagged neurons active in classical fear conditioning to identify the “learning engram”, our data suggests that the activity patterns during the learning stage reflects the sensory processing of cues and the shock. In contrast, tagging of neurons active during the retrieval of the learned fear revealed significantly more activity in hippocampal areas for encoding contextual and trace conditioned fear. Retrieval of trace conditioning also led to significantly more activity in the anterior cingulate cortex.

Together, this dataset reveals neural activation patterns that are highly dependent on the learning modality and underscores the importance of considering learning-paradigms that drive specific neural circuits. Further, the neural circuits active during learning may not represent a true memory, but rather an ensemble of activated neurons encoding sensory information.

id #22214

Morphometric assessment of a koala brain using MR mapping methods

Kristy RQ Goh[1], Timothy Stait-Gardner[2], Nyoman D Kurniawan[3], Marianne D Keller[1]

1. Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, Australia
2. Nanoscale Organisation and Dynamics Group, Western Sydney University, Sydney, NSW, Australia
3. Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia

Australia's isolated evolution resulted in a significant divergence of brain anatomy of marsupials compared to eutherians. As brain atlases do not need to use multiple specimens; this study's aim is to describe morphometric measurements and volumetry of a formalin-fixed brain of a naturally deceased mature female koala.[1] EPA-NSW Scientific license: SL102840.

The MRI dataset was acquired on a 9.4T Bruker MR system, using a 3D multigradient echo sequence, with TR/TE = 70msec/4msec, a field of view = 3x3x4cm, single excitation, with an isotropic image resolution of 0.2mm^3. For three-dimensional volume reconstruction AMIRA post-processing software was used.

The naturally lissencephalic brain of this koala had a volume of 15,549mm^3. For each structure, the volume and percent-ratio to the total brain volume (tbv) were: olfactory bulbs = 418mm^3 (2.85% tbv); cerebral hemispheres = 9337mm^3 (60% tbv); anterior commissure, collectively ventral hippocampal commissure and fasiculans abberans were 148mm^3 (1% tbv); The ventricular system = 172mm^3 (1% tbv); brainstem = 1874mm^3 (12.06% tbv); cerebellum = 2305mm^3 (14.82% tbv).

Interhemispheric connections in marsupials are of interest, as they do not have a corpus callosum but an alternative tract, the fasciculus aberrans, which requires further investigation using tractography.

The olfactory bulb in the koala occupies a smaller proportion of the tbv compared to the quokka, which has a more variable diet.[2] The caudate nucleus in the koala has been found to be larger than the putamen, which might be due to prioritisation of goal directed behaviour, over fine motor control

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id #22215

Mental health, personality and quality of life factors affecting mTBI recovery in an Australian cohort.

Amanda Jefferson[1, 2, 3], Sarah Hellewell[1, 2], Jemma Keeves[1, 2, 4], Rachel Singer[1, 2], Jacinta Thorne[1, 2, 5], Melinda Fitzgerald[1, 2]

1. Perron Institute for Neurological and Translational Science, Perth, WA, Australia
2. Curtin Health Innovation Research Institute, Perth, WA, Australia
3. Curtin Medical School, Curtin University, Bentley, WA, Australia
4. School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia
5. School of Allied Health, Curtin University, Bentley, WA, Australia

Background

17,700 Australians present to hospital annually with mild traumatic brain injury (TBI). 10-50% of people with mTBI experience poor recovery and deal with the sequelae of brain injury for months or years. Our objective was to gain insight into psychosocial factors of Australians with mTBI and identify those experiencing persistent post-concussion symptoms (PPCS), so that strategies can be employed to improve their recovery.

Methods

An online retrospective survey was conducted, inviting Australian residents aged 18-65 who experienced mTBI within the preceding 18 months. Results were analysed using Chi-squared tests or ANOVA using PPCS as the outcome variable. Logistic regression was used to determine the relationship between mental health (MH), personality traits (using the Big Five Inventory-10) and quality of life (QoL) and the presence of PPCS at 18m.

Results

Of 201 participants (47.6%F), 27(13.4%) had PPCS at 18m, with headache and nausea (physical disturbances), the most common complaints. Participants were 3x more likely to experience PPCS if they had a MH diagnosis pre-injury (p=0.048), and 5x more likely to remain symptomatic with a MH diagnosis post-injury (p=0.018). Of the five personality traits, neuroticism and extraversion were associated with anxiety, depression and/or panic attacks (p<0.025) and QoL was lower in the group with PPCS (p=0.002).

Conclusion

These findings suggest the presence of a MH disorder and scoring high in the neuroticism or low in the extraversion personality domains, may predispose people to poorer recovery. Incorporating these measures into clinical practice may allow early identification and personalised intervention to aid recovery.

id #22216

'Reaching for Robotics' : A mixed methods study of clinician perspectives on robot-assisted lower limb rehabilitation in Western Australia

Nikki Bakhtazad[1], Amanda Timler[1, 2], Matthew K Bagg[1, 3, 4], Rachel Buckingham [1], Taya Hamilton[3], Trish Leonard[5, 6], Stuart Hodgetts[3, 7], Barby Singer[8], Ann-Maree Vallence[9], Jessica Nolan [1, 10, 11]

1. School of Health Sciences, University of Notre Dame, Fremantle , Western Australia

2. Institute for Health Research, University of Notre Dame, Fremantle , Western Australia, Australia
3. Perron Institute for Neurological and Translation Science , University of Western Australia Medical School , Nedlands , Western Australia
4. Centre for Pain IMPACT, Neurosience Research Australia , Randwick , New South Wales , Australia
5. State Rehabilitation Service, Fiona Stanley Hospital , Murdoch , Western Australia, Australia
6. Australian Institute of Neuro-rehabilitation , Osbourne Park , Western Australia, Australia
7. School of Human Sciences, Anatomy, Physiology and Human Biology , University of Western Australia , Crawley , Western Australia, Australia
8. School of Medical and Health Sciences , Edith Cowan University , Joondalup , Western Australia, Australia
9. School of Psychology, College of Health and Education , Murdoch University , Murdoch, Western Australia, Australia
10. School of Allied Health , Curtin University , Bentley , Western Australia, Australia
11. Physiotherapy Department, Sir Charles Gairdner Osborne Park Health Care Group , Nedlands , Western Australia, Australia

Objectives

This study aims to investigate the perspective of Western Australian (WA) clinicians towards the use of robot-assisted lower limb rehabilitation alongside conventional therapy for people with neurological conditions. It also aims to explore the optimal service delivery for this technology in order to enhance neurorehabilitation in the future.

Methods

WA clinicians, working in neurorehabilitation, were recruited to participate in a survey and an optional interview. Fifty-six clinicians completed the survey. Eleven participated in an additional interview, 6 (54.5%) of whom had prior clinical experience using robotic rehabilitation. Survey data were summarised descriptively. Interview data were thematically analysed using a reflexive approach.

Key Findings

The majority of clinicians were interested in upskilling in robotic rehabilitation (n=49, 87.5%) and incorporating robotics into their clinical practice (n=44, 78.6%). Lack of access to devices, limited clinician confidence, and varied attitudes towards device usability and funding were identified as the main limiting factors to robotic implementation. Comprehensive clinician upskilling and improved access to robotic knowledge and support services were reported as key facilitators to robotic use.

Conclusion

Clinician perspectives of robot-assisted lower limb rehabilitation are varied. However, findings indicate strong interest in the clinical adoption of robotics statewide. Addressing current barriers and embracing identified facilitators could inform the creation and delivery of robot-assisted neurorehabilitation services in WA, optimised for this state. A centralised robotic service was proposed as a way to improve clinical outcomes and quality of life for clients throughout WA and encourage clinicians to incorporate using robotic assistance into neurorehabilitation programs.

id #22217

Repetitive Magnetic Stimulation Increases Intracellular Ca^{2+} Levels in Cultured Primary Oligodendrocytes

Joshua Sone[1, 2], Jamie Beros[1, 2], Aleks Miljevic[1, 2], Kaylene Young[3], Jennifer Rodger[1, 2]
1. UWA, Crawley, WA, Australia
2. Perron Institute, Perth, WA, Australia
3. Menzies Institute, Hobart, Tasmania, Australia

Objectives: Repetitive transcranial magnetic stimulation (rTMS) has been shown to promote oligodendrocyte (OL) survival, maturation and remyelination *in-vivo*, which has therapeutic potential in the treatment of demyelinating diseases. The neurobiological mechanism of rTMS-induced OL plasticity is not currently known. rMS *in-vitro* (no cranium) increases calcium (Ca^{2+}) signalling in neurons and astrocytes, and Ca2+ signalling in OLs is a key regulator of OL development and myelin sheath dynamics, thereby presenting as a possible candidate. Our preliminary study aimed to determine whether rMS increases intracellular Ca^{2+} levels in cultured primary OLs.

Methods: Postnatal day-1 C57Bl/6J mice cortices were dissociated, and OL-progenitor cells immunopanned, cultured and differentiated. OLs were incubated with Cal520-AM Ca^{2+}-indicator, and exposed to 600-pulses of rMS (intensity: 150mT) using 1Hz, 10Hz or sham stimulation for equivalent durations (10- and 1-minutes respectively). OLs were imaged every two minutes before and after rMS on a 2-photon microscope. Ca^{2+} was quantified as a percentage of average baseline fluorescence, with the fourth minute of baseline compared to the final minute of rMS.

Results: Ca^{2+} fluorescence intensity was unchanged from baseline in both sham treatments ($p>0.05$, Wilcoxon test, n=150/418). Ca^{2+} fluorescence intensity increased following 1Hz rMS to 134.1±19.3% of baseline ($p<0.0001$, Wilcoxon test, n=305) and following 10Hz rMS to 105.0±12.4% ($p<0.0001$, Wilcoxon test, n=674).

Conclusion: Our preliminary data suggests that rMS may alter Ca^{2+}-signalling and thus potentially induce OL plasticity. Future studies will investigate frequency-specific effects and the relative importance of pulse number vs duration of stimulation in regulating Ca^{2+} levels in primary OLs.

id #22218

Investigating the influence of *ABCA7* polymorphism on the lipidome in the Busselton Healthy Ageing Study (BHAS)

Luke Whiley[1, 2, 3], Samantha SY Lee[4], David A Mackey[4, 5], Bu B Yeap[6], Jennie Hui[7, 8], Michael L Hunter[7, 8], Hamid R Sohrabi[1, 9], Elaine Holmes[1, 3]
1. Health Futures Institute, Murdoch University, Murdoch, WA, Australia

2. Australian National Phenome Centre, Murdoch University, Murdoch, Western Australia, Australia
3. Centre for Computational and Systems Medicine, Murdoch University, Murdoch, Western Australia, Australia
4. Centre for Ophthalmology and Visual Science, University of Western Australia, Perth, Western Australia, Australia
5. School of Medicine, University of Tasmania, Hobart, Tasmania, Australia
6. Medical School, University of Western Australia, Perth, Western Australia, Australia
7. School of Population and Global Health, University of Western Australia, Perth, Western Australia, Australia
8. Busselton Population Medical Research Institute, Busselton, Western Australia, United Kingdom
9. Centre for Healthy Ageing, Murdoch University, Murdoch, Western Australia, Australia

Single nucleotide polymorphisms (SNPs) in the *ABCA7* gene region have been reported to elevate the risk of Alzheimer's disease (AD) by ~20%. However, their impact on fundamental biology remains unknown, as they do not appear to affect downstream protein function or metabolism. To elucidate any mechanistic links to disease, the impact of these polymorphisms on the biological system must be fully determined prior to disease onset.

Therefore, *ABCA7* SNP and metabolic phenotyping data were collected from 1953 individuals from the Busselton Healthy Ageing Study (BHAS) at the Australian National Phenome Centre (Murdoch University) using mass spectrometry lipidomics[1]. Data were acquired from samples collected at two timepoints, approximately six years apart. Baseline data underwent linear regression modelling against three *ABCA7* SNPs (rs3764650, rs4147929, and rs3752246) in the BHAS population, which have been associated with elevated AD risk in the literature.

Following quality control, the lipidomic dataset consisted of 860 lipid features. The minor allele frequency of the three *ABCA7* SNPs in the BHAS dataset ranged from 308-579 (heterozygous) and 17-69 (homozygous). Linear regression modelling of baseline data versus each lipid feature revealed a lipidomic signature indicative of perturbations in lactosylceramide metabolism for individuals carrying *ABCA7* polymorphisms associated with elevated AD risk.

These results align with previous reports of lactosylceramide perturbations linked to *ABCA7* polymorphism in independent cohorts[2]. Future work will focus on further elucidating the lipidomic signature of *ABCA7* polymorphism, including age-related trajectories and associations with additional AD risk factors such as biological sex, APOE status, BMI, and cognitive function.

1. Comprehensive Lipidomic Workflow for Multicohort Population Phenotyping Using Stable Isotope Dilution Targeted Liquid Chromatography-Mass Spectrometry, Ryan M et al. J Proteome Res. 2023 May 5;22(5):1419-1433. doi: 10.1021/acs.jproteome.2c00682.
2. Metabolome-wide association study on ABCA7 indicates a role of ceramide metabolism in Alzheimer's disease, Dehghan A et al. Proc Natl Acad Sci U S A. 2022 Oct 25;119(43):e2206083119. doi: 10.1073/pnas.2206083119.

id #21963

A Multi-Omic Approach for the Detection of Melanoma Brain Metastasis

Desiree Sexauer[1, 2], Russell Diefenbach[3, 4], Ashleigh Stewart[3, 4], Wei Yen Chan[3, 4], Anna L Reid[1, 2], Jenny H Lee[3, 4], Lydia Warburton[1, 2, 5], Luisa M Pinnel[1, 2], Rebecca Auzins[1, 2], Pauline Zaenker[1, 2], Alexander M Menzies[4, 6, 7], Richard A Scolyer[4, 7, 8], Aaron B Beasley[4, 6], Helen Rizos[1, 2], Elin S Gray[3, 4], Georgina Long[3, 9, 5]

1. Centre for Precision Health, Edith Cowan University, Perth, Western Australia, Australia
2. School of Medical and Health Sciences, Edith Cowan University, Perth, Western Australia, Australia
3. Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, New South Wales, Australia
4. Melanoma Institute Australia, Sydney, New South Wales, Australia
5. Department of Medical Oncology, Fiona Stanley Hospital, Murdoch, Western Australia, Australia
6. Department of Medical Oncology, Royal North Shore Hospital and Mater Hospitals, Sydney, New South Wales, Australia
7. Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia
8. Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital and NSW Health Pathology, Sydney, New South Wales, Australia
9. , Melanoma Institute Australia, Sydney, New South Wales, Australia

Context: Many melanoma patients with brain metastases present with large symptomatic tumours, leading to significant morbidity and poor prognosis. Early detection of brain metastases when they are small and asymptomatic is crucial for improving outcomes.

Method: In this project, microRNA (miRNA), tumour-associated autoantibodies, and brain-derived cell-free DNA methylation data were obtained from 34 melanoma patients with intracranial and extracranial metastases and 62 patients with only extracranial metastases (discovery cohort). We assessed the miRNA expression profile of the discovery cohort using a QIAseq miRNA library kit, followed by sequencing on an Ion Torrent Platform. The trimmed mean of M-values (TMM) method was used to normalise the miRNA expression count data, and lowly expressed miRNAs were filtered out using the *filterByExpr* function of the edgeR package. Library sizes were recomputed after filtering. Differential expression analysis was performed using the quasi-likelihood method of the edgeR package in R (v3.42.4). Differential expression with ≥1.5-fold changes and FDR ≤ 0.01 was considered significant.

Results: After expression filtering, 336/2633 miRNAs remained for further analysis. Of these 336 miRNAs, only one (miRNA-1246) was significantly enriched (logFC=2.028, FDR=0.0013) in patients with intracranial metastasis. To validate this miRNA target of interest, a TaqMan MicroRNA Assay will be performed using an independent validation cohort.

Conclusion: miRNA-1246 has pro-oncogenic functions in multiple cancers and is implicated in neuroinflammatory conditions. Further analysis is required for validation. A multi-omic model based on RNA-seq, methylation, and tumour-associated autoantibody data will be developed for more precise screening and prognostic assessment in metastatic melanoma patients.

id #21452

Potassium homeostasis during disease progression of amyotrophic lateral Sclerosis

Rebecca Stevenson[1], Evgeniia Samokhina[1], Sushmitha S Purushotham[1], Armaan Mangat[1], Ilaria Rossetti[1], Chandra S Malladi[1], John W Morley[1], Yossi Buskila[1]

1. Western Sydney University, Campbelltown, NSW, Australia

Objectives - Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder in which patients lose motor functions due to progressive loss of motor neurons in the cortex, brainstem, and spinal cord. Whilst the loss of neurons is central to the disease, it is becoming clear that glia, specifically astrocytes, contribute to the onset and progression of neurodegeneration. Astrocytes play an important role in maintaining ion homeostasis in the extracellular milieu and regulate multiple brain functions by altering their extracellular concentrations.

Methods - In this study, we have used electrophysiological recordings to investigate the ability of astrocytes to maintain K^+ homeostasis in the brain via direct measurement of the astrocytic K^+ clearance rate in the motor and somatosensory cortices of an ALS mouse model ($SOD1^{G93A}$).

Key findings - using electrophysiological recordings from acute brain slices, we show region-specific alterations in the K^+clearance rate, which was significantly reduced in the primary motor cortex but not the somatosensory cortex. This decrease was accompanied by significant changes in astrocytic morphology, impaired conductivity via Kir4.1 channels and low coupling ratio in astrocytic networks in the motor cortex, which affected their ability to form the K^+ gradient needed to disperse K^+ through the astrocytic syncytium.

Conclusion - these findings indicate that the supportive function astrocytes typically provide to motoneurons is diminished during disease progression and provides a potential explanation for the increased vulnerability of motoneurons in ALS.

id #22220

The Interplay Between Gut Microbiota, Inflammation, and Neurodevelopmental Disorders: A Focus on Autism Spectrum Disorder

Mohammed ALAMOUDI[1], Samantha Matta[1, 2], Chalystha Yie Qin Lee[1], Rachele Gore[1, 3], Edden SUBEJANO[3], Mitra Mohsenipour[1], Hithin VELAGAPUDI[3], Peter J Crack[2], Jennifer Wood[3], Anya Shindler[3], Ashley Franks[3], Elisa L. Hill-Yardin[1, 2]

1. School of Health and Biomedical Sciences, Royal Melbourne Institute of Technology , Bundoora, VIC, Australia

2. The University of Melbourne, Parkville, VIC, Australia

3. Department of Microbiology, Anatomy, Physiology and Pharmacology, School of Life Sciences, La Trobe University, Bundoora, VIC , Australia

Introduction: Autistic individuals commonly experience immune disorders and gastrointestinal (GI) symptoms and microbial dysbiosis. GI issues associated with altered gut microbiota may contribute to systemic inflammation and neurological dysfunction, potentially exacerbating core autism symptoms. We investigated impacts of acute inflammatory insult (dextran-sodium-sulphate-(DSS)-induced colitis), on the gut microbiome in *Nlgn3*^{R451C} mice with an *autism-associated* variant in the Neuroligin-3 cell adhesion protein expressed at neuronal synapses, and in *IFNAR1*$^{-/-}$mice (an immune-dysfunction mouse model).

Methods: 3%-DSS treatment for 7-days generated ulcerative colitis symptoms in 8-week-old *Nlgn3*^{R451C}, *IFNAR1*$^{-/-}$, *Nlgn3*^{R451C}*xIFNAR1*$^{-/-}$, and wild-type mice. We compared faecal microbial profiles using 16S rRNA sequencing.

Results: DSS treatment reduced microbial diversity in wild-type mice (P=0.004), particularly on Day-8 compared to SHAM-treated wild-type mice (P=0.03). Surprisingly, DSS-treated *Nlgn3*^{R451C} samples showed similar microbial diversity and richness compared to SHAM whereas microbial-community evenness was reduced in DSS-treated wild-type and *Nlgn3*^{R451C}*xIFNAR1*$^{-/-}$ mice. DSS treatment resulted in compositional dissimilarity of microbial communities in *Nlgn3*^{R451C} mice. Overall, the genus *Lachnospiraceae NK4A136-group* dominated, however, upon DSS treatment, *Clostridia UCG-014* and *Clostridia vadinBB60_group* increased on Day-4, and *Romboutsia* and *Akkermansia* increased on Day-8. Notably, *Akkermansia* was abundant in SHAM-treated $Nlgn3^{R451C}$ mice.

Conclusion: DSS-induced colitis reduced microbial diversity in Wild-type and *Nlgn3*^{R451C}*xIFNAR1*$^{-/-}$ mouse models, however $Nlgn3^{R451C}$ samples were unchanged. DSS-treated caused compositional differences across genotypes except for *IFNAR1*$^{-/-}$ mice. In response to inflammatory insult, bacterial genera associated with gut mucosal repair increased in abundance including in $Nlgn3^{R451C}$ mice. Here we show that a nervous system mutation associated with autism impacts gut microbiota in response to inflammation.

id #21453

Dopamine release in the ventral pallidum regulates reward processing

Matthew Williams-Spooner[1], Lauren Glozier[1], Kevin Chang[1], Asheeta Prasad[1]

1. Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia

The ventral pallidum (VP) is a key node for reward processing. However, the functional contribution of dopamine release in the VP is unknown.

Using a discriminative instrumental conditioning paradigm, male rats were first trained to press an active lever to obtain sucrose pellets. This was followed by extinction, reacquisition, and a progressive ratio test to assess motivation. We first performed circuit tracing to identify connections between the VP and midbrain dopamine neurons. We then mapped calcium activity (jGCaMP8m, n=8) and dopamine release ($GRAB_{DA}$, n= 10) in the VP using fibre photometry.

Lastly, we used chemogenetics and pharmacologically antagonised dopamine activity to causally demonstrate that VP activity and dopamine release in the VP support reward learning.

Circuit tracing confirmed that the VP receives input from midbrain dopamine. Fibre photometry found that the VP is involved in reward learning, and that reward learning involves dopamine release into the VP.

Using an inhibitory DREADD (Syn-hM4Di-mCherry, n=6) and control (Syn-mCherry, n=6), we then confirmed that inactivating the VP impairs reward learning and reacquisition. Finally, using fluphenazine, an antagonist of D1- and D2-like receptors, we showed that reward-related learning requires dopamine release into the VP.

Thus, using circuit tracing, fibre photometry, chemogenetics, and behavioural pharmacology, we provide converging lines of evidence that the VP is essential for reward learning in rats, and reveal a novel dopamine pathway in reward processing. We also demonstrate how VP activity and dopamine release into the VP map onto reward-related behaviours, such as working to obtain rewards vs consuming rewards.

id #21454

Unravelling the Neuropathology of Sudden Deaths During Infancy; Cell Death, Inflammation, Protein Regulation & Morphology

Rita Machaalani[1]

1. University of Sydney, University Of Sydney, NEW SOUTH WALES, Australia

Background: In Australia, on average, 130 babies per year, die suddenly and unexpectedly. For the majority, the cause is unknown and classified as Sudden Infant Death Syndrome (SIDS). The leading hypothesis for SIDS is a brainstem dysregulation in the face of hypoxic insult(s) causing failed cardiorespiratory responses succumbing with death, and often during a sleep period.

Aims: To determine whether the neuropathology of babies classified as SIDS differs to non-SIDS, and whether hypoxic related insults could have been contributory factors.

Methods: Formalin fixed and paraffin embedded brain tissue from n=160 infants were stained for various markers including caspase-3, TUNEL, acetylcholine, nicotinic receptors, Iba-1 (microglia), GFAP (astrocytes), BDNF and PACAP. Quantitative microscopic image analysis was undertaken to determine the proportion of cells expressing these markers in a total of 26 brain regions of interest. Levels were compared amongst the diagnostic groups and according to hypoxic related parameters of bedsharing, prone sleeping, cigarette smoke exposure and upper respiratory tract infection.

Results & Conclusion: Differences were evident for many of the markers in several of the brain regions of SIDS compared to non-SIDS, yet none were exclusive to SIDS and many changes were attributed to the presence of the hypoxic insult. Of the brain regions, the dorsal motor nucleus of the vagus is highlighted as a region for further in depth study given its involvement for many of the markers, particularly in relation to the cholinergic system.

id #21966

Characterising the onset and maturation of neocortical activity in marsupial joeys

Adam P Luff[1], **Dylan A Black**[1], **Laura R Fenlon**[1], **Rodrigo Suarez**[1]

1. The University of Queensland, Brisbane, Australia

Area-specific patterns of spontaneous neural activity emerge well before birth in humans and rodents. However, the spatial and temporal dynamics of early cortical activity have remained unknown due to the challenges of recording live brains in utero. Here, we combine in-pouch electroporation of the calcium indicator GCaMP6s with live widefield microscopy in marsupial joeys to visualise the onset and maturation of neuron activity patterns. By performing decompositions of optical flow data into principal modes and identifying sources and sinks of activity within and between cortical hemispheres during stages of cortical neurogenesis and wiring, we reveal the emergence of area-specific patterns of activity followed by mirror-like interhemispheric symmetry. Unbiased hierarchical clustering further revealed motifs of neural activity that coincide with functional maturation of specific cortical areas. Our findings are consistent with a pan-mammalian scheme of neural activity development that likely starts prior to the onset of sensory experience.

id #22223

Alteration of Brain White Matter Tracts and Plasma Free Fatty Acid Concentrations following Mild Traumatic Brain Injury

Ruby Gilroy[2, 1], **Amanda Jefferson**[2, 1, 3], **Melissa G. Papini**[2, 1, 3], **Jacinta Thorne**[1, 3, 4], **Caerwen S. Ellery**[2, 1, 3], **Andre N. Avila**[2, 1, 3], **Aleksandra K. Gozt**[1, 5], **Gill Cowen**[2, 1, 3], **Elizabeth Thomas**[6], **Alexander Ring**[4, 7], **HuiJun Chih**[8], **Glenn Arendts**[9, 10], **Antonia Celenza**[9, 11], **Ben Smedley**[12], **Sjinene Van Schwalkwyk**[13], **John Charles Illiff**[2, 14, 15, 16], **Philip Brooks**[2, 16, 17], **Nicole Ghedina**[17], **Russell Young**[18], **Monica Marton**[19], **Suzanne Robinson**[20], **Melissa Licari**[21], **Dan Xu**[2, 8, 22], **Stephen Honeybull**[23], **Michael Bynevelt**[24], **Carmela Pestell**[25], **Daniel Fatovich**[26, 27], **Melinda Fitzgerald**[1, 3], **Sarah C. Hellewell**[1, 28, 3]

1. Fitzgerald Lab, Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia

2. Curtin Medical School, Curtin University, Perth, WA, Australia

3. Curtin University, Curtin Health Innovation Research Institute, Bentley, WA, Australia

4. Curtin University, School of Allied Health, Bentley, WA, Australia

5. Connectivity Traumatic Brain Injury, Nedlands, WA, Australia

6. Centre for Clinical Research Excellence, School of Population Health, Curtin University, Bentley, WA, Australia
7. Institute for Immunology and Infectious Disease, Murdoch University, Murdoch, WA, Australia
8. School of Population Health, Curtin University, Bentley, WA, Australia
9. Division of Emergency Medicine, The University of Western Australia, Crawley, WA, Australia
10. Emergency Department, Fiona Stanley Hospital, Murdoch, WA, Australia
11. Emergency Department, Sir Charles Gardiner Hospital, Nedlands, WA, Australia
12. Emergency Department, Rockingham General Hospital, Rockingham, WA, Australia
13. Emergency Department, Joondalup Health Campus, Joondalup, WA, Australia
14. Emergency Department, St John of God Hospital Murdoch, Murdoch, WA, Australia
15. Royal Flying Doctor Service - Western Operations, Perth, WA
16. School of Medicine, University of Notre Dame, Fremantle, WA, Australia
17. Emergency Department, St John of God Midland Public Hospital, Midland, WA, Australia
18. Emergency Department, Albany Regional Hospital, Albany, WA, Australia
19. Emergency Department, Geraldton Regional Hospital, Geraldton, WA, Australia
20. School of Health and Development, Deakin University, Melbourne, VIC, Australia
21. The Kids Research Institute, Nedlands, WA, Australia
22. The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China
23. Statewide Director of Neurosurgery, Department of Health, Perth, WA
24. The Neurological Intervention & Imaging Service of Western Australia, Sir Charles Gardiner Hospital, Crawley, WA
25. School of Psychological Science, The University of Western Australia, Nedlands, WA
26. Emergency Medicine, Royal Perth Hospital, Perth, WA
27. Centre for Clinical Research in Emergency Medicine, Harry Perkins Institute of Medical Research, Nedlands, WA
28. Centre for Neuromuscular and Neurodegenerative Disorders, The University of Western Australia, Crawley, WA, Australia

Objectives: White matter (WM) tracts in the brain are susceptible to subtle alterations resulting from mild traumatic brain injury (mTBI), although these are not readily detectable using conventional MRI or fluid biomarkers. Given that WM is enriched with lipids which can cross the blood-brain barrier, lipids may be promising biomarker candidates and adjuncts to more sophisticated research MRI techniques to assess WM. This study aimed to determine whether there are significant differences in WM alteration and plasma lipid concentration in participants with mTBI compared to healthy control participants.

Methods: The Concussion REcovery STudy (CREST) recruited participants with mTBI and healthy controls from the Western Australian community. 48 participants (23 mTBI, 25 control; 40% female) were included with a mean age of 35y. Plasma samples collected within 7d of injury were analysed for free fatty acid (FFA) concentrations using a colorimetric assay. Diffusion MRI scans were acquired within 9d, and fractional anisotropy (FA) values for mTBI participants were compared to controls using tract-based spatial statistics for voxel-based analysis.

Key findings: Participants with mTBI had significantly elevated FFA concentrations compared to controls, with mean concentrations of 0.035 and 0.027 nmol/μL respectively. ($p<0.001$). Significant clusters of reduced WM FA were found in the corpus callosum, corticospinal tract, optic radiations and internal capsule.

Conclusion: mTBI results in concomitant increases in plasma FFA and WM alterations in major commissural and projection fibres of the brain. The essential role of FFAs in membrane integrity suggests this may be a promising complementary biomarker to WM diffusion MRI.

id #21968

Morphometric brain changes in an ovine CLN6 neuronal ceroid lipofuscinosis model

Marianne D Keller[1], Amelia C Nanni[2], Marina G Gimeno[1, 3], Imke Tammen[1]

1. Sydney School of Veterinary Science, University of Sydney, Sydney, NSW, Australia
2. School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia
3. Elizabeth Macarthur Agricultural Institute, Department of Primary Industries, Menangle, NSW, Australia

Neuronal ceroid lipofuscinoses (NCL) or Batten Disease, is a group of genetic fatal paediatric neurodegenerative lysosomal storage disorders. The autosomal recessive subtype CLN6, occurs in humans as a late-infantile onset disease. It also naturally occurs in Merino and South Hampshire sheep, making them an ideal model for human disease. This study aimed to identify morphometric changes that occur in brains of 15-month-old Merino sheep which were homozygous for the Merino CLN6 variant (c.184C>T; p.(R62C)), compared to healthy, Merino control brains. The formalin-fixed brains of seven affected and three control sheep were scanned in a 9.4T Bruker MR scanner with a T_1-weighted gradient echo (TE/TR=17.5/2500ms; voxel size= 0.4x0.4x0.8mm^3). To facilitate soft tissue segmentation and volume quantification 'Amira-avizo' was used. Volumetric and statistical analysis found that the cerebellum and arbor vitae of affected sheep had an 11% and 9% reduction of their volume respectively, which was not significant compared to controls. However, the corpus callosum, striatum and thalamus of affected sheep experienced a significant loss of volume; 77%, 54% and 73% respectively, compared to controls. These findings are consistent with previous *in-vivo* studies of the South Hampshire CLN6 model, which is caused by a different genetic variant. Our study showed that MRI analysis of formalin-fixed tissues is more cost effective and allows for high resolution imaging. The affected brain regions can be used as biomarkers in the evaluation of therapeutic interventions for NCL as well as in routine assessments of disease progression through *in-vivo* MRI scans.

id #22224

Prognostic Biomarkers for Childhood Dementia

FRAULEIN DENISE ARIGO[1], **Prashant Bharadwaj**[1], **Ralph Martins**[1, 2], **Steve Pedrini**[1, 2]

1. Centre of Excellence for Alzheimer's disease Research and Care, School of Medical and Health Sciences, Sarich and Patricia Neuroscience Research Institute, Edith Cowan University, Joondalup, Western Australia, Australia

2. School of Biomedical Science, Macquarie University, Sydney , New South Wales, Australia

Childhood dementia (CD) is a collective term for rare paediatric conditions characterized by severe degeneration of the CNS. Around one in every 2,900 babies are born with a condition that causes CD. Around 21% of CD is caused by lysosomal storage disorders, a group of 70+ rare genetic conditions that cause a systemic build-up of toxic materials in cells. Although diagnosis of most LSDs is relatively well established, there is a need for prognostic biomarkers that can improve disease monitoring and assess therapeutic efficacy of new and proposed treatments. A reliable and easily administered blood test to assess neurodegeneration is currently a health care priority for people with CD. The main aim is to assemble a cohort of individuals with CD caused by LSDs and to assess their blood biomarkers at baseline and again at 1 year. This project proposes the use of state-of-art biomarker technologies for discovery of protein biomarkers of neurological decline in CD. This study also aims to investigate genes associated with neurodegeneration and identify differentially expressed genes in CD. Our analyses will evaluate the relationships between protein, gene biomarkers, and standard diagnostic markers to identify a disease signature associated with neurodegeneration. Additionally, this will evaluate if drugs targeting the autophagy-lysosomal pathway can alleviate disease phenotypes in a neuronal cell model of CD. Findings of this research will advance knowledge into the neurological deterioration and novel treatment strategies to alleviate neuronal damage and will reveal the overlapping disease mechanisms between the subgroups highlighting potential common therapeutic targets.

id #21969

Using statistical genetics to investigate the relationship between stress-related traits and Alzheimer's disease.

Ayeisha Milligan Armstrong[1, 3, 4, 2], **Tenielle Porter**[1, 3, 4], **Giuseppe Verdile**[1, 2, 5], **David M Groth**[1, 2], **Emmanuel O Adewuyi**[3, 4], **Simon M Laws**[1, 3, 4]

1. Curtin Medical School, Curtin University, Bentley, WA, Australia

2. Curtin Health Innovation Research Institute, Curtin University , Bentley , WA, Australia

3. Centre for Precision Health, Edith Cowan University , Joondalup, WA, Australia

4. Collaborative Genomics and Translation Group, School of Medical and Health Sciences, Edith Cowan University , Joondalup, WA, Australia

5. School of Medical and Health Sciences, Edith Cowan University , Joondalup , WA, Australia

Epidemiological studies have shown that chronic life stress or stress-related disorders such as depression and anxiety are associated with an increased risk of developing Alzheimer's disease (AD). Underlying mechanisms remain poorly understood. To begin to identify potential biological pathways involved, the study aimed to investigate the shared genetic architecture between stress-related traits (cortisol secretion, stress-response, depression, anxiety and neuroticism scores) and AD-related phenotypes (AD incidence, cognitive measures, hippocampal volume, cerebral spinal fluid (CSF) amyloid-beta (Aβ) and phosphorylated tau levels) using statistical genetic methods. Data from large genome-wide association studies (GWAS) for each of these traits was utilised to explore genome-wide and local genetic correlation in a pair-wise manner (stress-related traits to AD-related traits). Mendelian randomisation was also undertaken to investigate potential causal relationships between stress-related traits and AD-related phenotypes. Finally, gene-level overlap assessments were undertaken to investigate whether there were biological pathways implicated in the risk that were shared between stress-related traits and AD-related phenotypes. A negative global genetic correlation between depression, anxiety and neuroticism with cognition was observed, along with a positive genetic correlation between depression and AD incidence. Local genetic correlation analyses also revealed shared genetic correlations between these traits and allowed for regions of the genome driving these associations to be identified. Finally, at the gene-level, there was significant overlap between neuroticism and AD incidence, cognition and CSF-Aβ. Taken together this study highlights that there is shared genetic architecture with implications in identifying potential biological pathways between stress-related traits and AD-related phenotypes.

id #22225

Biological Resilience in Stress Environments: The impact of Mild Traumatic Brain Injuries on Saliva Biomarkers

Grace A Bliesner[1, 2], **Ibolja Cernak**[3], **Sarah C Hellewell**[1, 2]

1. Curtin Health Innovation Research Institute, Curtin University, Bentley, WA, Australia

2. Perron Institute for Neurological and Translational Science, Nedlands, Western Australia, Australia

3. Thomas F. Frist, Jr. College of Medicine, Belmont University, Nashville, TN, USA

Modern warfare has resulted in a high percentage of military personnel experiencing mild traumatic brain injury (mTBI) and blast exposure due to training accidents and other occupational risks. These 'mild' injuries are thought to decrease mental resilience and increase the risk of developing maladaptive physiological processes. This study compared saliva neuroendocrine biomarkers in Canadian Armed Forces Personnel (n=108) over three unique stress environments: pre-deployment, deployment in Afghanistan and post-deployment reintegration. At each timepoint, participants provided saliva samples which were assessed for amylase, Chromogranin A (CgA) C-reactive protein (CRP), cortisol and testosterone. Participants were grouped based on their baseline TBI history (i.e. mTBI vs none), with further subgrouping for (a) both blast+mTBI: (b) blast only: (c) mTBI only: (d) no history of blast or mTBI. Two-way repeated measures ANOVAs were used to compare groups over time. Significant differences were found in amylase, CgA, CRP and testosterone concentrations (all $p<0.05$), with significant interactions between group, timepoint and sex ($p<0.05$). Notably, these differences were driven by the blast and blast+concussion subgroups for amylase and CgA, while CRP and testosterone levels were elevated over time only in the mTBI subgroup. No differences were found in cortisol concentrations at any timepoint. These findings suggest that blast and mTBI may cause differential responses to stress and influence biological resilience or susceptibility. Our findings provide unique insight into the mechanistic changes which occur during and following high stress military environments in at-risk populations, suggesting that blast and mTBI should be considered and treated as separate entities.

id #21458

Investigating Klotho gene expression patterns in healthy, Alzheimer's disease (AD) and treated human monocyte-derived microglia (MDMi)

Alan M Henrique[1, 2], Hazel Quek[1, 3], Juliana CS Chaves[1, 3], Merari FR Ferrari[2], Anthony White[1]

1. QIMR Berghofer Medical Research Institute, Brisbane/Wilston, QUEENSLAND, Australia

2. Institute of Biosciences, University of São Paulo, São Paulo, Brazil

3. Faculty of Medicine, University of Queensland, Brisbane, Queensland, Australia

Klotho is a neuroprotective protein associated with neurodegenerative disorders. Klotho mRNA is spliced into canonical and alternate transcripts. Expression ratio between these was associated with pathological conditions in kidney. While Klotho's functions in the kidney are well established, its mechanisms of action in the brain remain elusive. Klotho modulates brain neuroinflammation and mediates the crosstalk between immune system and central nervous system.

Total Klotho expression and alternative/canonical ratio were compared in monocyte-derived microglia (MDMi), neural progenitor cells (ReNcell VM) and peripheral blood mononuclear cells (PBMCs). MDMi cultured from PBMCs were subjected to differentiation using growth factors. MDMi generated from Healthy control (HC), AD, HC treated with β-amyloid (Aβ) and bushfire (BF), untreated ReNcells and PBMCs were used.

Overall, Klotho expression varied among groups. ReNcell showed low expression, while HC and AD MDMi showed similar levels, except for one high-expression AD individual. Similarly, MDMi treated with Aβ and BF had comparable Klotho expression with one outlier each. PBMCs showed great variability, with one individual showing minimal Klotho expression and other having the highest. Transcript ratio analysis indicated predominance of canonical over alternative Klotho in almost all samples. AD, Aβ and ReNcell showed lower alternative/canonical ratios. This suggests potential differences in Klotho splicing patterns across conditions.

Klotho expression in human microglia in highly variable, particularly under pro-inflammatory conditions. While kidney alternative/canonical Klotho ratio is higher under pathological conditions, the opposite trend was observed in microglia. These results raise questions Klotho's mechanism in the brain and its implications for brain health and disease.

id #21970

SOX transcription factors as LINE-1 retrotransposon modulators in the human brain

Juan M. Botto[1], M. Eugenia Ferreiro[1], Gabriela O. Bodea[1, 2], Sandra R. Richardson[2], Geoffrey J. Faulkner[1, 2]

1. Queensland Brain Institute, The University of Queensland, Saint Lucia, Queensland, Australia

2. Mater research institute, The University of Queensland, Woolloongabba , Queensland, Australia

Long interspersed element-1 (L1) is the only autonomously mobile human retrotransposon. The L1 sequence comprises a 5′ untranslated region (5′UTR) containing a promoter that drives the expression of the L1 retrotransposition machinery proteins ORF1p and ORF2p. The brain has been identified as a hotspot for somatic L1 expression and retrotransposition but the neuronal factors regulating L1 activity remain unclear. The L1 promoter contains two SOX transcription factor binding sites and thus, SOX proteins have been proposed as L1 modulators. We detected a high L1 signature in Parvalbumin interneurons, which are characterized by high SOX6 expression. Hence, we focused on studying the potential role of SOX6 as an L1 transcriptional activator in neurons.

To tackle this, we generated novel L1 retrotransposition and L1 transcriptional reporter assays. We found that SOX6 overexpression significantly enhanced endogenous L1 expression, as well as L1 retrotransposition and promoter reporter activity in HeLa cells. Mutating the first SOX binding site of the L1 5′UTR significantly reduced these effects. Moreover, deletion of the DNA binding domain of SOX6 decreased L1 transcriptional reporter activation. Lastly, we translated these findings into an *in vitro* human neuronal model (i3Neurons). We demonstrated that i3Neurons express endogenous L1 mRNA and ORF1p, and can accommodate L1 retrotransposition. Overexpression of SOX6 in mature i3Neurons increased ORF1p expression and L1 reporter activity.

These results present SOX6 as a novel transcription factor modulating L1 activity in the brain. Understanding L1 regulation in the brain is essential to comprehend its role in physiological neuronal function and neurological disease.

id #22227

Unlocking the potential for single-cell RNA sequencing and spatial analysis at scale with massively parallelized barcoding

Kyle Hatton-Jones[1, 2]

1. Vizgen, Inc, Cambridge, MA, United States

2. Scale Biosciences, San Diego, California, United States

Single-cell sequencing technologies, such as single-cell RNA sequencing (scRNA-seq) and single-cell methylation (scMET) profiling, have transformed our understanding of cellular heterogeneity by revealing gene expression and regulation at unprecedented resolution. However, these methods lack spatial context necessary for understanding tissue organization and cellular interactions. Spatial transcriptomics adds spatial information but is often limited in the number of genes or methylation sites profiled. Integrating spatial and single-cell genomic data can bridge this gap, offering multi-modal insights into complex tissues.

In this study, we integrated single-cell genomic data using the ScaleBio scRNA kit, ScaleBio Methylation kit, and Vizgen MERSCOPE® platform on adjacent brain slices from three regions in one mouse. Samples were profiled using Vizgen's MERSCOPE workflow with a 500-gene pan-neuron panel and ScaleBio's kits for transcriptome-wide RNA and methylation profiling. Integration of data from both platforms showed high-quality, spatially resolved information on gene expression and methylation states.

Our analysis revealed distinct spatial localization of scRNA clusters, high correlation between gene expression in Vizgen and scRNA data, and alignment with the Allen Brain Atlas for imputed spatial gene locations. Additionally, scMET data provided insights into epigenomic states, mapping methylation patterns onto

spatial transcriptome data.

This study demonstrates that integrating spatial gene expression, scRNA-seq, and scMET data enables comprehensive spatial genomic analysis, revealing gene expression and regulatory landscapes within complex neuronal tissues.

id #21460

Inflammatory Demyelination Disrupts the Cellular Integrity of the Neurovascular Unit

Jake M Cashion[1], Thomas S Lewis[2], Jessica Fletcher[2], Catherine G Foster[1], Gary P Morris[1], Lachlan S Brown[1], Kalina Makowiecki[2], Kaylene Young[2], Brad Sutherland[1]

1. Tasmanian School of Medicine, University of Tasmania , Hobart, Tasmania, Australia

2. Menzies Institute of Medical Research, University of Tasmania , Hobart, Tasmania, Australia

In multiple sclerosis (MS), immune cells breach the blood-brain barrier (BBB) and attack the central nervous system (CNS), causing significant neuroinflammation and demyelination. People with MS also experience neurovascular pathology, including BBB leakage, reduced blood flow and neurovascular uncoupling. In the CNS, these vascular functions are maintained by a multicellular structure known as the neurovascular unit, comprised primarily of endothelial cells, pericytes and astrocytes. The impact of inflammatory demyelination on cells within the neurovascular unit remains poorly understood.

To investigate the impact of MS-like pathology on the neurovascular unit, we utilised the cuprizone-autoimmune encephalomyelitis (CAE) model of inflammatory demyelination. CAE was induced by feeding mice 0.2% (w/w) cuprizone in powdered chow for two weeks to biochemically alter myelin, followed by stimulation of the peripheral immune system with complete Freund's adjuvant and pertussis toxin. After 21 days, immunohistochemistry confirmed peripheral immune cell (T cell) infiltration, oligodendrocyte loss, and demyelination in the brains of CAE mice. Immunohistochemistry also revealed the disruption of neurovascular cells following CAE. There was a reduction in $AQP4^+$ astrocytic endfeet coverage of the endothelium. Furthermore, $CD13^+$ pericyte soma density was significantly decreased, despite an increase in pericyte coverage of blood vessels. $CD31^+$ vessel morphology was also impacted, with vessels exhibiting reduced density, length and width, suggesting significant endothelial cell changes following CAE induction. These findings suggest immune-mediated demyelination in the brain significantly disrupts the cellular integrity of the neurovascular unit. Protecting cells of the neurovascular unit may be an attractive therapeutic approach to maintain vascular integrity in MS.

id #22228

Hippocampal function is altered in a model of chronic neuropathic pain

Bryony Winters[1]

1. The University of Sydney, Sydney, New South Wales, Australia

Pain is a complex sensory and emotion experience that typically facilitates a learning event so that an individual may avoid future harm. However, in a chronic pain context, pain that is experienced at inappropriate times no longer provides a useful function and may impair learning. Cognitive deficits including difficulties with learning and memory recall have been well reported in the clinic, but preclinical data is lacking and the neural basis remains poorly understood. Using a chronic constriction injury (CCI) model of neuropathic pain in mice, we found marked differences in basal synaptic transmission at CA3-CA1 synapses of the hippocampus compared to sham controls. In addition, two forms of long-term potentiation were markedly impaired in CCI mice compared to controls. Intriguingly, we found this impairment was lateralised to specific hemispheres, which were dependent on injury site and the type of synaptic plasticity studied. Consistent with altered hippocampal function, we also found mice with the CCI injury displayed impaired spatial recognition memory on the Y-maze task. Together these data provide evidence that neuropathic pain alters the function of a key memory centre of the brain, suggesting neuroadaptations associated with chronic pain are not limited to conventional pain circuits.

id #21717

Creating a micro-exon splicing map of early human brain development with genetically engineered pluripotent stem cell derived brain organoids

Pallavi Gupta[1, 2, 3], Hannah Leeson[1], Ishaan Gupta[2], Ernst J Wolvetang[1]

1. AIBN, The University of Queensland, Brisbane, Queenland, Australia

2. DBEB, Indian Institute of Technology, Delhi, India

3. UQ - IITD Research Academy, Delhi

Micro-exons are exons, 3-27nt in length, that modulate protein-protein interactions and are specifically spliced-in in neurons, affecting mRNAs involved in neuronal development and synaptic function. Differential inclusion of micro-exons has been reported in post-mortem brains of individuals diagnosed with autism spectrum disorder (ASD). This was attributed to a reduced expression of the splicing factor SRRM4. However, when, during development, mis-splicing occurs in ASD and which cell types are affected remains unclear. Brain organoids, derived from human embryonic or induced pluripotent stem cells, recapitulate early human brain development to a large extent and can therefore be used to elucidate the extent and impacts of dysregulated micro-exon splicing. In support of this idea, our bioinformatic analyses of in-house brain organoid models of neurodevelopmental disorders identified consistent patterns

across several conditions, including Down Syndrome (DS), Ataxia-Telangiectasia (AT), and Hypomyelination with Brainstem and Spinal cord involvement and Leg Spasticity (HBSL). To systematically investigate the role of micro-exon splicing we utilized the CRISPR-Cas9 system to knockout (KO) the micro-exon splicing factors SRRM4 and its paralog, SRRM3, in H9 embryonic stem cells and established clonally derived lines with validated frameshift mutations. We are currently utilizing these lines and their isogenic controls to generate cortical brain organoids that will be subjected to full-length single-cell RNA sequencing (scRNA-seq) across multiple timepoints during brain organoid maturation. This systematic analysis should unravel how disruptions in SRRM4 and SRRM3 function are associated with the pathophysiology of neurodevelopmental disorders and elucidate which mRNA species are affected in each cell type across development.

id #21974

LncRNA regulation of lysosome function in brain disease

Priyal Dass[1], Robert Day[1], Indranil Basak[1], Stephanie M. Hughes[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

Lysosomal function is essential for cellular homeostasis. This is highlighted by the involvement of dysfunctional lysosomes in Alzheimer's and Batten disease. Understanding regulatory networks involved in neuronal function is key. Recently, lncRNAs have emerged as a contributor to cellular regulation including autophagy, the degradation process reliant on lysosomes.

This project focuses on the lncRNA *DANCR* and its impact on neuronal lysosomes. Initially described as a regulator of differentiation, DANCR is known to regulate autophagy. However, no studies have investigated the influence of DANCR on lysosomes in human neurons.

Transcript variants of *DANCR* remain unexplored. To address this, Ensembl was used to select the four most biologically relevant *DANCR* transcripts. This also revealed *DANCR* shares a transcriptional start site with the uncharacterised lncRNA *LINC01618*. A qPCR panel was developed to differentiate between four *DANCR* and four *LINC01618* transcripts. In iPSCs, five out of eight transcripts were detected. CRISPRi guides were designed to target transcripts of *DANCR* or *LINC01618*. The knockdown iPSC lines showed 99.6% (P=0.0093) and 99.2% (P=0.0500) reduction in the expression of targeted *DANCR* and *LINC01618* transcripts respectively. Interestingly, the *DANCR* knockdown line showed almost 2-fold increase (P=0.0378) in proliferation compared to a control line.

Neurons derived from knockdown iPSC lines will be used to investigate the impact of *DANCR* and *LINC01618* on differentiation and lysosomal function. RNAseq will uncover molecular pathways impacted by *DANCR* and *LINC01618* in the knockdown lines. This will reveal the relationship between these lncRNAs and lysosomal regulation, and point towards potential therapeutic strategies.

id #21975

How do you feel? Neuroimaging Correlates of Cognitive Affective Bias in Healthy Humans

Ofir Gurfinkel[1, 2], Twain Dai[1, 3], Troy Visser[2], Jennifer Rodger[1, 3]

1. Perron Institute for Neurological and Translational Science, Nedlands, WA, Australia

2. School of Psychological Science, The University of Western Australia, Crawley, WA, Australia

3. School of Biological Sciences, The University of Western Australia, Crawley, WA, Australia

Objectives: Cognitive affective bias (CAB) refers to the influence of emotions on cognitive processes. While most healthy individuals exhibit a neutral or slightly positive CAB, some display a negative CAB which is associated with elevated risk for developing affective disorders. Critically, despite its prevalence in the population, the relationship between anxiety and CAB remains largely unexplored. To address this gap, we explored associations between CAB and cognitive and somatic symptoms of anxiety while investigating potential underlying neural correlates of CAB using MRI.

Methods: Twenty-four healthy individuals (12 female;17-25yrs) participated. To assess CAB, auditory and visual perceptual discrimination tasks were used which measured participants' tendency to process ambiguous information in a positive or negative manner. The State-Trait Inventory for Cognitive and Somatic Anxiety was then administered followed by a multimodal MRI session.

Findings: CAB is related to trait but not state anxiety. Notably, trait-somatic anxiety is related to the proportion of negative CAB responses, however, an association with reaction time of those negative CAB responses was not observed. In contrast, trait-cognitive anxiety is unrelated to the proportion of negative CAB responses, however, it is related to faster endorsements of negative CAB responses. Analysis exploring the relationship between CAB and structural MRI data is underway.

Conclusion: Our data suggests that the relationship between CAB and anxiety varies as a function of anxiety dimension, which can assist with identification and management of anxiety symptomatology. In conjunction with our MRI data, this research could contribute to improving diagnosis, treatment and prevention of affective disorders.

id #21464

Heterogenous thirst sensitive neurons within the anterior cingulate cortex modulate water intake in response to dehydration in mice

Khalid Elsaafien[2, 1], Lei A Wang[3], Jesus D Penaloza-Aponte[3, 4], Scott W Harden[3, 5], Karen A Scott[2, 1], Charles J Frazier[3, 5], Annette D de Kloet[2, 1], Eric G Krause[2, 1]

1. Neuroscience Institute, Georgia State University, Atlanta, Georgia, United States

2. Center for Neuroinflammation and Cardiometabolic Diseases, Georgia State University, Atlanta, Georgia, United States
3. Department of Pharmacodynamics, University of Florida, Gainesville, Florida, United States
4. Department of Neuroscience, University of Florida, Gainesville, Florida, United States
5. Center for Integrative Cardiovascular and Metabolic Diseases, University of Florida, Gainesville, Florida, United States

The anterior cingulate cortex (ACC) has recently emerged as a cortical structure involved in fluid homeostasis. Extracellular dehydration increases the synthesis of angiotensin II, which acts on receptors in the brain to mediate fluid ingestion. This study investigated the role of angiotensin receptor-containing neurons within the ACC in the regulation of fluid homeostasis. We found an abundance of neurons within the ACC that express angiotensin type 2 receptors (referred to as ACC^{AT2R}). To determine whether ACC^{AT2R} are thirst sensitive, we delivered Cre-inducible adeno-associated virus synthesizing the calcium indicator, GCaMP6f, into the ACC of male AT_2R-Cre mice. Following implantation of a GRIN lens targeting the ACC, we used a head-mounted miniscope to record calcium events in conscious freely-moving mice. Dehydration excited a subset of ACC^{AT2R} but these neurons became inhibited upon rehydration with water ingestion. RNAscope *in situ* hybridization revealed that ACC^{AT2R} neurons are either GABAergic or glutamatergic. Follow-up anatomical and electrophysiological studies confirmed that GABAergic ACC^{AT2R} are inhibitory inter-neurons that make inhibitory synapses onto neighboring neurons, whereas glutamatergic ACC^{AT2R} form functional glutamatergic projections to thalamic nuclei. Next, we utilized intersectional genetics to selectively target GABAergic ACC^{AT2R} and reveal that optogenetically exciting this subset of neurons attenuates dehydration-induced water intake. Conversely, optogenetically exciting the subset of ACC^{AT2R} fibers that project to the thalamus increases water intake. The collective implication of the studies is that the excitatory/inhibitor balance of ACC^{AT2R} modulates the sensation of thirst by mediating fluid homeostasis.

id #21465

Treatments for those who have none platform: from Western Australia to nationwide

May Aung-Htut[1, 2], Jessica Cale[1, 2], Vanessa Fear[3], Timo Lassmann[3], Gareth Baynam[4, 5], Steve Wilton[1, 2]
1. Murdoch University, Perth, WA, Australia
2. Perron Institute for Neurological and Translational Science, Perth, WA, Australia
3. Telethon Kids Institute, Perth, WA, Australia
4. Rare Care Centre, Perth Children's Hospital, Perth, WA, Australia
5. University of Western Australia, Perth, WA, Australia

Despite individual low prevalence, rare diseases (>7,000) collectively affect between 263 million and 446 million people worldwide. Most (95%) rare diseases do not have approved treatment and approximately 45% cause neurological disorders. We initiated “Treatment for those who have none” platform for Western Australian children with a rare disease in 2021, with support from a Channel 7 Telethon Trust research grant. The purpose of the platform is to accelerate treatments from bench to bedside for rare diseases using antisense oligomer-mediated modulation of gene expression.

Through Rare Care Centre, Perth Children Hospital, we recruited patients diagnosed with a rare disease, and analysed the consequences of the mutation at the RNA levels. Based on these observations and known pathogenic mechanisms, we selected three patients, two with neurological disorders and one with kidney disease, for ASO design and assessment. We designed ASOs to either increase levels of gene expression for the children with neurological problems or redirect pre-mRNA splicing to induce a truncated protein with some functionality for the child with kidney disease.

Within 18 months, we have designed ASOs and performed proof-of-concept studies in patient-derived cells for three rare diseases Kleefstra syndrome, Birk-Landau-Perez Syndrome and Alport syndrome. With this experience, we are now extending this platform nationwide through collaborations with Royal Brisbane Hospital, Queensland University Technology, University of New South Wales, Garvan Institute, Sydney Children Hospital network, Murdoch Children Research Institute, and Royal Hobert Hospital. We aim to integrate this program into the healthcare system as a treatment route for rare diseases.

id #21977

Developmental vitamin D deficiency increases DNA methylation in embryonic mesencephalon

Xiaoying Cui[1, 2], Renata Pertile[1], Paul Marshall[3], Darryl Eyles[1, 2], Suzy Alexander[1, 2], Asad Ali[1]
1. Queensland Brain Institute, The University of Queensland, St Lucia, QLD, Australia
2. Queensland Centre for Mental Health Research, Wacol, QLD, Australia
3. ANU College of Health and Medicine, Canberra, ACT 2601

Schizophrenia, a complex neurodevelopmental disorder, is characterized by aberrant dopamine neurotransmission. Previous research has established a link between developmental vitamin D (DVD) deficiency and an increased risk of schizophrenia in humans. Our rodent model further supports this association, demonstrating impaired dopamine neuron maturation. Given vitamin D’s role in DNA methylation, a critical epigenetic regulator, we investigated its potential involvement in dopamine development.

We observed a selective upregulation of a DNA methyltransferase (DNMT3A) from a panel of DNA methylation enzymes in the embryonic day 14 mesencephalon of DVD-deficient embryos. In primary mesencephalic neural cultures, DNMT3A overexpression and silencing decreased and increased CCND1 (Cyclin D1) and CDKN1A (P21) expression, respectively, without altering dopamine-associated transcript expression. Furthermore, hypermethylation of the CCND1 and CDKN1A promoters was detected in DVD-deficient embryos. These findings suggest that DVD deficiency induces aberrant DNA methylation, impacting cell cycle regulation in the developing mesencephalon.

Our results provide evidence that DVD deficiency may contribute to the pathogenesis of schizophrenia by disrupting early brain development. The observed hypermethylation of cell cycle genes in the mesencephalon could underlie the delayed differentiation of dopaminergic neurons, ultimately leading to the long-

term dopamine dysfunction characteristic of schizophrenia. Our study highlights the importance of vitamin D in early brain development and suggests a possible epigenetic mechanism by which DVD deficiency can contribute to schizophrenia.

id #21978

Theta Burst Transcranial Magnetic Stimulation to Treat Premenstrual Dysphoric Disorder: An Open Label Pilot Study

Elizabeth HX Thomas[2, 1], Caroline Gurvich[2], Leo Chen[2, 1, 3]

1. Monash Alfred Psychiatry Research Centre, Alfred Health, Melbourne , Victoria, Australia

2. Department of Psychiatry, School of Translational Medicine, Monash University, Melbourne, Victoria, Australia

3. Alfred Mental and Addition Health, Alfred Health, Melbourne , Victoria, Australia

Background: Premenstrual Dysphoric Disorder (PMDD) is a depressive disorder that occurs in a cyclical pattern due to hormonal changes in the menstrual cycle. Symptoms are most severe during the premenstrual phase, with existing treatments providing limited relief. Theta burst stimulation (TBS) has demonstrated efficacy in treating depression and may be a promising approach to treating mood and other psychological symptoms of PMDD.

Methods: Twelve women with PMDD completed the Daily Record of Severity of Problems (DRSP), a measure of daily PMDD symptoms, every day for a three-month period. Months 1 and 2 were defined as the pre-treatment phase. During Month 3, the treatment phase, participants received daily 10-minute sessions of prolonged intermittent TBS (piTBS) over 5 continuous days during their premenstrual phase (determined by an ovulation test), delivered at an intensity of 120% of their resting motor threshold.

Results: There was a decrease in premenstrual symptom elevation of the total DRSP from pre-treatment (25.03%) to treatment phase (9.96%). Looking at each DRSP symptom domain, premenstrual symptom elevation decreased from pretreatment to treatment phase across all domains; depression (23.67% to 9.62%), anxiety (23.96% to 10.93%), mood lability (30.21% to 12.86%), anger (26.28% to 14.39%), interest (29.16% to 17.32%), concentration (27.29% to 10.20%), lethargy (31.65% to 3.59%), appetite (27.99% to 7.92%), sleep (24.58% to 1.95%), overwhelmed (21.26% to 12.21%) and physical symptoms (20.47% to 9.91%).

Conclusion: These preliminary findings demonstrate that piTBS reduces symptom elevation (i.e. improves symptoms) during the premenstrual phase and may be an effective treatment for women with PMDD.

id #22234

Analysis of gender and institutional representation of invited speakers at the ANS annual meeting

Heather McPherson[1], Lizzie Manning[2], Michelle Rank[3], Lyndsey Collins-Praino[4], Johanna Montgomery[5], Ethan Scott[3], Melissa Sharpe[6], Adele Woodhouse[7]

1. Queensland Brain Institute, The University of Queensland, Queensland

2. University of Newcastle, Newcastle

3. The University of Melbourne, Melbourne

4. The University of Adelaide, Adelaide

5. The University of Auckland, Auckland

6. The University of Sydney, Sydney

7. University of Tasmania, Tasmania

Invited presentations at large major conferences like ANS are critical for career progression and promotion; however, historically, the scientific program of conferences worldwide has poorly reflected the diversity of those scientific fields. In 2017, the ANS Equity and Diversity committee proposed a new policy: gender balance in invited and selected talks at the ANS annual meeting should reflect the overall gender balance of ANS membership. This was ratified by council in 2019.

Here, we analyse publicly available data on invited speakers at the ANS conference, focussing on plenaries (1981-2024) and symposia (2008-2024), to assess the impact of this policy on gender balance in representation at the annual meeting. In addition to gender equity, analysis of institutional and geographic representation was also performed.

Analysis of plenary speakers shows that representation of women has increased significantly across the last 40 years. Before 2004, women represented <10% of plenary speakers, whereas across the next decade, this increased to an average of ~20% women. More recently, including following the introduction of the gender equity policy, this has continued to increase, with women speakers averaging ~40% between 2014 and 2019, which subsequently further improved to 50%. We also noted that while around 60% (n=113 of 178 total) of plenary speakers were from Australia, only 5 were from New Zealand, in comparison to 32 from the USA, 7 from the UK, and 6 from Germany.

These data suggest that, over time, gender balance amongst plenary speakers has shifted to better reflect the gender equity in membership. Nevertheless, geographic representation remains heavily skewed. We also recognise that several groups may be under-represented amongst ANS invited speakers (e.g. LGBTQIA+, First Nations, People with disabilities), which will be important to understand to meet the Society's goals around equity and inclusion.

id #21979

Effects of psilocin on neuronal networks of cortical brain organoids using a high-density multi-electrode assay (HD-MEA)

Selin Pars[1], Dara Daygon[1], Huiwen Zheng[1], Jessica Mar[1], Ernst Wolvetang[1]

1. AIBN, Brisbane, QLD, Australia

Psilocybin is a neuromodulatory compound found in psychedelic mushroom genus *Psilocybe*. For the past decade, psilocybin, along with other psychedelic compounds such as lysergic acid diethylamide (LSD) and N,N-Dimethyltryptamine (DMT) were found to have therapeutic effects for neuropsychiatric diseases such as major depressive disorder, anxiety, and drug addiction. It is known that psilocybin binds to 5-HT2A receptor in cortical neurons and increases synaptic density and causes changes in default mode network (DMN). However, the mechanism of action of psilocybin in human neurons are incompletely understood.

In order to assess neuromodulatory effects of psilocybin in human neurons, we used human iPSC-derived cortical organoids. Cortical organoids include the key cellular subtypes of human cortex; excitatory and inhibitory neurons, neural stem cells, astroglia and oligodendrocyte precursor cells, which we have confirmed in 6-month-old organoids using single cell RNA sequencing. Additionally, we found *HTR2A* (5-HT2A gene) expression to be the highest in excitatory neurons among other neuronal subtypes. To assess neural activity changes with psilocybin, we employed high density multi electrode assay and exposed cortical organoids with 6 µM of psilocin (active compound in psilocybin). Our analysis indicated that psilocin increased the number of network bursts and network connectivity, however, decreased the overall burst rate, as well as peak-to-peak amplitude of spikes in cortical organoids.

Our results show that cortical brain organoids are a suitable in vitro human neuronal model to study the effects of psilocybin at molecular level. This in vitro model can be utilised for safety and neurotoxicity effects of psychedelic substances.

id #21980

The Effect of a Novel Poly-Arginine Peptide on α-Synuclein Pathology Implicated in Parkinson's Disease

Emma Robinson[1, 2], Bruno Meloni[1, 3, 4], Anastazja Gorecki[2, 5], Ryan Anderton[2]

1. Neurotherapeutics Group, Perron Institute for Neurological and Translational Science, Perth, WA, 6009

2. School of Health Sciences, Univeristy of the Notre Dame, Fremantle, Perth, WA, 6160

3. Department of Neurosurgery, Sir Charles Gairdner Hospital, WA, 6009, Australia

4. University of Western Australia, Perth, 6009

5. Curtin Health Innovation Research Institute, Ralph and Patricia Sarich Neuroscience Research Institute, 6009, WA, Australia

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder, affecting over 8.5 million people worldwide. The aggregation of α-Synuclein (α-syn) into pathological oligomers and fibrils, known as Lewy bodies, is central to the pathogenesis and progression of PD. Pathologic α-syn seeds induce further aggregation, propagating throughout neurons and causing cytotoxicity and neurodegeneration. Cationic arginine-rich peptides (CARPs) are gaining attention due to their diverse neuroprotective effects and protein anti-aggregatory properties, establishing them as a promising therapeutic avenue for PD. Available PD therapies are palliative, failing to address the underlying a-syn pathology. This study aims to characterise both intracellular a-syn aggregation and intercellular a-syn propagation (uptake) *in vitro*, and investigate the therapeutic potential of the CARP, R18D (18-mer of D-arginine), to prevent these processes. To mimic PD pathology, a-syn aggregate overexpression is induced in the rodent neuronal cell-culture model through two approaches: administration of a-syn seed and MG-132, a proteasome inhibitor. To investigate a-syn aggregation inhibition by R18D, neuronal cultures receive R18D treatment at various concentrations and are monitored 48 hours later. Any changes induced by R18D are quantified using homogenous time-resolved fluorescence. To investigate a-syn uptake inhibition by R18D, α-syn seeds are treated with R18D and introduced to neuronal cultures where uptake is directly visualised using confocal microscopy. Preliminary HTRF results and confocal image analysis suggest R18D may reduce a-syn aggregation and uptake. R18D represents a novel therapeutic approach to reduce key processes of α-syn pathology and may be critical in halting or slowing the aggressive progression of PD.

id #21469

Unveiling Cholinergic Vulnerability During Aging and Chronic glial activation in Alzheimer's Disease Pathogenesis

Erika Gyengesi[1], Yossi Buskila[1], Rose Chesworth[1], Ingrid Wagnon[1], Ilaria Rossetti[1], Rashmi Gamage[1]

1. Western Sydney University, Penrith, NSW, Australia

Chronic neuroinflammation, a multifaceted response of the central nervous system (CNS) to various disruptive stimuli, encompasses reactions to infections, autoimmune insults, and protein misfolding, implicated in neurodegenerative disorders, notably Alzheimer's disease (AD). We hypothesize that chronic exposure to neuroinflammatory mediators exacerbates glial reactivity leading to cholinergic vulnerability during aging, and proposing a link between cholinergic transmission alterations and glial activation.

A comprehensive approach combining behavioral studies, in vitro electrophysiology assessing cholinergic neuron firing patterns, and immunohistochemistry combined with stereology and single-cell morphological analysis and qPCR was employed to investigate the excitability profile of single basal forebrain cholinergic neurons (BFCNs) in a transgenic mouse model of chronic neuroinflammation (GFAP-IL6).

Across age groups (4-, 12-, and 18-months), significant increases in microglial numbers within the medial septum were observed, accompanied by a notable decrease in cholinergic cell numbers at 12 and 24 months. Aging and chronic glial activation impacted septal cholinergic function, evident through alterations in passive and active membrane properties, firing patterns, and morphological changes. Cholinergic neuron reductions and increased astrocyte numbers during aging and chronic neuroinflammation, particularly evident in adult mice, were noted. The firing activity of cholinergic neurons was compromised, possibly due to altered membrane properties.

Neuroinflammation is a pivotal factor in neurodegenerative disorders, that involves astrocytes and microglia, and CNS homeostasis disruptions. Brain region-specific analyses revealed diverse responses to IL-6 overexpression, emphasizing the influence of age on neuroinflammatory impact and cholinergic vulnerability. These findings offer valuable insights into potential therapeutic strategies for mitigating neuroinflammatory-driven neurodegeneration, particularly in AD.

1. Chronic neuroinflammation during aging leads to cholinergic neurodegeneration in the mouse medial septum. Gamage R, Rossetti I, Niedermayer G, Münch G, Buskila Y, Gyengesi E. J Neuroinflammation. 2023 Oct 13;20(1):235. doi: 10.1186/s12974-023-02897-5.PMID: 37833764
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id #21981

Completion of Massive Open Online Course Improved Global Distribution of Knowledge about Traumatic Brain Injury

Yasmine V Doust[1], Hannah Fair[1], Claire Eccleston[1], Christine Padgett[1], Kathleen Doherty[1], Peta S Cook[1], Jenna M Ziebell[1], Jenna Ziebell[1]

1. University of Tasmania, Hobart, TASMANIA, Australia

The understanding traumatic brain injury massive open online course (TBI MOOC) was developed to improve awareness and reduce misconceptions of TBI in the community. This study assessed the impact of the TBI MOOC on participant's misconceptions of TBI. Participants (n=1,267) were from 45 different countries that represented all 6 continents except for Antarctica where 91.7% were from high income countries. Most of the study population were women (82.6%) with a mean age of 45.8 years (18 – 83 years) and have completed tertiary education or above (65.8%). In the March 2023 iteration of the TBI MOOC, 10.5% of the respondents indicated that that they had sustained a TBI whilst 45.7% responded that a family member is living with a TBI. Additionally, 48.9% of all study participants stated that they are currently providing paid care for individuals living with a TBI. Participants who exhibited the lowest scores on the misconceptions about TBI survey prior to completing the MOOC had either lower educational levels, never provided paid care, are currently providing unpaid or voluntary care, have never personally sustained a TBI or had a family member who has sustained a TBI. However, after completing the MOOC all participants, regardless of group, had similar scores indicating that the MOOC is an effective tool to reduce misconceptions of TBI in the community.

id #21471

Identifying Connectome-based Neuroimaging Biomarkers Associated with Behavioral Changes in An Animal Model of Depression: An Exploratory Analysis

Twain Dai[1, 2], Shannon Algar[3], Michael Small[3], Jennifer Rodger[1, 2]

1. School of Biological Sciences, The University of Western Australia, Perth, WA, Australia

2. Perron Institute for Neurological and Translational Science, Perth, WA, Australia

3. The Complex Systems Group, Department of Mathematics and Statistics, The University of Western Australia, Crawley, WA, Australia

Background: Traditional analyses to identify resting-state functional MRI (rs-fMRI) biomarkers for depression have relied on uncovering group-level patterns in cross-sectional data. This approach fails to adequately capture individual behavioral and neurobiological variability in core symptoms (anxiety and psychomotor disturbance). Therefore, the present study aimed to identify rs-fMRI biomarkers associated with the development of anxiety-related behaviors and locomotion disturbance following chronic restraint stress (CRS) at an individual level in rats.

Methods: 96 male Sprague Dawley rats underwent two sessions of MRI and behavioral tests, before and after CRS. Behaviors were assessed with elevated plus maze and forced swimming test. Hierarchical clustering was applied to construct functional clusters of the brain. Ridge-regularized partial correlation was used to compute connectivity between each pair of functional clusters. Regression was employed to identify rs-fMRI biomarkers associated with behavioral changes following CRS.

Results: Following CRS, alteration of functional connectivity between the salience-orbitofrontal and lateral-temporal cortical cluster was associated with anxiety-related behaviors. Connectivity changes in the hippocampal-cortical and default mode-somatomotor clusters with the lateral-temporal cortical cluster were associated with locomotor disturbance. High individual variability was presented in these functional alterations.

Conclusions: The present study is the first to identify connectome-based biomarkers in the brain associated with anxiety and psychomotor disturbance using an animal model of depression. It provides another piece of evidence supporting the idea that biomarker research would benefit from shifting the focus from group to individual level. Our results shed light on biomarkers to aid diagnosis and treatment of depression in humans.

id #21984

Exploring the relationship between Alzheimer's disease, oral health, and the oral microbiome: A narrative review

Thilini Jayasinghe[1], Smitha Sukumar[1], Tihanna Divnic-Resnik[1], Binosha WMAD fernando[2], Axel Spahr[1]

1. The University of Sydney, Camperdown, NSW, Australia

2. School of Medical and health Sciences, Edith Cowan University, Joondalup, WA, Australia

Alzheimer's disease (AD) is a complex neurodegenerative disorder with potential links to oral health. Recent studies suggest that dysbiosis of the oral microbiome may influence the progression of cognitive decline in AD. This narrative review aims to systematically examine our current understanding of the relationship between oral health, the oral microbiome, and Alzheimer's disease. It focuses on identifying key mechanisms, and gaps in knowledge with the aim to direct future research and develop potential therapeutic interventions. The review includes a comprehensive search of peer-reviewed articles from 2014 to 2024, databases such as PubMed, Scopus, and Web of Science), focusing on clinical studies, systemic reviews, and meta-analyses. Preliminary findings indicated a consistent link between poor oral hygiene and elevated levels of specific pathogenic bacteria, such as *Porphyromonas gingivalis*, in the oral

microbiome of Alzheimer's patients. These studies also suggest that individuals with greater oral dysbiosis exhibit more pronounced cognitive decline, as measured by various cognitive assessment tools. Furthermore, evidence from animal models supports the hypothesis that oral bacteria may contribute to neuroinflammation and AD pathology. These preliminary findings highlight the potential role of oral microbiome dysbiosis in the progression of Alzheimer's disease.

id #21473

Turnover dynamics of CNS border-associated macrophages in adult mice

Yi Xu[1], Jana Vukovic[1], Seung Jae Kim[1], Emily Willis[1], Marc Ruitenberg[1]

1. Faculty of Medicine, The University of Queensland, Brisbane, Queensland, Australia

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. We adopted a transgenic model, SclCreERT2 x R26-ZsGreen 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 invasive surgeries like whole-body irradiation followed by Bone marrow transplantation inducing strong immune responses. Using this method, we quantitatively analysed the peripheral monocyte-based BAM turnover of adult mice under the homeostatic conditions.

id #21985

Decoding SARM1's role in cortical axon degeneration: a live imaging study

Ensieh Izadi[1], Bill Bennett[1], Anna King[1], Alison Canty[1]

1. Wicking Dementia Research and Education Centre, University of Tasmania, HOBART, TAS, Australia

Despite the widespread occurrence of axon degeneration in the injured and diseased nervous system, our understanding of axon degeneration remains incomplete. Sterile Alpha and TIR motif-containing protein 1 (SARM1) has been identified as important for regulating axon degeneration in many parts of the peripheral nervous system. The role of SARM1 in cortical axon plasticity and degeneration remains unclear. Here we use live imaging through a cranial window to directly visualise excitatory axons in the cortex of adult Thy1-GFP-M mice (WT-GFP) crossed with SARM1 null mutants (SARM1KO-GFP). Long synapse-rich axons are targeted with the imaging laser to create a microlesion, disconnecting the distal axon segment from the cell body. Imaging of the surviving axon at 48h intervals allows synapses to be identified as stable, gained, or lost (pre/post-lesion). In the absence of SARM1, synaptic density is maintained, but synaptic turnover (gains and losses) is higher in SARM1KO-GFP axons (WT-GFP:10axons; SARM1KO-GFP:10axons; $p=<0.05$). In disconnected axon segments undergoing Wallerian degeneration, we identify and compare key steps in the degenerative process. Preliminary analysis shows disconnected SARM1KO-GFP axons undergo axon thinning and reactive blebbing, remain intact for a period of time, and then commence similar fragmentation processes as WT-GFP axons (WT-GFP:10axons, SARM1KO-GFP:10axons). Our data highlight the key role of *in vivo* imaging in providing new insights on how SARM1 contributes to synaptic plasticity and axon degeneration in the injured central nervous system and open the door for targeting SARM1 in therapeutic interventions in the injured brain.

id #21474

Superior colliculus anatomy and neurochemistry remain intact following primary visual cortex lesion

Melissa Chong[1], Katrina Worthy[1], Marcello Rosa[1], Nafiseh Atapour[1]

1. Monash University, Clayton, VIC, Australia

Visual input from retina to primary visual cortex (V1) is necessary for proper vision[1]. Induced lesions in V1 in non-human primates cause blindness[2] and have been shown to lead to widespread damage to the structures along the visual pathway, including lateral geniculate nucleus (LGN) and retina[3, 4]. Both of them undergo neurodegeneration and volume loss post-V1 lesion[3, 4], and LGN in particular displays additional neurochemical changes[5]. The superior colliculus (SC), a layered midbrain structure facilitating polysensory and sensorimotor integration, has direct and indirect V1 connections[6-9]. We investigated in adult marmoset monkeys (*Callithrix jacchus*) if degenerative changes could be extended to SC. We also investigated the expression of orthodenticle homeobox 2 protein (Otx2) – a retinal protein that can be transported across axons towards other visual structures[10, 11] – as a measure of change in SC neurochemistry following V1 lesion. Our study revealed that ipsilateral lesioned SC volume was not significantly different from contralateral or control/healthy SC, and SC volume shrinkage had little correlation with that observed in LGN. Estimates of total neuronal density and density of Otx2-positive neurons were similar between lesioned and control SC. Lesion induction age had no impact on Otx2 expression in the SC. Our data supports the idea that SC may be protected from detectable degeneration and volume loss as a result of V1 lesion by its widespread connectivity with structures other than V1. The absence of any detectable change in Otx2 staining supports the idea that SC neurochemistry remains unaffected by V1 lesion.

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id #21986

A novel probucol analogue lowers amyloid-beta level and prevents blood-brain barrier dysfunction in diabetic mice

Arazu Sharif[1, 2], Ryu Takechi[2, 3], John Mamo[2, 3, 4], Virginie Lam [2, 3]

1. Curtin Medical School, Curtin University, Bentley, WA, Australia

2. Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia, Australia

3. Curtin School of Population Health, Curtin University, Perth , Western Australia, Australia

4. Neurovascular and Metabolic Disease, Perron Institute for Neurological and Translational Science, Perth, WA, Australia

Heightened vascular exposure to elevated levels of free amyloid-beta (Aβ) and lipoprotein-Aβ contributes to blood-brain barrier (BBB) dysfunction and the development of neurodegenerative processes in the brain. Alterations in lipoprotein metabolism can affect the circulating levels of lipoprotein-Aβ by increasing its production in lipogenic organs, such as the intestine, and reducing its clearance in the blood. Therefore, BBB disruption in diabetes may result from increased capillary exposure to lipoprotein-Aβ due to disturbances in lipoprotein metabolism. Previously, probucol, a historic lipid-lowering drug, was shown to mitigate the intestinal production and secretion into blood of lipoprotein-Aβ and prevent neurovascular pathologies in murine models of dietary-induced BBB dysfunction. However, the high hydrophobicity of probucol limits its bioavailability and therapeutic potential. To address this, a novel probucol analogue has been developed by our lab. In this study, using the db/db mouse model of diabetes, we investigated whether probucol and its novel analogue, through modulation of peripheral lipoprotein-Aβ, protect BBB integrity and prevent neurovascular inflammation. Our results indicated elevated levels of enterocytic Aβ, and plasma Aβ42 and Aβ oligomers, concomitant with increased BBB permeability in control db/db mice. Both probucol and equimolar administration of the probucol analogue reduced intestinal Aβ production. However, only probucol analogue lowered plasma Aβ42 and Aβ oligomer levels and prevented BBB dysfunction, oxidative stress, and neuroinflammation. Collectively, this data suggests that the novel probucol prodrug has high potential in modulating peripheral Aβ levels and preventing BBB disturbances in diabetes.

id #22242

Proteomic Profiling of the Ovine Brain: Insights for Translational Neuroscience Research

Merce Fuentes Amell[2, 1], Kirsten Coupland[2, 1], Neil Spratt[2, 1, 3], David Skerrett-Byrne[4], Renee Turner[1], Annabel Sorby-Adams[4]

1. School of Biomedical Sciences and Pharmacy, The University of Newcastle, Newcastle, NSW, Australia

2. Heart and Stroke Research Program, Hunter Medical Research Institute, Newcastle, NSW, Australia

3. Department of Neurology, John Hunter Hospital, Hunter New England Local Health District, Newcastle, NSW, Australia

4. School of Biomedicine, Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide

Background: The sheep (*Ovis aries*) is a powerful, translational large animal model in neuroscience. The sheep brain has several characteristics in common with the human brain that rodent models lack including comparable brain size, the presence of gyri and sulci and white matter proportion that more closely resemble those of the human brain. Despite these benefits, the proteomic profile of the disease-free ovine brain remains under-characterized, limiting the use of this model in characterising the on- and off-target effects of trial therapies.

Aims: characterise the proteome of disease-free sheep brain using cutting-edge mass spectrometry.

Methods: Naïve Sheep brain tissue from each hemisphere was collected and digested into peptides. Unbiased, unlabelled mass spectrometry analysis was performed using an Orbitrap Exploris 480 coupled with high-field asymmetric waveform ion mobility spectrometry (FAIMS) at 40, 50, 60 and 70 compensation voltages. Peptide matches were be adjusted for 1% false discovery rate, and bioinformatic analysis was conducted using Perseus and Ingenuity Pathway Analysis (IPA, QIAGEN) to identify activity of canonical molecular pathways. Comparative analysis between the two hemispheres was conducted to explore differences in their proteomes.

Results: Biological replicates were highly correlated (Pearson correlation >0.89). There is no significant difference in protein abundance between biological

replicates (p = 0.902), and no significant differences in protein abundance between brain hemispheres within an individual. IPA analysis of proteins in the top quartile of abundance showed significant enrichment in the synaptogenesis, EIF2 and Rho GTPase cycle pathways.

Conclusion: We present, to date, the most in-depth characterisation of the sheep brain proteome. The results of this study are a valuable resource for future research in translational neuroscience and will assist in the testing of new diagnostic and therapeutic strategies for brain-related disorders.

id #21992

Investigating a novel dopaminergic projection from Ventral Tegmental Area to Ventral Hippocampus in associative learning

Kiruthika Ganesan[1], Masakazu Taira[1], Melissa Sharpe[1]

1. School of Psychology, University of Sydney, Camperdown, NSW, Australia

Dopamine neurons in the ventral tegmental area (VTA_{DA}) have been traditionally viewed as assigning value to cues and context paired with rewards (e.g., food). However, recent work has revealed that VTA_{DA} neurons contribute in more complex ways to learning and behaviour than previously thought. This has catalysed efforts to understand how distinct dopamine circuits might contribute to different types of learning. Here, we investigated how an understudied dopamine circuit comprising the ventral hippocampus (vHP) contributes to associative learning. First, we established a novel projection from VTA_{DA} neurons to vHP by performing retrograde tracing. We then took advantage of fibre photometry of a genetically encoded dopamine sensor, $GRAB_{DA}$, to precisely record dopamine released in vHP as rats experienced a neutral cue (e.g., tone) paired with a reward. We compared dopamine signals across this reward learning task with that of fear conditioning, where rats experienced a new neutral cue (e.g., light) with mild footshock. In both cases, a dopamine signal developed to both the reward- and shock-paired cues across learning sessions. This signal showed a unique and interesting temporal dynamic, which does not reflect a quintessential dopamine prediction error (i.e., phasic increase of dopamine at cue onset). Specifically, we saw a delayed and slow increase in the dopamine response at cue onset, which reduced below baseline before cue offset. This mirrored the dopamine response during reward and shock receipt, which was reminiscent of a 'stimulus-stimulus' association. This reveals a unique role for dopamine in vHP in associative learning.

id #21481

A genetic vaccination approach to generating cognition-enhancing antibodies in mice

Conor Nelson[1], Angela Wu[1], Deborah Young[1]

1. University of Auckland, Auckland, AUCKLAND, New Zealand

N-methyl-D-aspartate receptor (NMDAR) activity is involved in the processes of learning and memory. A novel antibody-based therapy developed by our lab targeting the NMDAR GluN1 subunit has previously been found to differentially modulate NMDAR function to improve learning and memory in 6-month-old and 22-month-old mice following passive transfer of IgG antibodies. We investigated genetic vaccination approaches using adeno-associated viral vectors (AAVs) as a mechanism of antigen delivery to produce therapeutic anti-GluN1 antibodies *in vivo* in mice and compared its effect to our existing polyclonal therapy. C57/BL6j mice received an intramuscular injection of AAV8 or AAV6 vectors expressing a recombinant GluN1 antigen (recGluN1) cDNA, with a luciferase transgene included as a control. Western blot analysis and ELISA assays were used to monitor the development of serum recGluN1 and luciferase antibodies at 8 weeks and 12 weeks post-vector injection. Learning and memory were assessed via the Morris Water Maze, Open Field Test, and Novel Object Recognition Test. Results from our pilot study demonstrated that AAV6 but not AAV8 vectors led to long-term antibody production following treatment. Serum antibodies reacted to recGluN1 epitopes similar to those produced in rats immunised with recGluN1 protein. Analysis of behavioural data is underway. However, current results suggest that this alternate approach to antibody production could facilitate long-term therapeutic studies and offer a method to rapidly screen similar immunotherapies in disease models.

id #21993

Expression profile of the clustered protocadherins in the mouse hippocampus and their role in engram formation and memory processes

Olivier Clement[1], Clara Ortega-de San Luis[2, 3], Charles Herring[1], Mariia Yurova[2, 3], Esteban Urrieta[2, 3], G Guillaume[2, 3], Lydia Marks[2, 3], Ryan Lister[1, 4], Tomas Ryan[2, 3, 5, 6]

1. Harry Perkins Institute of Medical Research, The University of Western Australia, Nedlands, WA, Australia

2. School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland

3. Trinity College Institute for Neuroscience, Trinity College Dublin, Dublin, Ireland

4. ARC Centre of Excellence in Plants for Space, The University of Western Australia, Nedlands, WA, Australia

5. Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria

6. Child & Brian Development Program, Canadian Institute for Advanced Research, Toronto, Ontario, Canada

Memories are critical for our survival and are stored in engram cells through plastic changes. Upon memory acquisition, engram cells are thought to refine their synaptic connections to permanently store the newly acquired information. In this process, cell adhesion molecules play a crucial role by mediating precise cell-to-cell interactions and synapse formation. With 57 different genes, the clustered protocadherins (cPcdhs) represent the largest subgroup of the cadherin

superfamily of cell surface protein and function as a molecular barcode to mediate highly specific self-recognition, playing a pivotal role in neuronal self-avoidance. If the role of cPcdhs during brain development is well established, their contribution to the regulation of engrams and memory function is poorly understood, which is the focus of this study.

Using single-nucleus sequencing, we first examined the precise expression profile of cPcdhs in engram and non-engram cells in the hippocampus and explored their dynamics during memory processes. Then, using a combination of genetic tools, optogenetics and behavioural tests, we investigated how the manipulation of cPcdhs in the mouse hippocampus alters engram formation and function, leading to memory deficits. Altogether, our results demonstrate the important role for cPcdhs in engram physiology and memory processes.

id #21483

Transcription Factor-based Induced-differentiation of Immortalised Neuronal Cell Lines

Madeline Meli[1], Kristy Swiderski[2], Marissa Caldow[2], Gordon Lynch[2], Huseyin Sumer[1], Brett Cromer[1]

1. Swinburne University of Technology, Melbourne, VIC, Australia

2. The University of Melbourne, Melbourne, VIC, Australia

In-vitro investigation of neuronal development and function was initially performed via the isolation and short-term culture of primary neuronal cells. Since then, immortalised neuronal cell lines have allowed for more efficient and reproducible *in-vitro* neuronal models, bypassing the limited life span of primary neuronal cells. These cell lines can be rapidly differentiated into neuronal cells by altering the cell-culture environment, but do not reach the maturity or diversity of neurons. More recently, the induction of pluripotent stem cells (PSCs) has allowed for *in-vitro* differentiation of several types of mature neurons, either by directed differentiation media or more efficiently through recombinant expression of transcription factors (TFs). Whilst PSCs offer advantages for mature neuronal models, immortalised neuronal cell lines are widely used and are much simpler to culture. Here we investigated an intermediary option, testing if the same TFs used to induce neurons using PSCs would enhance the differentiation of immortalised neuronal cells. We found that induced differentiation by TF Ngn2 using the piggyBac transposon system improved the differentiation potential of NG108-15 and SH-SY5Y cells towards a cholinergic phenotype. The co-culture of C2C12-derived myotubes showed improved neuromuscular junction formation and myotube differentiation. Alternatively, Ngn2 insertion at the AAVS1 site in SH-SY5Y cells resulted in a mixed cholinergic and glutamatergic phenotype. Furthermore, induced differentiation of NG108-15 cells by TFs Ascl1/Dlx2 resulted in a mixed cholinergic and GABAergic phenotype. Therefore, the use of transcription factors to differentiate immortalised cell lines offers a convenient and efficient alternative for in-vitro models of the nervous system.

id #21996

Small molecule TrkB agonists enhance the generation of striatal neurons.

Linh Nguyen[1], Amy Chapman[1], Brad Raos[2], Darren Svirskis[2], Bronwen Connor[1]

1. Department of Pharmacology, The University of Auckland, Auckland, New Zealand

2. School of Pharmacy, The University of Auckland, Auckland, New Zealand

Brain-derived neurotrophic factor (BDNF) binds to the high-affinity tropomyosin-related kinase B (TrkB) receptor to mediate neuronal survival and maturation and provide neuroprotection. Activating the BDNF-TrkB pathway has significant potential for treating several neurological diseases, including Huntington's disease (HD). However, exogenous BDNF or monoclonal antibody agonists as therapeutic agents have potential limitations for therapeutic use. To overcome this, two BDNF small molecule mimetics, LM22A-4 and 7,8- dihydroxyflavone (7,8-DHF), with specific affinity for the TrkB receptor, were developed and are potential candidates for treating HD. This study investigated whether LM22A-4 or 7,8-DHF could support the generation of MSNs from directly reprogrammed human-induced striatal precursor cells (hiSNPs) as effectively as BDNF. Briefly, human dermal fibroblasts were reprogrammed into hiSNPs by transfecting with lipid nanoparticle *SOX2* and *PAX6* mRNA. During differentiation, cells were treated with either 1nM BDNF, LM22A-4 (5μM, 10μM and 20μM) or 7,8-DHF (5μM, 10μM and 20μM), then fixed for immunocytochemistry and Sholl analysis or collected for ELISA. Treatment of hiSNPs with either LM22A-4 or 7,8-DHF generated the same number of DARPP32+ MSNs as quantified following treatment with BDNF. We also observed no difference in neurite length or branch number of MSNs following LM22A-4 or 7,8-DHF treatment compared to BDNF. Phosphorylation of the TrkB receptor following LM22A-4 or 7,8-DHF treatment was equivalent to that produced by BDNF as determined by ELISA analysis. This research demonstrates that small molecule TrkB agonists can promote the generation of MSNs from hiSNPs comparable to BDNF, indicating these compounds have the potential for the treatment of HD.

id #21487

Title: Evaluation of anti-inflammatory molecule in restoring K^+ homeostasis during neurodegeneration

Sushmitha Somanahalli Purushotham[1], Yossi Buskila[2, 1], Erika Gyengesi[3]

1. Department of Cellular Neurophysiology, School of Medicine, Western Sydney University, Campbelltown, New South Wales, Australia

2. The MARCS Institute, Western Sydney University, Penrith, NSW, Australia

3. Department of Neuropharmacology, School of Medicine, Western Sydney University, Campbelltown, New South Wales, Australia

Background - Neuroinflammation and glial cell dysfunction are associated with neurodegenerative diseases and are central to disease pathology. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease associated with a specific loss of motor neurons (MNs) leading to motor dysfunctions. The $SOD1^{G93A}$

mouse model of ALS is accompanied by increased levels of neuroinflammation, reactive astrocytes and an impaired ability of astrocytes to maintain K^+ homeostasis. This study aims to evaluate the therapeutic effect of phytosomal curcumin, a cytokine suppressive anti-inflammatory drug in ameliorating ALS disease progression and motor symptoms in $SOD1^{G93A}$ mice.

Methods – In this study, we used various behavioural tests, extracellular electrophysiological recordings and imaging techniques to assess motor functions, dysregulation of K^+ ion homeostasis, and neuroinflammation levels.

Key findings – Through extracellular electrophysiological recordings, we observed a significant reduction in the astrocytic K^+ clearance rate in layer 2/3 of the primary motor cortex of aged $SOD1^{G93A}$ mice fed with a normal diet. This decrease was accompanied by astrocytic hypertrophy and increased reactivity. Additionally, our preliminary behavioural results indicate significant motor deficits in $SOD1^{G93A}$ mice that were fed with a normal diet in the open field test and the accelerod test, while a curcumin-enriched diet had a modest impact on $SOD1^{G93A}$ mice motor functions.

Conclusions – Our preliminary findings indicate that curcumin had a limited effect on rescuing motor deficits in $SOD1^{G93A}$ mice. However, the ability of curcumin to reduce neuroinflammation and restore K^+ homeostasis in $SOD1^{G93A}$ mice is still under investigation.

id #22000

SARS-CoV-2 impact on oligodendrocyte lineage cells

Raphael P Ricci[1], Cheng Xiang X Foo[1], Katharina Ronacher[1], Carlie L Cullen[1]

1. Mater Research Institute, The University of Queensland, Brisbane, Queensland, Australia

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the virus responsible for coronavirus disease 2019 (COVID-19). Beyond acute respiratory distress, mild SARS-CoV-2 infection can also result in neural inflammation and dysregulation of neurons and glial cells, ultimately leading to neural circuit dysfunction and cognitive impairment. Oxidized derivatives of cholesterol (oxysterols) drive inflammation during SARS-CoV-2 infection. Pharmacological antagonism of oxysterol receptor Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2 or Gpr183) attenuates infection severity and viral load in the mouse lungs. Oligodendrocytes and their immature precursors (OPCs) express Gpr183 receptors as well as angiotensin converting enzyme 2 (ACE2) receptor used by SARS-CoV-2 to invade host cells, conferring OPCs an extra vulnerability to SARS-CoV-2 infection. This project investigates the impact of SARS-CoV-2 infection in oligodendrocyte lineage cells using $Gpr183^{-/-}$ mice and wildtype controls that received intranasal SARS-CoV-2 infection. Immunohistochemistry and proteomics analysis showed myelin proteins in the mouse cortex were unaffected by $Gpr183^{-/-}$. However, differential gene expression and pathway analysis revealed alteration in synaptic network pathways of $Gpr183^{-/-}$ mice, highlighting oxysterol signalling involvement in synaptic communication. Immunohistochemistry to detect oligodendrocyte lineage cells 4 days post SARS-CoV-2 infection uncovered an OPC ($Pdgfr\alpha^+$) cell density increase by 11.3% in the motor cortex that could be rescued by $Gpr183^{-/-}$. Meanwhile, the density of mature oligodendrocytes ($ASPA^+$) was unaffected by SARS-CoV-2 infection or $Gpr183^{-/-}$. Altogether, these data highlight the impact of SARS-CoV-2 infection in oligodendrocyte lineage cells and a possible mechanism of brain protection against viral infection.

id #21489

Targeted Delivery of the NgR-(1-310)ecto-Fc-Myc Protein Enhances Neurorepair during Experimental Autoimmune Encephalomyelitis

Zahra Rashidbenam[1], Ezgi Ozturk[1], Maurice Pagnin[1], Steven Petratos[1]

1. Department of Neuroscience, School of Translational Medicine, Monash University , Melbourne , Victoria, Australia

Multiple sclerosis (MS) is a neuro-inflammatory disorder resulting in neuroaxonal damage in the central nervous system (CNS). Immune-mediated mechanisms triggered during chronic-active demyelination in MS leads to accumulation of myelin debris in the extracellular environment. It's well-characterized that Nogo-A, a myelin-associated inhibitory factor, promotes axonal injury by signaling through its receptor Nogo receptor 1 (NgR-1) in MS. Our group utilized the experimental autoimmune encephalomyelitis (EAE) model of MS-like disease, where inflammatory monocytic cells infiltrate CNS lesions. We demonstrate that removing myelin debris using directed delivery of NgR(1-310)ecto-Fc-Myc therapeutic via transplantable hematopoietic stem cells (HSCs) promotes neurorepair. In this study we aimed to specifically define the population of transplanted HSCs that contribute to monocytic cell populations that primarily exert neuro-regenerative effects through their capacity to clear myelin debris. To develop a robust cellular delivery system, we isolated HSCs from wild-type mice and genetically modified them with lentivirus carrying NgR(1-310)ecto-Fc-Myc. We assessed the profile of isolated HSCs and the impact of lentiviral transduction on HSC phenotypic profiles using single-cell RNA sequencing. Transduced HSCs were transplanted into mice, then EAE induced. In the *in-vivo* phase, we analyzed peripheral blood by flow cytometry and single molecule array to determine immunomodulatory effects of HSC transplantation on recipient mice and to assess levels of axonal degeneration, respectively. Levels of axonal degeneration were coupled with evaluating locomotor performance of mice during the treatment course. Our findings demonstrate that cellular delivery of NgR(310)ecto-Fc-Myc significantly reduced disease severity. This treatment promoted axonal regeneration, remyelination and recovery following clinical decline.

id #21745

Probabilistic modeling reveals coordinated social interaction states and their multisensory bases

Sarah Stednitz[1], Andrew Lesak[2], Philip Washbourne[2], Luca Mazzucato[2], Ethan Scott[1]

1. University of Melbourne, South Yarra, VIC, Australia

2. Institute of Neuroscience, University of Oregon, Eugene, OR, USA

Social behavior across animal species ranges from simple pairwise interactions to thousands of individuals coordinating goal-directed movements. Regardless of the scale, these interactions are governed by the interplay between multimodal sensory information and the internal state of each animal. Here, we investigate how animals use multiple sensory modalities to guide social behavior in the highly social zebrafish (*Danio rerio*) and uncover the complex features of pairwise interactions early in development. To identify distinct behaviors and understand how they vary over time, we developed a new hidden Markov model with constrained linear-model emissions to automatically classify states of coordinated interaction, using the movements of one animal to predict those of another. We discovered that social behaviors alternate between two interaction states within a single experimental session, distinguished by unique movements and timescales. Long-range interactions, akin to shoaling, rely on vision, while mechanosensation underlies rapid synchronized movements and parallel swimming, precursors of schooling. Altogether, we observe spontaneous interactions in pairs of fish, develop novel hidden Markov modeling to reveal two fundamental interaction modes, and identify the sensory systems involved in each. Our modeling approach to pairwise social interactions has broad applicability to a wide variety of naturalistic behaviors and species and solves the challenge of detecting transient couplings between quasi-periodic time series.

id #22001

Positive experience shifts the fear circuit away from the basolateral amygdala

Arvie Rodriguez Abiero[1], Lauren DiFazio[2], Zara Greer[2], Ruiting Jia[2], Melissa Sharpe[1, 2]

1. Department of Psychology, University of Sydney, Camperdown, NSW, Australia

2. Department of Psychology, University of California, Los Angeles, California, USA

One of the most replicable findings in the behavioural neuroscience literature is that lesions or pharmacological inactivation of the basolateral amygdala (BLA) abolishes the encoding and recall of fear memories. This foundational work in rodents has been replicated in correlative studies in humans. Thus, the basolateral amygdala (BLA) is conceptualised as the brain's fear center, necessary for formation and storage of fear memories. Yet we recently showed that GABAergic neurons in the lateral hypothalamus (LH_{GABA}) can also become critical for formation of fear memories if rats have had a recent positive experience. That is, LH_{GABA} neurons are not necessary to form fear memories in naïve rats. However, if rats have had a recent positive experience, LH_{GABA} neurons suddenly become necessary to encode the fear memory. Here, we investigated how this recruitment of LH_{GABA} neurons to encode fear memories influences the role of BLA fear memory encoding. Using optogenetic and lesion manipulations of BLA activity, we replicated the existing findings that BLA is necessary for the formation of fear memories in experimentally-naïve rats. However, if rats had a recent positive experience, the BLA was no longer necessary to form the fear memory. Our findings reveal that the recruitment of hypothalamic circuits to encode fear memories shifts the fear memory away from BLA. This begs the question as to the physiological mechanisms underlying this shift in the fear circuit. Together, this work shows that prior experience changes the way we encode memories and suggests a more fluid approach to conceptualising mnemonic processing.

id #21490

The role of LINE-1 retrotransposons in Amyotrophic Lateral Sclerosis

Abigail Pfaff[1, 2], Sulev Koks[1, 2]

1. Perron Institute for Neurological and Translational Science, Perth

2. Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Perth

An individual's genetics contributes to their risk of developing amyotrophic lateral sclerosis (ALS), however there is still a large proportion of the heritability of ALS to be understood. Part of this missing heritability may lie in complex variants yet to be evaluated, such as the long interspersed element-1 (L1) retrotransposon which are a source of polymorphic insertions in the population. The majority of L1s are no longer able to retrotranspose, however to date 276 retrotransposition competent (RC) L1s have been reported. We hypothesise that L1s could influence disease development either through their effects on endogenous genes or due to the properties that enable them to retrotranspose. Therefore, using whole genome sequencing data from the New York Genome Center ALS consortium we characterised L1 variation and then focused on the subset of RC-L1s.

In 4393 whole genomes we identified 205 reference and 2598 non-reference polymorphic L1 elements. Association analysis did not identify any individual L1 elements associated with disease, however we did identify an increased number of RC-L1s in the genomes of individuals diagnosed with ALS compared to controls ($p=6.02x10^{-5}$). The presence of ≥51 RC-L1s showed the strongest association with ALS (padj=0.0005, OR=1.14 (1.07-1.21)). Age at onset analysis identified a L1 whose presence was significantly associated with a lower age at onset (52.7 years) compared to homozygous absent individuals (59.2 years) (padj=0.009).

Our study has identified novel genetic factors for both disease risk and age at onset in ALS providing further evidence for the role of L1 retrotransposons in neurodegenerative disease.

id #21746

Pathological links between lipid dyhomeostasis in tau proteinopathy

Pawat Laohamonthonkul[1], Samaneh Mirzaei[1], Pippa Pringuer [1], Aggie Hansen [1], Yi Ling Clare Low[1], Emily Hart[1], Seth Masters[2], Chien-Hsiung Yu[1]

1. Demetia, The Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, Australia

2. Inflammation, Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia

Background: Lipid dyshomeostasis and inflammation co-aggregate as common features in neurodegenerative diseases including tauopathies. Emerging evidence suggests an intertwined relationship between lipid metabolic processes and type-I interferon (IFN-I) inflammation, indicating that intervening in either of these processes can modulate the pathogenesis of neurodegenerative conditions. However, the relationship between lipid metabolism, IFN-I inflammation in the context of tau-mediated neurodegenerative processes remains unclear. This project aims to investigate the relationship between lipid metabolic processes, IFN-I inflammation in the pathogenesis of tau proteinopathy.

Methods: SH-SY5Y cells stably expressing tau-WT or disease-associated tau-mutants (P301L, P301S, V337M, K369I, R406W) were assessed for disease-associated phosphorylation of tau and IFN-I inflammation via western blot analysis. Fluorescent imaging was performed to assess lipid droplet (LD) formation and analysed via Imaris. 100 μM of oleic acid was introduced to induce LD formation. 5-month age-matched WT-mice and transgenic mice harbouring human-tau-K369I (K3 mice) were assessed for LD formation via fluorescent imaging.

Key findings: Increased p-tau-S262 and PHF-13 were consistently observed in tau-mutant cells harbouring mutation within microtubule binding domain. Interestingly, LD formation was consistently reduced in tau-mutant cells, a phenotype that is associated with elevated IFN-I inflammation (p-TBK1 and p-STAT1) which ameliorated when LD was reconstituted with oleic acid. Importantly, reduced LD formation was recapitulated in K3 mice.

Conclusions: Reduced LD formation may reflect altered lipid metabolic processes. Interestingly, induced LD formation ameliorated IFN-I inflammation suggesting the interplay relationship between lipid metabolism and IFN-I inflammation. Further investigation is warranted to understand how tau abnormalities mechanistically lead to lipid metabolic disturbances.

id #22003

Characterising α-Synuclein Related Gut Microbial Dysfunction in a Transgenic Synucleinopathy Model of Parkinson's disease

Divya Miss Mondhe[1], Zizheng Xian[1], Robert J Dr Adams[2], John D Dr O'Sullivan[2], Alexander C. Dr Lehn[1], Richard Dr Gordon[1]

1. Queensland University of Technology, Wooloongabba, QLD, Australia

2. University of Queensland, UQ Centre for Clinical Research, Brisbane, Queensland, Australia

Parkinson's Disease (PD) is the fastest growing neurodegenerative disease and manifests as a synucleinopathy with reduced motor output. Non-motor symptoms often precede or coincide with the hallmark motor symptoms in PD. Multiple studies have identified altered gut-microbial composition in PD, but the underlying pathological and function significance of these changes remains unknown. In this study, we identified the changes in the gut-microbial composition relevant to PD in an α-synuclein transgenic mouse model of PD at early and late stages of the disease. M83-transgenic mice express the full length A53T human-α-synuclein variant, making it an excellent model to study PD-synuclein pathology. We utilised high-resolution functional metagenomics with ultra-deep sequencing to ensure identification of low-abundance and novel taxa. The microbial community and metabolic pathways were profiled using Microba community and pathway profiler respectively. Although the microbial alpha-diversity remained unchanged in the M83 transgenic and WT group across different stages of PD a difference in microbial composition was observed. M83-transgenics occupied two separate clusters across both the stages of the disease. Gut dysbiosis in the early stages of PD was characterised with an increase in *Staphylococcus*, *Hungatella, and kineothrix species* and a decrease of *Duncaniella* and *Muribaculum* species. A decrease in genera *lactobacillus* was observed in later stages of PD. Microbes associated with blood-glucose levels, metabolic inflammation, SCFA production decreased whereas butyrate producers and opportunistic pathogens increased in abundance in our dataset. Our results provide evidence to support the hypothesis that the gut-microbial changes could contribute to the onset and progression of PD.

id #21492

Inflammatory responses and impaired BBB integrity are required for the neurobehavioral changes observed in Acute Rheumatic Fever

Riya Thapa[1], Rukshan AM Rafeek[1], Natkunam Ketheesan[1], Adam S Hamlin[1]

1. University of New England, Armidale, NSW, Australia

Group A streptococcal (GAS) infection is associated with autoimmune disease, including Acute Rheumatic Fever (ARF). Sydenham's chorea (SC) is the neurological manifestation of ARF occurring in up to 30% of patients and is characterised by random involuntary movements, irritability, attention deficit, and obsessive-compulsive disorder (OCD). Previous research has shown that a compromised blood brain barrier (BBB) is essential in allowing cross-reactive antibodies to enter the CNS. However, it is not yet known whether infection with GAS alone is enough to disrupt the BBB or if a secondary insult is needed to trigger disease. We hypothesised that a secondary insult is required for the development of neurological symptoms following a GAS infection.

In this study, Bordetella pertussis toxin (PT) was used as a secondary insult following immunisation with GAS recombinant M5 protein (rM5). The presence of antibodies against GAS rM5 was confirmed through an indirect ELISA. Neurobehavioral assessments showed significant abnormalities in fine motor control and the presence of OCD-like behaviour in rats immunised with GAS rM5 and administered PT but not GAS rM5 alone. Evaluation of BBB permeability, using Evans Blue Dye, confirmed that the BBB was only disrupted in rats injected with GAS rM5 and administered PT. We concluded that a secondary insult is required to disrupt the BBB and allow GAS rM5 antibodies to access the brain leading to the development of post-streptococcal neurological symptoms. Understanding the precise conditions and molecular basis under which SC develops will be critical to the development of future treatments of the disorder.

id #22004

Investigating rTMS Interventions in an Adolescent Model of Mood Disorders

Will B Johnsen[1], Nhu Nguyen[1], Holly Dear[1], Alex D Tang[2, 3], Jennifer Rodger[4, 3], Jamie L Beros[4, 3]

1. School of Human Sciences, University of Western Australia , Perth , Western Australia , Australia

2. School of Biomedical Sciences , University of Western Australia , Perth , WA, Australia

3. Perron Institute for Neurological and Translational Science, Perth , Western Australia , Australia

4. School of Biological Sciences , University of Western Australia , Perth , Western Australia , Australia

Major depressive disorder is one of the most prevelant mental health disorders affecting adolescents.Unfortunately, approximately 50% of individuals do not respond to conventional antidepressant treatments, highlighting the need for alternative interventions. Repetitive transcranial magnetic stimulation (rTMS) is a form of non-invasive brain stimulation widely used as an intervention for adults with treatment resistant depression. However, rTMS use in younger populations is underdeveloped and outcomes such as efficacy and the underlying neurobiological mechanisms remain poorly characterised.

To address these concerns, we investigated the neurobiological and behavioural outcomes of rTMS in a mouse model of adolescent mood disorders. Time-mated mice underwent daily restraint stress until birth to induce anxiety and depression-like behaviours in offspring. When offspring reached adolescence (4 weeks post-birth), rTMS (10Hz, 140mT) was administered for 3 minutes a day, 5 days a week for 4 weeks. Anxiety and depression-like behaviours were assessed before and after rTMS with multiple behavioural tests including the light/dark emergence test and markers of neuroplasticity (e.g. hippocampal neurogenesis) were assessed in post-mortem brain sections.

Experiments are currently ongoing but preliminary evidence suggests that rTMS increased exploratory behaviour in prenatally stressed animals compared to sham ($p<0.05$). Immunofluorescent imaging suggests that rTMS increased neurogenesis in the dentate gyrus of the hippocampus in stressed animals ($p<0.05$) and was accompanied with changes in brain derived neurotrophic factor protein levels. Our results provide important insight into the mechanisms of rTMS in adolescents and may help to expand the therapeutic reach of rTMS in this population.

id #21493

Development of Electroconductive Nerve Guidance Conduits with Gradient Conductivity

Chi Pang Tai[1], Iyer Swaminathan[2, 3], Ming-Hao Zheng[2, 1], Priya Kaluskar[2, 1, 4]

1. Bone and brain axis group, Perron Institute, Nedlands, WA, Australia

2. Univesity of Western Autralia, Nedlands, WA, Australia

3. School of Molecular Sciences, the University of Western Australia, ARC Training Centre for Next-Gen Technologies in Biomedical Analysis , Perth, Western Australia, Australia

4. Department of Biochemistry and Pharmacology, School of Biomedical Sciences, ARC Training Centre for Personalised Therapeutics Technologies, University of Melbourne, Melbourne, Victoria, Australia

Objective:

The efficacy of peripheral nerve repair (PNR) remains inconsistent. External stimulation and guidance improves regeneration. This study developed electroconductive gradients using PEDOT: PSS and Gold nanoparticles (AuNPs) on topologically cued nerve guidance conduits (NGC) for electrical stimulation.

Methods:

AuNPs were generated in situ, and PEDOT: PSS were deposited on the conduit. Electroconductive gradients were achieved, conductivity was evaluated using a four-point probe, live dead, and MTS assays assessed biocompatibility. SEM, TEM, and MS-ICP were used to characterise the particles.

Results:

AuNPs generated by $HAuCl_4$, and sodium citrate (SC) were ~50nm (TEM) with 5.8×10^{11} particles/mL concentration (ICP). As particles were unevenly distributed (SEM), AuNPs were generated in situ on the collagen conduit. 0.01M SC was added with 2.5 mM $HAuCl_4$ and incubated on collagen conduit overnight at 37°C. With a $6.5 \times 10^6/mm^2$ concentration, spherical NPs of size ~18-30nm (SEM) formed. < 0.01 ppm (ICP) particles released over 13 hrs. The sheet resistance of the AuNPs collagen conduit was ~8KΩ/square. Biocompatibility assay showed no significant difference in viability. The morphology of the cells in both the conduits was elongated. Electroconductive gradients showed four distinct conductivity regions from 0.75 S/m - to 2.0 S/m, depending on the AuNPs concentration.

Conclusion:

An epineurium-like electroconductive NGC was generated to hold electrical stimulation and align neuronal cells. A novel in situ method to develop NPs generated electroconductive gradients. This study will explore the behaviour of neuronal cells on electroconductive gradients and topological cues for PNR.

id #21495

Striatal Mechanisms of Punishment Learning

Bixuan Lin[1], Philip Jean-Richard-dit-Bressel[1], Gavan P McNally[1]

1. School of Psychology, University of New South Wales, Sydney, New South Wales, Australia

The role of dopamine and the dorsal striatum in instrumental reward learning is well studied but its role in instrumental punishment learning remains poorly understood. Reinforcement learning accounts identify punishment as the opposite of reward. It predicts that punishers should reduce dopamine release in the striatum and increase the activity of dopamine 2 receptor-expressing neurons to support avoidance learning. To assess this, we used dLight fibre photometry to record dopamine binding in the dorsal striatum (dorsolateral [DLS], dorsomedial [DMS], tail [TS]) as well as GCaMP fibre photometry to record activity of dopamine 1 receptor (*Drd1)* and dopamine 2 receptor (*Adora-2a*) expressing neurons during punishment learning. Rats were trained to press two levers for food under a VI-30 schedule. Then one response was punished, via footshock, on an FR-10 schedule whereas the other response was not. There were action-related dopamine transients for both the punished and unpunished lever press. Punishment itself caused robust dopamine transients across the dorsal striatum. Within the DMS, punishment was associated with increased activity of *Drd1* neurons but only a transient activation of *Adora-2a* neurons during early punishment sessions. Interestingly, punishment augmented both dopamine release and *Drd1* activity in DMS during reward retrieval. These findings suggest that the profile of dopamine and neuronal activity during punishment learning is similar to instrumental reward learning. We suggest dopamine binding and *Drd1* activity in the DMS report a teaching signal for instrumental learning that is unsigned with respect to the value of the outcome being learned about.

id #22008

Morphology of thinly myelinated sensory and autonomic axons in a mixed visceral nerve revealed by 3D electron microscopy, deep learning segmentation and quantitative computational image analysis

Ziying Yang[1], Afshin Khadankishandi[1], Adam J Blanch[1], John-Paul Fuller-Jackson[1], Eric Hanssen[2], Janet R Keast[1], Peregrine B Osborne[1]

1. Anatomy and Physiology, University of Melbourne, Victoria, Australia

2. Ian Holmes Imaging Center, Bio21 and Department of Biochemistry and Pharmacology, University of Melbourne, Melbourne, Victoria, Australia

Pelvic nerves are required for normal baseline functions of the pelvic organs and provide a target for therapeutic neuromodulation (neurostimulation). Unlike somatic nerves (e.g. sciatic), pelvic and other visceral nerves mostly comprise small diameter myelinated or unmyelinated axons (i.e. afferent Adelta- and C-fibers, or efferent fibers of autonomic spinal or ganglionic visceral motor neurons). In recent studies we found quantitative measurements of primary morphological parameters that define action potential conduction in visceral nerves is limited relative to somatic nerves. To address this, we studied 3D ultrastructural properties of myelinated axons in the rat pelvic nerve. First, a 93 micrometer segment of a rat pelvic nerve fascicle was imaged using scanning block-face electron microscopy. We then developed a custom pipeline (3D-VAx) to analyze the resulting large 3D image and obtain morphological parameters from all myelinated axons, including measurements of nodes of Ranvier and paranode regions. Myelin, myelinated and unmyelinated axons were auto-segmented with a novel machine learning approach trained with a small number of human annotations. To validate model accuracy, we compared 3D spatial morphometric characteristics measured from 3D-VAx segmentation results and human annotation of the node region of selected myelinated axons. This demonstrated a significant advance over currently used U-Net models. Additional capabilities of 3D-VAx include node auto-detection, total count estimation and computation of the centroid B-spline and perpendicular cross section in tortuous axons. Development of this pipeline provided new insights into the structure of the pelvic nerve and established a powerful approach for analyzing peripheral nerves in 3D.

id #21497

The role of neuroinflammation in delayed neurodegeneration following photothrombotic stroke in rats

Shannon Stuckey[1], Rosie Costigan-Dwyer[1], Isabella Bilecki[1], Madeleine Homes-Vickers[2], Amy Poyzer[1], Lin Ong[3], Rebecca Hood[1], Lyndsey Collins-Praino[2], Renee Turner[1]

1. Translational Neuropathology Laboratory (TNL), School of Biomedicine, The University of Adelaide, Adelaide, South Australia

2. Cognition Ageing and Neurodegenerative Disease Laboratory (CANDL), School of Biomedicine, The University of Adelaide, Adelaide, South Australia

3. School of Health and Medical Sciences, University of Southern Queensland, Toowoomba, Queensland

Background: Delayed neuronal death, in areas distal from the infarct site, termed secondary neurodegeneration (SND), is linked with long-term functional deficits following stroke. Increasing evidence demonstrates neuroinflammation as a potential driver of SND, although few studies have looked long-term post-stroke, which is essential in understanding the timeline of disease progression and identifying treatment targets. As such this study sought to investigate the spatiotemporal profile of neuroinflammation and functional changes long-term post-stroke.

Method: Male Sprague-Dawley rats (n=88; 12-weeks) underwent photothrombotic stroke or sham surgery. Motor outcomes (step test), anxiety (open field), and cognitive decline (Barnes maze) were assessed throughout the post-stroke period, with brain tissue and serum collected (n=30/stroke gp; n=14/sham gp) at 12- or 15-months post-stroke. Peripheral (serum) and neuroinflammation (thalamus, hippocampus) were analysed using a cytokine/chemokine multiplex (Millipore).

Results: Motor deficits were greater at 15-months post-stroke than 12-months post-stroke ($p=0.0016$), although higher levels of anxiety ($p=0.001$), and cognitive decline ($p=0.03$) were observed at 12-months. Within the thalamus and hippocampus, pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IL-10, IFN-γ and MCP-1) were significantly decreased in stroke groups ($p<0.05$) compared to shams, but most pronounced in the 15-month group. Serum showed the opposite pattern, with pro-inflammatory cytokine levels elevated at 15-months post-stroke ($p<0.05$).

Conclusion: Given that we observed changes both beyond the understood timeline and in known regions of SND, our study suggests that disease progression continues >12-months. Indeed, suggesting that SND has a prolonged therapeutic window wherein treatments at these delayed time-points may be beneficial in improving patients' quality of life.

id #21753

More than making amyloid-β: Defining the roles for Presenilins in microglial function.

Melissa Eccles[1, 2], Amy Woodfield[1], Ayeisha Milligan Armstrong[1], Mark Agostino[1, 3], David Groth[1], Giuseppe Verdile[1, 4]

1. Curtin Medical School, Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia, Australia

2. Centre for Genetics and Experimental Medicine, University of Edinburgh, Edinburgh, Scotland

3. Curtin Institute for Computation, Curtin University, Bentley, Western Australia, Australia

4. School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia

Background: Presenilins, of which there are two homologues, Presenilin 1 (PS1) and presenilin 2 (PS2), are the catalytic components of the γ -secretase enzyme, most well-known for its essential role in generating amyloid-beta (Aβ) associated with Alzheimer's disease (AD). The enzyme is also essential in signalling pathways within microglia and murine studies suggest that presenilins have a role in microglia activity. However, whether there are specific PS1/PS2 roles or associated pathways in microglia remain to be determined.

Objective: To investigate microglia activity and function in human microglia lacking PS1 or PS2.

Methods: PS1/PS2 CRISPR knockout human microglia (HMC3) cells were generated and underwent functional assays that included, expression analysis, morphology, phagocytosis, inflammatory response, and transcriptomics. Computational homology modelling was used to identify potential binding preferences of relevant γ-secretase substrates.

Results: Cells retaining PS2 have increased Aβ phagocytosis, and a specific cytokine response to lipopolysaccharide treatment. However, cells retaining PS1 have a specific cytokine response to Aβ treatment. Transcriptome analysis of these microglial cells revealed distinct transcription profiles between cells with either a PS1+/PS2+, PS1 only or PS2 only genotype, further indicating the functional distinction between PS1 and PS2. Modelling revealed that PS1- and PS2-γ-secretase typically demonstrate specific substrate binding preferences.

Conclusion: In addition to Aβ production, presenilins have essential roles in microglial function and activity. We provide novel evidence that PS1 and PS2 have differential roles in specific microglia functions. These differential roles should be considered when therapeutically targeting γ-secretase in AD.

id #22009

Actin dysregulation in transgenic FUS animal models and its novel isoform EC-FUS

Sara Assar Kashani[1], Cyril Jones Jagaraj[1], Bradley Turner[2], Julie D Atkin[1, 3]

1. MND Research Centre, Macquarie Medical School, Faculty of Medicine, Health and Human Sciences, Macqurie University, Sydney, NSW 2109, Australia

2. The Florey Institute of Neuroscience and Mental Health, University of Melbourn, Parkville, VIC 3010, Australia

3. La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Melbourne, VIC 3086, Australia

Background: FUS is an RNA/DNA binding proteins that mislocalized to the cytoplasm and causes a severe form of Amyotrophic Lateral Sclerosis (ALS). Recently, we identified a novel isoform of FUS, termed extracellular FUS (EC-FUS). The actin cytoskeleton is a key determinant of neuronal function. Rapid assembly and disassembly of actin filaments, involving the polymerisation from monomeric G-actin to polymeric F-actin, regulates vital responses in neurons. This is mediated by actin binding proteins, including the phosphorylation of cofilin, which promotes the formation of F-actin. However, the interplay between actin dynamics, FUS and EC-FUS pathology has not been characterized previously.

Methods and Materials: This study used three homozygous and three non-transgenic brain mice. G-actin and F-actin *in vivo* assay and western blotting for anti pCofilin/Cofilin, pLIMK/LIMK and profilin-1 used to measure actin dysregulation. We also used two days zebrafish lysates in three groups: EGFP-FUS (n=3), EGFP-WT (n=3) and non-transgenic controls (n=3). HA-stable HEK cells expressing EC-FUS and their conditioned media, used to monitor the actin dynamics in this cell line.

Key Findings: More F-actin (polymerized actin) relative to G-actin (monomeric actin) were present in FUS transgenic mice and zebrafish brains in comparison to non-transgenic controls. Also increased phosphorylation of Cofilin, pLIMK1/2 and profilin-1 were also detected, providing mechanistic insights into these observations. FUS mislocalisation resulted after pharmacological actin polymerization, linking dysregulation of actin dynamics to FUS pathology in ALS. These findings reveal dysregulation of actin dynamics as one of the novel mechanisms in the pathophysiology of FUS and EC-FUS in ALS.

id #21498

Exploring the long-term blood-brain-barrier alterations associated with post-stroke secondary neurodegeneration in an ovine model.

Rosie A Costigan-Dwyer[1], Shannon M Stuckey[1], Rebecca J Hood[1], Samantha L Joubert[1], Tracy D Farr[2], Mustafa Almuqbel[3], Georgia Williams[4], Mark Jenkinson[5, 6], Renee J Turner[1]

1. University of Adelaide, Adelaide, SA, Australia

2. School of Life Sciences , The University of Nottigham, Nottigham, United Kingdom

3. Department of Medical Radiography, University of Doha for Science and Technology, Doha, Qatar

4. Preclinical, Imaging & Research Laboratories, South Australian Health and Medical Research Institute, Adelaide, Australia

5. School of Computer and Mathematical Sciences, Australian Institute for Machine Learning, Adelaide, Australia

6. Nuffield Department of Clinical Neuroscience, University of Oxford, Oxford, United Kingdom

Background: >70% of stroke survivors experience long-term cognitive impairments, which has been linked to secondary neurodegeneration (SND). SND

involves the progressive death of neurons in regions distal to the stroke site i.e., thalamus and hippocampus. Within these regions blood-brain-barrier (BBB) dysfunction has been observed out to 3-months with links to cognitive decline, however long-term investigations remain scarce.

Aim: This study sought to explore the spatio-temporal profile of chronic BBB permeability at SND sites post-stroke using two different magnetic resonance imaging (MRI) sequences in a clinically-relevant ovine model.

Methods: 30 Merino sheep (2-3 years) underwent 2h middle cerebral artery occlusion, with MRI (3T Siemens) performed at baseline, and 1-, 3- or 6-months post-stroke (n=10/timepoint; 5F, 5M). BBB permeability was assessed by looking at the difference in gadolinium signal on T1-weighted MRI [MPRAGE, TR/TE: 2000/2.8ms] and Dynamic Contrast Enhance MRI (DCE) [TR/TE: 5.06/1.98]. SND regions of interest were extracted and registered with the FMRIB Software Library (v6.0.7.9; T1W) and Quantiphyse (v0.9.9; DCE).

Results: Processing and analysis protocols have been established for DCE MRI for the first time in sheep, however DCE appears to be highly sensitive and associated with high variability across animals within time-points. T1 pre- and post-gadolinium analysis is currently underway but is predicted to be more reliable given the scan acquisition time and a simpler post-processing procedure.

Conclusion: We anticipate these findings will both help characterise the profile of BBB permeability changes in post-stroke SND and inform the best MRI sequence to reliably detect and quantify BBB permeability alterations.

id #21499

Trajectories of Outcomes in Neurological Conditions (TONiC): Motor Neuron Disease & Parkinson's disease

Sulev Koks[1], Abigail Pfaff[1], Denise Howting[1], Jack Price[1], Carolyn Young[2]

1. Perron Institute for Neurological and Translational Science, Nedlands, WESTERN AUSTRALIA, Australia

2. The Walton Centre NHS Foundation trust, Liverpool, United Kingdom

Background and aims

Based on the Trajectories of Outcomes in Neurological Conditions (TONiC) methodology from the Walton Centre, Liverpool, we are exploring trajectories of patient experience in PD and MND. Both MND and PD are complex diseases where genetic predisposition and environmental factors interact. One aim is to characterise the less well-understood non-motor symptoms of MND and PD, their influence on quality of life (QoL), and investigate any possible influence upon non-motor symptoms and translate findings into more comprehensive clinical care.

Methods

A longitudinal design with 6 to12 monthly administration of questionnaires of validated patient-reported outcome measures (PROMs) to people with MND and PD. These encompass domains such as physical functioning, dyspnoea, fatigue, sleep, pain, anxiety, apathy, depression, disability, quality of life, coping, hope, social withdrawal, self-efficacy, self-esteem, stigma, worry, clinical and community communication, and health-related costs.

Results

Recruitment is ongoing. Currently have recruited 30 MND and 76 PD participants.

There is no difference between the two groups in sex and age.

Longitudinal analysis (MND, 7 timepoints, baseline to 36 months; PD 3 timepoints, baseline to 24 months) showed differences between the 2 groups. The MND group had statistically significant differences across time points for physical functioning, handicap, disability, fatigue, anxiety, depression, worry, self-esteem, coping, hope, social withdrawal, self-efficacy, and stigma. The PD group showed statistically significant differences across timepoints for disability and function.

Discussion

The differences between patient reported outcomes for MND and PD are largely due to physical functioning associated with disease trajectory.

id #21500

Effect of extreme light/dark cycles on monoamine levels, physiological indices, and emotional behaviors in rats

Takayuki Ishiwata[1, 2, 3], Akira Kawata[3], Yuta Kaneda[3], Daisuke Matsunaga[4], Hikaru Nakagawa[1], Fumiharu Togo[5], Mikinobu Yasumatsu[1, 2, 3]

1. Department of Sport and Wellness, RIkkyo University, Niiza, Saitama, Japan

2. Graduate School of Sport and Wellness, Rikkyo University, Niiza, Saitama, Japan

3. Graduate School of Community & Human Services, Rikkyo University, Niiza, Saitama, Japan

4. Department of Health-Promotion & Sports Science, Osaka Electro-Communication University, Shijonawate, Osaka, Japan

5. Graduate School of Education, The University of Tokyo, Bunkyo, Tokyo, Japan

Aberrant light/dark (LD) cycles are prevalent in modern society due to the widespread use of electric lights, causing mood disorders due to circadian disruption. This study investigated the potential effects of an extreme LD cycle on body weight (BW), core body temperature (T_{core}), locomotor activity (ACT), emotional behaviors, and the levels of monoamines in several brain regions of rats, including noradrenaline (NA), dopamine (DA), and serotonin (5-HT). Male Wistar rats were kept in conventional physical parameters apart from their exposure to one month of either long light phase (Long-LP; 20 h:4 h), long dark phase (Long-DP; 4 h:20 h), or normal (Norm-LD; 12 h:12 h) LD cycles. Compared to Norm-LD rats, the Long-LP and Long-DP rats showed distinct differences in T_{core} and ACT rhythms with increments in BW gain. The NA and 5-HT levels of the Long-LP rats decreased in the suprachiasmatic nucleus and amygdala, respectively. Long-DP rats showed increased DA and 5-HT levels in the caudate putamen and dorsomedial hypothalamus, respectively. In the emotional behavior tests, both the Long-LP and Long-DP rats showed anxiety-like behaviors in both the open field test and social interaction test. These results suggest that an extreme LD cycle affects T_{core} and ACT circadian rhythms and monoamine levels across brain regions and induces anxiogenic responses.

id #22012

Three-dimensional characterisation of pelvic ganglia development in the human embryo

Aymeric Nadjem[1, 2], Chad Moore[2], Raphael Blain[2], Peregrine Osborne[1], Janet Keast[1], Alain Chedotal[2, 3, 4]

1. Department of Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, Australia

2. Institut de la Vision, Sorbonne Universite, INSERM, CNRS, Paris, Ile-de-France, France

3. Institut de pathologie, groupe hospitalier Est, hospices civils de Lyon, Lyon, France

4. MeLiS, CNRS UMR5284, INSERM U1314, University Claude Bernard Lyon 1, Lyon, France

Pelvic ganglia contain autonomic neurons essential for urogenital and lower bowel function. They are atypical autonomic ganglia in their sexual dimorphism, steroid hormone sensitivity and anatomy, being a mixture of neurons with inputs from thoracolumbar (sympathetic) or sacral (parasympathetic) spinal cord. In humans, these neurons are located bilaterally within a large plexus, the inferior hypogastric plexus (IHP). The development of the human IHP is poorly understood.

We aimed to elucidate the development of the IHP in both male and female human embryos, specifically to construct the first 3D atlas of connections between the IHP and pelvic organs from the seventh to twentieth post-conceptional weeks (PCW7-PCW20). This work comprises part of the Human Cell Atlas (HCA) and Human Developmental Cell Atlas (HuDeCa) consortia.

In 29 samples, we have applied tissue clearing, immunofluorescence and light sheet microscopy to visualise combinations of markers for all neurons (beta-tubulin), transmitter class (tyrosine hydroxylase), transcription factors (e.g., Phox2b) and/or organ morphology (smooth muscle actin). Using a 3D virtual reality tool (syGlass), in 12 samples the entire IHP and its primary neural connections have been segmented. This has revealed distinct patterning of neural aggregates in the IHP, the hypogastric nerves, specific spinal nerves contributing to the pelvic and sacral splanchnic.

id #21501

Plastic Perils: An *In Vitro* Study Investigating Microplastic Exposure in the Gut-Brain-Axis

Charlotte E Sofield[1, 2], Anastazja M Gorecki[1, 2], Ryan S Anderton[1, 3]

1. School of Health Sciences, University of Notre Dame Australia, Fremantle, WA, Australia

2. Curtin Health Innovation Research Institute, Ralph and Patricia Sarich Neuroscience Research Institute, Curtin University, Nedlands, WA, Australia

3. Institute for Health Research, University of Notre Dame Australia, Fremantle, WA, Australia

Microplastics are ubiquitously present in the environment, and growing research shows that microplastics can affect cells of the nervous system, but the potentially protective effect of the gastrointestinal barrier has been neglected. This project aims to model biologically relevant intestinal microplastic exposure and consequent neuronal effects *in vitro,* using intestinal and neuronal cells to study microplastic uptake, toxicity and functional consequences. Intestinal epithelial cells and neurons will be exposed to microplastics for 48 hours to examine size-dependent microplastic uptake via confocal microscopy, and to study the functional effects of microplastic exposure using protein assays. In pilot experiments, enteroendocrine and intestinal epithelial cells were exposed to upto 80 µg/mL of 2 µm microplastics for 48 hours. Microplastic uptake was determined using fluorescent absorbance and confocal microscopy, and toxicity was determined by lactate dehydrogenase release. Microplastic internalisation by both intestinal cell lines was identified. This was a novel finding in enteroendocrine cells; however, uptake was greater in intestinal epithelial cells. Critically, this pilot evidence indicates that intracellular transport of microplastics through intestinal cell populations may be an important route by which microplastics cross from the gut lumen into peripheral circulation. Microplastics in the circulatory system are transported throughout the body becoming lodged in various organs and potentially, the brain. Additionally, microplastic presence within cells is predicted to disturb the homeostatic function of cells particularly in the gut and brain. Thus, robust characterisation of how microplastics affect the gut and brain is critical to understanding the risk of microplastics to human health.

id #22013

Investigating the effect of the oxytocin-receptor agonist LIT-001 on the expression and extinction of conditioned avoidance in female rats

Elise M Cleary[1], Fergus Scott[1], John Lin[1], William Connelly[1], James W Crane[1]

1. Tasmanian School of Medicine, University of Tasmania, Hobart, TAS, Australia

Oxytocin is a neuropeptide with known anxiolytic properties. However, little is known about the role of OT in avoidance behaviour, a hallmark of anxiety disorders. Using a platform-mediated avoidance model, we investigated the impact of LIT-001 non-peptide OT agonist on the expression and extinction of conditioned avoidance behaviours in female rats. Female Sprague Dawley rats underwent 10-days of training to learn to avoid a tone-signalled footshock (75dB, 4kHz, 28s tone, 0.4mA 2s shock, 9 tones/day) by stepping on a non-conductive platform. On day 11, rats received either a single dose of LIT-001 (10mg/kg i.p, n=8), or vehicle (i.p, n=8) 1 hour prior to an extinction protocol (9 tones presented without footshock). Analysis revealed no significant difference between LIT-001- and vehicle-treated rats in measures of time with food, time on platform, or time spent reaching off the platform during either tones or inter-tone periods. The following day, rats were exposed to a further 3 tone presentations without footshock. There was no significant difference between LIT-001- and vehicle-treated animals in time on platform or time with food during tone or inter-tone periods. However, LIT-001-treated rats spent significantly less time reaching off the platform during inter-tone periods than vehicle-treated rats ($p<0.0001$). These results suggest that while systemic administration of LIT-001 does not influence expression or extinction of conditioned avoidance, it may have subtle effects on the expression of metastability-like behaviours during motivational conflict.

id #22014

Associations of Plasma Lipid Correlation Network Profiles with Global Cognition in Cognitively Normal Older Adults

Maria Kadyrov[1, 2, 3], Luke Whiley[1, 4], Kirk I Erickson[5], Elaine Holmes[1, 2, 3], Belinda Brown[6, 2, 7]

1. Australian National Phenome Centre, Health Futures Institute, Murdoch University, Perth, WA, Australia

2. Centre for Healthy Ageing, Murdoch University, Perth, WA, Australia

3. Centre for Computational and Systems Medicine, Health Futures Institute, Murdoch University, Murdoch, Perth, WA, Australia

4. Perron Institute for Neurological and Translation Science, Nedlands, WA, Australia

5. Neuroscience Institute, AdventHealth Research Institute, Orlando, Florida, United States of America

6. Discipline of Exercise Science, Murdoch University, Perth, WA, Australia

7. School of Medical Science, Sarich Neuroscience Research Institute, Edith Cowan University, Nedlands, WA, Australia

Objectives: Cognitive decline is a common phenomenon associated with ageing and has been linked to disruptions in lipid metabolism. Lipidomics, the comprehensive analysis of all lipids, is a useful tool for investigating cognitive decline due to the ability of lipidomics to provide a “snapshot” of current physiological state of an individual. This study aimed to use a lipidomics approach to investigate the associations between plasma lipids and global cognition in cognitively normal older adults.

Methods: Mass spectrometry based targeted lipid profiling of 926 plasma lipids from 94 participants (aged 60-80, 54% female, MMSE > 26) was performed. Participants underwent neuropsychological assessments, and from this data a composite score for global cognition was generated. Weight Gene Co-Expression Network Analysis was performed to identify lipid clusters that co-vary, suggesting similar functions and involvement in common metabolic pathways. Pearson correlation analysis between each cluster and global cognition was performed.

Key Findings: From the 11 lipid clusters, one was significantly correlated (r = 0.37, FDR p = 0.03) with global cognition. This cluster contained 44 phospholipid species, of which 75% were plasmalogens, a subclass of lipids that are highly enriched in neuronal membranes. Plasmalogens are increasingly recognised for their potential role in neuroprotection as they have been shown to suppress amyloid and tau accumulation, promote neurogenesis and reduce inflammation.

Conclusion: Our study has demonstrated that plasmalogen lipid species are positively associated with global cognition in cognitively normal older adults, highlighting a possible target for future healthy cognitive ageing interventions.

id #21402

Preclinical iPSC drug screen and machine learning predict neuroprotective agents for a form of childhood dementia

Zarina Greenberg[1], Ella McDonald[1, 2], Alejandra Norena-Puerta[1], Manam Inushi De Silva[1, 2], Cade Christensen[1], Robert Adams[1], Jenne Tran[1, 2], Paris Mazzachi[1, 2], Sebastian Loskarn[1], Siti Mubarokah[2], Megan Maack[3, 4], Kristina Elvidge[3, 4], Mark Hutchinson[5, 6], Kim Hemsley[2], Lisa Melton[4], Nicholas Smith[7, 8], Cedric Bardy[1, 2]

1. Hopwood Centre for Neurobiology, South Australian Health and Medical Research Institute, Adelaide, SA, Australia

2. College of Medicine and Public Health, Flinders University, Adelaide, SA, Australia

3. Childhood Dementia Initiative , Sydney, NSW

4. Sanfilippo Children’s Foundation, Sydney, NSW

5. Institute for Photonics and Advanced Sensing, University of Adelaide, Adelaide, SA

6. School of Biomedicine, University of Adelaide, Adelaide, South Australia

7. Department of Neurology and Clinical Neurophysiology, Women’s and Children’s Health Network, Adelaide, South Australia

8. Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, South Australia

Childhood dementia is an umbrella term for rare paediatric neurological conditions with symptoms and pathophysiology overlapping the common adult forms of dementia. Sanfilippo syndrome or Mucopolysaccharidosis type IIIA (MPSIIIA) is a progressive neurodegenerative form of childhood dementia caused by a genetic mutation in lysosomal enzyme sulfamidase. Currently, there is no cure for MPSIIIA with the primary area of research focusing on treating enzyme deficiency, however evidence demonstrates that downstream neurophysiological impairments can drive neurological symptoms and accelerate neurodegeneration. Here, we developed a patient-derived iPSC preclinical model to screen 63 repurposed drugs used in adult neurodegenerative and CNS disorders and target downstream neuropathological pathways. We found that our iPSC-derived patient neurons revealed lysosomal dysfunction and heparan sulfate build-up, characteristics of MPSIIIA. Furthermore, patient-derived neurons were particularly prone to stress, astrocytic reactivity, and chronic neurodegeneration. Combining machine learning, high-content imaging and single-cell transcriptomics, we found nine compounds that ameliorated MPSIIIA phenotypes within two weeks of treatment *in vitro*. Our study introduces a novel preclinical model for childhood dementia and reveals repurposable agents that may help slow neurodegeneration and neuroimmune dysregulation in children with MPSIIIA and related disorders.

1

Cholinergic basal forebrain neurons regulate glymphatic-like flow and BOLD-CSF coupling: relevance to Alzheimer's

disease.

Elizabeth (Lizzie) J Coulson[1], Zengmin Li[1], Ling-Yun Fan[1], Kai-Hsiang Chuang[1]

1. The University of Queensland, Brisbane, QLD, Australia

The cholinergic neurons of the basal forebrain (BFCNs) are known to degenerate early in Alzheimer's disease. Their functional loss underpins aspects of cognitive decline and is coincident with amyloid beta (Aβ) plaque deposition. Studies using Alzheimer's disease mice models of amyloid deposition in which BFCNs are activated or inhibited result in reduced or enhanced amyloid plaque deposition respectively, indicative of a possible causal relationship.

There are several mechanisms by which Aβ can be removed from the brain, including clearance through the hypothesized perivascular glymphatic system. Animal studies have demonstrated that cholinergic activity in the brain controls artery dilation modulating the blood oxygen level-dependent (BOLD) signal, and providing a mechanism to change the perivascular space that could promote cerebral spinal fluid (CSF) exchange.

We hypothesize that BFCNs can control glymphatic flow and thus clearance of soluble amyloid from the brain.

BFCNs in mice were manipulated by lesioning using the toxin p75-saporin. To measure glymphatic flux, a gadolinium contrast agent was injected into the cisterna magma of mice and longitudinal MRI including imaging sequences for BOLD, arterial pulsation, and spin labeling was undertaken. We have also undertaken analyzed human MRI scans of people living with dementia who have also undergone cholinergic PET tracer FEOVB imaging.

Our results support the hypothesis that BFCNs can control glymphatic flow. The implication is that in situations of reduced cholinergic function such as aging, the resultant reduced glymphatic flux could contribute to Aβ accumulation and deposition, hastening the development of Alzheimer's dementia.

2

Synergistic Expression of Human Apo E4 and Amyloid Restricted to the Liver: A Novel Pathway for Alzheimer's Disease Onset and Neurodegeneration.

Ryu Takechi[1], Virginie Lam[1], John Mamo[1]

1. Curtin University, Perth, WA, Australia

Mice expressing human amyloid precursor protein restricted to the liver (HSHA strain) and secreted as apoprotein of nascent triglyceride-rich lipoproteins develop blood-brain barrier dysfunction, neurodegeneration, cerebral amyloid deposition, and cognitive dysfunction. In this study, we developed a novel transgenic mouse strain to examine the synergistic effects of human APOE genotype in HSHA-murine APOE knock-out-human APOE knock-in mice. By having human apoE4 gene, the cognitive function in HSHA-E4 mice was significantly worse, compared to HSHA-E3 and wild-type controls. Concomitantly, the HSHA-E4 mice exhibited substantial neurovascular dysfunction and Alzheimer's-like pathophysiology, including barrier breakdown (IgG), astrogliosis (GFAP), inflammation (Iba1), oxidative stress (8OHdG) and neurodegeneration (NeuN). Furthermore, spatial whole RNA transcriptomics indicated significant alterations in lipid metabolism, inflammation and cellular survival in HSHA-E4, which are similar to clinical Alzheimer's. These findings suggest that peripheral metabolism of lipoprotein-amyloid may be a major risk factor for a neurodegenerative phenotype consistent with early Alzheimer's and may explain the present novel pathway and therapeutic target for Alzheimer's in individuals carrying apoE4 allele.

3

Identification of *APOE4*-driven lipid accumulation in the aging brain with compromised myelin integrity

Gerald W.Y. Cheng[1], Jianpan Huang[2], Shirley Zhang[3], Martin H.Y. Yeung[1], Pei Cai[2], Zilin Chen[4], Sunny H.S. Yeung[1], Henry K.F. Mak[2], Kannie W.Y. Chan[4], Karl Herrup[5], Kai-Hei Tse[7, 1, 6]

1. Department of Health Technology and Informatics, , The Hong Kong Polytechnic University , Kowloon, Hong Kong

2. Department of Diagnostic Radiology, The University of Hong Kong , Hong Kong

3. Department of Rehabilitation Sciences, The Hong Kong Polytechnic University , Kowloong, Hong Kong

4. Department of Biomedical Engineering, City University of Hong Kong, Kowloon, Hong Kong

5. Department of Neurobiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

6. Brain and Mind Centre, University of Sydney, Camperdown, NSW, Australia

7. Royal Prince Alfred Hospital, Sydney, NSW, Australia

Apolipoprotein E allele ε4 (*APOE4*) is the most significant genetic risk factor for sporadic Alzheimer's disease (sAD). We reported earlier that myelinating oligodendrocytes (OL) are lost in *APOE4* carrier brain and APOE4 protein impairs astrocyte-to-OL lipid transport and OL differentiation, independent of amyloid plaque formation. Here, we hypothesize that APOE4 compromises intrinsic myelin integrity by causing lipid buildup in the aging brain. First, we examined the magnetic resonance spectroscopy (MRS) of a retrospective cohort of 80 healthy individuals (mean age=51 [20-84] years; 62.5% female).In human, MRS detected a significant increase of lipid (0.9-ppm, +4.41-folds, P=0.0174; 1.3-ppm, +4.81-folds, P=0.0002) in the anterior cingulate of *APOE4* carriers above 40 years of age, but not in young cases. These human findings were then verified in a cohort of humanized *APOE* knock-in mice (hAPOE3 and hAPOE4) at 6, 10 and 16 months old (n=46). While body composition was unchanged, hAPOE4 mice showed a significantly reduced basal metabolic rate, activity and energy expenditure than hAPOE3. At 10-month-old, but not earlier. Importantly, MRS revealed substantial lipid buildup in hAPOE4 mouse brains (0.9-ppm, +17.7%, P=0.0066; 1.3-ppm, +22.9%, P=0.043), concurrent with compromised myelin integrity detected by diffusion kurtosis imaging(mean, axial and radial diffusivity, P<0.05). Liquid chromatography-mass spectrometry identified the accumulated lipids as being enriched in the ceramide pathway, a key metabolite for myelination. Further MALDI-TOF imaging and transcriptomic analyses are underway to confirm these results. In conclusion, APOE4 may lead to abnormal lipid accumulation and myelin degradation in the aging brain, potentially increasing the risk for sAD.

4

Molecular and cellular mechanisms underpinning disruptions to neuronal signalling and vulnerability in Alzheimer's disease neurons and novel applications of machine learning

Lezanne Ooi[1]

1. University of Wollongong, Wollongong, NSW, Australia

Disruption to neuronal signaling and alterations in neuronal excitability have been identified as early phenotypes in Alzheimer's disease (AD). Induced pluripotent stem cell (iPSC) derived neurons generated from AD donors were used as an in vitro model to interrogate the mechanisms underlying dysfunctional signaling and neuronal activity and increased vulnerability. Using a combination of calcium imaging, patch clamp and microelectrode arrays, with molecular and cellular techniques and machine learning approaches, we identified alterations in neuronal signaling in AD neurons in neuronal cultures and organoids.

AD neurons exhibited an increased susceptibility to nitrosative/oxidative stress damage, with an altered distribution of the lipid soluble antioxidant alpha-tocopherol and decreased levels of the antioxidant enzyme, glutathione peroxidase. This decreased resilience to redox dyshomeostasis increased neuronal vulnerability to the cell death pathway, ferroptosis. Induction of ferroptosis significantly reduced cell viability, multiple phosphatidylethanolamine polyunsaturated fatty acid lipid species and abolished glutamatergic calcium signaling. Supplementation with alpha-tocopherol, which can prevent ferroptosis, reduced the proportion of spontaneously signaling AD neurons and partially protected against the loss of select fatty acid species, implicating alterations to the lipid membrane in the breakdown of neuronal calcium signaling. Following on from this, novel machine learning tools were applied to identify alterations in AD neuron activity and function that can be ameliorated by targeting specific cell signaling pathways.

Together, the data indicate that the AD neuronal signaling phenotype is linked to both neuronal function and survival.

5

Investigating the mediating impact of insulin resistance on the physical activity and brain beta-amyloid relationship in cognitively unimpaired older adults.

Michael Slee[1], **Stephanie R Rainey-Smith**[2, 1, 3, 4], **Victor L Villemagne**[5, 6, 7], **Hamid R Sohrabi**[2, 8, 1, 3], **Kevin Taddei**[2, 3], **David Ames**[9, 10, 11], **Vincent Doré**[5], **Paul Maruff**[12, 13], **Simon M Laws**[7, 14], **Colin L Matsers**[10], **Christopher C Rowe**[5, 10], **Ralph N Martins**[2, 8, 3], **Kirk I Erickson**[16, 15], **Timothy J Fairchild**[1], **Belinda M Brown**[2, 1, 3]

1. Centre for Healthy Aging, Healthy Futures Institute, Murdoch University, Murdoch, WA, Australia

2. Australian Alzheimer's Research Foundation, Perth, WA, Australia

3. School of Medical and Health Sciences, Edith Cowan University, Perth, WA, Australia

4. School of Psychological Science, University of Western Australia, Nedlands, WA, Australia

5. Department of Molecular Imaging & Therapy, Austin Health, Melbourne, VIC

6. Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

7. Centre for Precision Health, Edith Cowan University, Perth, WA, Australia

8. Department of Biomedical Sciences, Macquarie University, Sydney, NSW, Australia

9. National Ageing Research Institute, Melbourne, VIC, Australia

10. The Florey Institute of Neuroscience and Mental Health, Melbourne, VIC, Australia

11. Academic Unit for Psychiatry of Old Age, St George's Hospital, University of Melbourne, Melbourne, VIC, Australia

12. Cogstate Ltd, Melbourne, VIC, Australia

13. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC, Australia

14. Collaborative Genomics and Translation Group, School of Medical and Health Sciences, Edith Cowan University, Perth, WA, Australia

15. Neuroscience Institute, AdventHealth, Orlando, FL, USA

16. Department of Psychology, University of Pittsburgh, Pittsburgh, PA, USA

Objective: The association between Type 2 diabetes and Alzheimer's disease (AD) suggest common pathological mechanisms in the brain; insulin resistance (IR) can impair insulin pathway signalling, that aids in reduced beta amyloid (Aβ) accumulation. Studies have shown that physical activity (PA) is associated with lower brain Aβ burden and can reduce IR. To date no study has assessed the mediating impact that IR has on the PA ~ brain Aβ relationship in humans. Observational data was used to examine any mediation impact of IR on the PA ~ brain Aβ load relationship through statistical mediation methods.

Methods: PA, Aβ imaging and metabolic measures, from 367 cognitively unimpaired older adults were used. IR was assessed using the Homeostatic Model Assessment for Insulin Resistance. Mediation analysis statistically examined the relationships between physical activity and Aβ directly and then indirectly via IR impact. Participants were also stratified into diabetic and non-diabetics groups.

Result: No mediation effect of IR on the PA and brain Aβ relationship was observed [95% CI -0.0001 to 0.0000]. No direct effect was observed between PA and brain Aβ (p = 0.0544) or PA and IR (p = 0.4905) or IR and brain Aβ (p = 0.4052). These results were also seen within the two diabetic stratified groups except for a direct effect observed between PA and brain Aβ (p = 0.0154) in the diabetic group only.

Conclusion: Our study did not show that IR mediated the relationship between PA levels and brain Aβ in cognitively unimpaired older adults.

6

The Effects of Hearing Loss on Spatial Learning and Memory in Middle-Aged Guinea Pigs

Kristin Barry[1, 2], **Carlos Jimena**[1], **Hadeel Tarawneh**[1], **Will Johnsen**[1], **Adnana Osmanbaasic**[1], **Jennifer Rodger**[1, 3], **Wilhelmina Mulders**[1]

1. University of Western Australia, Nedlands, WA, Australia

2. Ear Science Institute Australia, Crawley

3. Perron Institute for Neurological and Translational Research, Crawley, WA

Hearing loss (HL) in mid-life has been identified as a risk factor for cognitive decline. It is unclear whether this relationship is due to the deprivation of auditory input, neurodegenerative processes, or a combination of both. Animal models are useful for investigating the underlying neural mechanisms of cognitive decline as they can control for confounding factors that may arise in human studies. However, most animal studies investigating HL and cognition use young animals and often exclude females.

Methods: We used middle-aged guinea pigs of both sexes to investigate whether 8 weeks of auditory deprivation due to conductive HL caused spatial learning and memory impairments. Forty guinea pigs (20 M, 20 F, ~12 months) were tested in the Morris Water Maze (MWM) to assess baseline spatial learning and memory. In 20 of these animals (10 M, 10 F) the ear canal was plugged and 8 weeks later, animals were again assessed in MWM.

Key Findings: No deficits in spatial learning or memory were observed in either sex. HL caused a small decline in body weight suggesting some stress associated with conductive HL, although adrenal/body weight did not change.

Conclusion: Our data suggest that auditory input deprivation alone does not affect spatial cognition in middle-age, in line with recent human data suggesting that additional risk factors need to be present.

7

Executive control fluctuation underlies intra-individual behavioural variability.

Farshad Mansouri[1], **Mark Buckley**[2], **Keiji Tanaka**[3]

1. Monash University, Melbourne, VIC, Australia

2. Experimental Psychology, University of Oxford , Oxford, United Kingdom

3. Cognitive Brain Mapping Laboratory, RIKEN Centre for Brain Science, Wako, Saitama, Japan

Publish consent withheld