Oral Presentations

Sunday 4th December

Plenary 1 – International Plenary, Prof Michael Hausser, University College London

ALL-OPTICAL INTERROGATION OF NEURAL CIRCUITS

Michael Häusser, University College London

Neural circuits display complex spatiotemporal patterns of activity on the millisecond timescale during behavior. Understanding how these activity patterns drive behavior is a fundamental problem in neuroscience, and remains a major challenge due to the complexity of their spatiotemporal dynamics. The ability to simultaneously image and manipulate patterns of activity in neural circuits at cellular resolution would open up new frontiers in neuroscience. I will describe a strategy for "all-optical" interrogation of neural circuits in vivo with single-spike and single-neuron precision. Two-photon calcium imaging is combined with two-photon optogenetic activation using coexpression of a red-shifted opsin and a genetically encoded calcium indicator. A spatial light modulator allows tens of user-selected neurons to be targeted for spatiotemporally precise optogenetic activation, while simultaneous fast calcium imaging provides high-resolution network-wide readout of the manipulation with negligible optical cross-talk. I will describe experiments in mouse barrel cortex and visual cortex which demonstrate how this approach can be used to target neuronal ensembles based on their functional signature, and enable real-time closed-loop interrogation of neural circuits during behaviour.

Monday 5th December
Symposia 1: The cytoskeleton: leading the way in development and disease (LOC)

ACTIVITY-DEPENDENT REGULATION OF A DENDRITIC KINESIN INVOLVED IN LEARNING AND MEMORY

Erika Holzbaur
University of Pennsylvania

Neurons are highly polarized cells that depend on active and specific trafficking of cargos to and from axons and dendrites. While the axon is specialized for long distance trafficking from soma to synapse and back, trafficking in dendrites is more complex. Microtubule organization is also more complicated, as dendritic microtubules are organized with mixed polarity as opposed to unipolar organization in axons. Further, the dendritic cytoskeleton undergoes activity-dependent remodeling during learning and memory. However, the mechanisms that dynamically regulate these processes remain largely unknown. Here, we focus on the dendritic kinesin, KIF21B. Using both single molecule assays in vitro and live cell imaging of organelle and microtubule dynamics in primary rat hippocampal neurons, we identified the kinesin-4 motor KIF21B as a key regulator of both activity-dependent trafficking and microtubule dynamicity in dendrites. We find that KIF21B is essential for the retrograde trafficking of BDNF/TrkB complexes, and loss of KIF21B leads to altered BDNF signaling to the nucleus. KIF21B also regulates microtubule dynamics, via a separable, non-motor C-terminal microtubule-binding domain. Critically, neuronal activity enhances the motility of KIF21B at the expense of effects on microtubule dynamics. Thus, KIF21B is the first example of a kinesin whose function is directly tuned to neuronal activity state. Consistent with these observations, knockout of KIF21B in mice leads to defects in learning and memory (Muhia et al., 2016). Together, these data support a model in which KIF21B navigates the complex dendritic cytoskeleton by differentially regulating motor and microtubule dynamicity functions in an activity-dependent manner.

RIDING THE WAVE - LINKING THE ACTIN CYTOSKELETON TO CORTICAL STEM CELL MORPHOLOGY AND FUNCTION.

Dr Conor O'Leary[1], Ms Natalie Lee[1], Ms Amanda White[1], **A/Prof Helen Cooper[1]**
[1]*Queensland Brain Institute*

The fidelity of neocortical development is dependent on the highly polarized morphology of the neuroepithelial Radial Progenitors (RPs) within the ventricular zone. Loss of RP polarity disturbs the delicate balance between self-renewing and neurogenic divisions and also prevents the migration of newborn neurons into the emerging cortical layers. Establishment of RP polarity requires the formation of cadherin-based cell-cell junctions (adherens junctions) between RPs. Junctional stability is critically dependent on the closely apposed actin cytoskeleton and failure to maintain this actin network results in junctional collapse. Loss of RP junctional integrity has now been linked to cortical malformations, including neuronal heterotopias. Therefore, elucidating the signaling events that precisely control actin remodeling at the RP junction is essential to our understanding of corticogenesis.
Actin nucleation at adherens junctions is mediated by the Wave Regulatory Complex (WRC). However, it is not understood how the WRC is spatially restricted to the junctional membrane and subsequently activated. Here we describe a novel function for the guidance receptor, Neogenin, as a key component of the actin nucleation machinery. We show that in the absence of Neogenin, the WRC fails to localize to the junction and initiate actin nucleation, leading to the abrogation of junctional integrity. Therefore Neogenin is essential for the stabilization of the actin cytoskeleton at RP junctions by recruiting the WRC. We further show that in the embryonic mouse cortex the consequence of disrupting the Neogenin-WRC pathway in RPs is the generation of neuronal heterotopias, thereby implicating Neogenin in the aetiology of cortical malformations.

TARGETING THE ACTIN CYTOSKELETON TO MANIPULATE NEURITE OUTGROWTH IN CENTRAL NERVOUS SYSTEM NEURONS.

Fath T[1].
[1]*Neurodegeneration and Repair Unit, School of Medical Sciences, University of New South Wales, Sydney, Australia*

Regeneration of neurons in the adult central nervous system (CNS) after injury is limited due to a hostile extracellular environment at the site of injury, lack of trophic factors and a limited intrinsic potential of adult CNS neurons to regenerate. Neurite outgrowth is highly dependent on the underlying actin cytoskeleton. A central regulator of the actin cytoskeleton in all eukaryotic cells is the actin cytoskeleton-associated protein tropomyosin. Tropomyosins form a multi-gene protein family. Association of different tropomyosins with actin filaments bestows these filaments with different structural and dynamic properties, and defines distinct actin filament populations.
Using a peripheral nerve injury model in mice, we found a strong upregulation of Tpm3 and Tpm4 gene products in peripheral nerves, 6 hours after injury. Inhibitory factors of axon outgrowth, such as Nogo, are known to impact on the local cellular architecture of extending axons, inhibiting neuronal regeneration in the central nervous system. Our data show that overexpression of the major Tpm3 gene product Tpm3.1 promotes axonal outgrowth from cortical neurons, grown on a surface, coated with Nogo66, a well characterised inhibitor of neurite outgrowth. We therefore propose that increasing the expression

of neurite growth promoting tropomyosins in CNS neurons may provide a new strategy to overcome inhibitory cues in the CNS after injury and to promote neuronal repair.

STIM1, the intersection of calcium signaling and the cytoskeleton in navigating growth cones

Pavez M, Thompson A, Gasperini R, **Foa L**
University of Tasmania, School of Medicine, Hobart, Australia

The precise connectivity that underlies the neural circuitry of the brain begins with axon guidance. Growth cones, the sensory tip of extending axons, respond to environmental guidance cues as they navigate the embryonic milieu and guide axons to appropriate targets. Spatially-restricted cytosolic calcium transients are central to most growth cone responses to guidance cues. However, the mechanisms that regulate the spatial and temporal nature of growth cone calcium signals are not clear. We hypothesize that the calcium-sensing protein Stromal Interacting Protein 1 (STIM1) is necessary for the spatiotemporal regulation of calcium within the growth cone. STIM1 is located in the membrane of the endoplasmic reticulum (ER), a major internal source of growth cone calcium. We have previously shown that STIM1 is necessary for growth cone navigation in vitro and we have extended this to zebrafish motor axons navigating in vivo. Importantly, our data indicate that STIM1 is not only required for growth cone calcium signaling, but that STIM1 expression is also essential for microtubule assembly and organization during growth cone turning. Reduction of STIM1 expression disrupted the dynamics of microtubule polymerisation ($p<0.0005$) as well as the asymmetric distribution of microtubules in growth cones as they turned towards a source of BDNF ($p<0.0005$) or away from a source of Sema3a ($p<0.05$). The data support the hypothesis that STIM1 is critical in regulating the spatial localisation of growth cone calcium signals. Our work significantly builds on our current understanding of how calcium regulates the growth cone navigation that underlies early neuronal connectivity.

Monday 5th December
Symposia 2: Neuron-glial interactions and gliogenesis in the CNS

REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION INCREASES OLIGODENDROGENESIS IN THE ADULT MOUSE BRAIN

Dr Carlie Cullen[1], Dr Matteo Senesi[1], Mr Alex Tang[2], Ms Megan O'Rourke[1], AProf Jennifer Rodger[2], **Dr Kaylene Maree Young[1]**
[1]University of Tasmania, [2]University of Western Australia

Elevated neuronal activity promotes oligodendrogenesis in the adult mouse brain. As repetitive transcranial magnetic stimulation can induce electrical currents in the underlying brain tissue, we hypothesised that repetitive TMS (rTMS) would also promote oligodendrogenesis. To examine this possibility we used *Pdgfrα-CreER^{T2} :: Rosa26-YFP* transgenic mice to perform lineage tracing of oligodendrocyte progenitor cells (OPCs) in adulthood. The mice received 3 minutes of sub-threshold, intermittent theta burst rTMS (120mT coil) or sham stimulation (coil not turned on) for daily for up to 14 days, and received EdU continuously in their drinking water to label proliferating cells. Mice were perfused after 1, 3, 7, 10 and 14 days of treatment. Coronal brain sections were processed to determine the proportion of OPCs that underwent division (PDGFRα$^+$ EdU$^+$ / Total PDGFRα$^+$), as well as the number of new oligodendrocytes added to the cortex (YFP$^+$ OLIG2$^+$ PDGFRA-negative cells). rTMS treatment did not alter OPC proliferation ($p>0.05$), however it significantly increased the number of new oligodendrocytes detected in the cortex. For example, within 14 days of treatment, approximately twice as many new oligodendrocytes were detected in the motor cortex (M1; $p=0.03$) and visual cortex (V2; $p=0.02$) of rTMS mice relative to sham stimulated controls. These data suggests that rTMS does promote oligodendrogenesis in the healthy adult mouse central nervous system.

NEW STRATEGIES TO STUDY MYELINATION, DEMYELINATION AND REMYELINATION IN THE CNS

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The optic nerve (ON) has been used extensively as an experimental model of axon injury in the central nervous system as it contains only one type of axons, which originate from retinal ganglion cells (RGCs). It also contains endothelial cells and all types of CNS glial cells (oligodendrocytes, astrocytes and microglia). A single oligodendrocyte myelinates multiple axons and therefore, even a minor axonal injury might have a consequence on a large number of axons and the death of a small number of oligodendrocytes might demyelinate many axons. I will present new genetic and imaging strategies to study demyelination and remyelination after injury in the ON and brain. We have been using various transgenic mice (such as brainbow lines) in which the three-dimensional morphology of single oligodendrocytes could be reconstituted. We have also been able to adapt the 3DISCO brain clearing method to the 3D analysis of oligodendrocytes in the normal and injured CNS. Our data show that oligodendrocytes can survive without axons for at least one year after optic nerve crush. We also obtained preliminary evidence suggesting that they might remyelinate regenerating axons. We are also assessing the role of neuronal activity in myelination and demyelination.

T-REGULATORY CELL MODULATION OF GLIAL CELL ACTIVATION AND NEURODEGENERATION IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

Dr Bradley Turner[1], Dr Rebecca Sheean[1], Dr Fiona McKay[2], Dr Erika Cretney[3], Prof Stephen Nutt[3], Ms Karlene Scheller[4], Ms Nirma Perera[1], Dr Christopher Bye[1], Dr Justin Yerbury[5], Prof Steve Vucic[6]
[1]Florey Institute of Neuroscience And Mental Health, [2]Westmead Millennium Institute, University of Sydney, [3]Walter and Eliza Hall Institute of Medical Research, [4]School of Life Sciences, La Trobe University, [5]Illawarra Health and Medical Research Institute, University of Wollongong, [6]Western Clinical School, University of Sydney

The peripheral immune system is strongly implicated in modulating glial cell activation, motor neuron survival and disease progression in amyotrophic lateral sclerosis (ALS). Activation of circulating CD4+ FoxP3+ T-regulatory cells (Tregs) positively correlates with progression rate in ALS patients, while transplantation of Tregs is neuroprotective in mutant SOD1 mice by modulating glial cell inflammation. Here, we employed a pharmacological approach to stimulate Tregs and suppress glial cell activation in the CNS by treating mutant SOD1 mice with an IL-2/IL-2 mAb complex. Co-treatment with IL-2/IL-2 mAb complex and rapamycin improved rotarod performance and significantly prolonged survival of SOD1^{G93A} mice ($p<0.01$), compared to rapamycin alone and vehicle groups. IL-2/IL-2 mAb complex significantly elevated circulating CD4+ FoxP3+ Treg numbers and FoxP3 expression in spinal cords of SOD1^{G93A} mice. Furthermore, these Tregs showed increased CTLA4 and GITR expression, consistent with a suppressor phenotype. Importantly, astrocyte and microglial activation were significantly reduced in spinal cords of SOD1^{G93A} mice treated with IL-2/IL-2 mAb complex ($p<0.05$). Inflammatory cytokine expression analysis by qPCR revealed a delay in M2 to M1 transition of microglial phenotype in spinal cords of SOD1^{G93A} mice treated with IL-2/IL-2 mAb complex. This study demonstrates that IL-2/IL-2 mAb complexes stimulate selective expansion of suppressor Tregs in mutant SOD1 mice, leading to attenuated glial cell activation, neuroinflammation and clinical improvement in mutant SOD1 mice. These data suggest a feasible possible therapeutic strategy for ALS based on expansion of neuroprotective Tregs from the periphery to modulate central glial cell activation.

REGULATION OF GLIAL DIFFERENTIATION BY NUCLEAR FACTOR I IN CORTICAL DEVELOPMENT, DEVELOPMENTAL DISORDERS AND BRAIN CANCER

J.Bunt[1], J.W.C. Lim[1], K.S. Chen[1], Z. Liu[1], C.Bridges[1], I. Gobius[1], , T.J. Edwards[1], L.J. Richards[1,2]
The University of Queensland, [1]Queensland Brain Institute, [2]School of Biomedical Sciences, Brisbane 4072, Australia.

The Nuclear Factor I (NFI) family of transcription factors are important for normal development of the brain, and specifically the cortex. In humans, NFI deletion or mutation results in congenital disorders characterized by intellectual disability and structural cortical abnormalities, such as agenesis of the corpus callosum and macrocephaly.

To understand the underlying aetiology in these patients, we performed immunohistochemical analyses on a developmental time course of NFI knockout and wildtype mouse brains. We determined that disruption of NFI results in retention of proliferative radial glial cells and delayed differentiation into neurons and mature glia in the cortex. To better understand how this persistent delay in glial maturation alters the function of the cortex, we are now using a cortical-specific NFI knockout model to investigate brain wiring and behaviour.

As glial cells with NFI deletion remain proliferative, we hypothesized that reduction of NFI results in a similar outcome in brain tumours of glial orgin. Genomic loss and low NFI expression were associated with reduced tumour differentiation and decreased survival in astrocytic tumours. Furthermore, high NFI protein and mRNA expression correlated with tumour differentiation and overexpression of NFI resulted in decreased proliferation. Using mouse models, we are currently determining whether loss of NFI contributes to glial cells transforming to tumour cells and whether NFI activation can be translated into therapy for brain cancer. Together our data indicates a broad role of NFI in normal glial differentiation and alterations of its function are associated with disease**.**

Monday 5th December
Symposia 3: Connecting the Dots: Understanding integrative brain function

COMPONENTS, COMPUTATION, COGNITION: THE ALLEN INSTITUTE FOR BRAIN SCIENCE 2020 VISION

Allan Jones
Allen Institute for Brain Science, Seattle

The Allen Institute for Brain Science is a non-profit research organization dedicated to providing tools and data for the larger research community. Launched in 2003, the Allen Institute has created a suite of large-scale data efforts along with a web portal to view and analyze the data. In 2012, the Allen Institute launched an ambitious $1B, 10-yr program to systematically characterize the structure and function of the circuitry of the mouse visual system, along with characterization of cell types in the human brain. The presentation will cover an overview of the Allen Institute, its current projects and infrastructure, a few data highlights, and a look at future directions.

ATTENTIONAL MODULATION OF PREFRONTAL CIRCUITS THAT MEDIATE FEAR EXTINCTION

Roger Marek[1], Jingji Jin[2], Li Xu[1], Stephen Maren[2], Pankaj Sah[1]
[1]The University of Queensland, [2]Texas A&M University

It is well established that the amygdala, the 'core region' for emotional processes, forms extensive connections with the hippocampus (HPC) and the prefrontal cortex (mPFC) to control extinction learning. In rodents, the prelimbic (PL) and infralimbic (IL) prefrontal cortices both play distinct roles in regulating emotional learning, whereby the hippocampus is crucially involved in regulating the contextual information in which extinction occurs. By using an optogenetic and pharmacogenetic approaches, combined with single cell recordings or behavior, we investigated the neuronal circuit underlying fear extinction both ex vivo and in vivo.

Ex vivo investigation within the mPFC revealed gutamatergic unidirectional deep-layer projections from the PL to the IL. Moreover, activation of this PL to IL connection during extinction learning was found to enhance extinction learning. This novel finding redefines the role of prefrontal sub-regions in fear extinction.

Hippocampal projections were found to target both pyramidal cells and interneurons in the IL, with a predominant innervation of interneurons. Characterization of these interneurons revealed strongest innervation of fast-spiking, parvalbumin-positive interneurons, which form local feed-forward inhibition of pyramidal cells, some of which project to the amygdala. On the behavioral side, pharmacogenetic modulation of this HPC-IL projection altered context-dependent recall of fear extinction, thereby illustration the importance of this projection in regulating the recall of extinction memory.

Taken together, these findings improve our understanding about the mPFC-driven neural circuitry that underlies the extinction of learned fear.

PREDICTIVE CODING IN RECEPTIVE FIELD FORMATION IN VISUAL CORTEX

Dr Hamish Meffin[1,2], Mr Ali Almasi[2,3], Prof Michael Ibbotson[1,2]
[1]ARC Centre of Excellence for Integrative Brain Function, Department of Optometry and Vision Sciences, University of Melbourne, [2]National Vision Research Institute, Australian College of Optometry, [3]Department of Electrical and Electronic Engineering, University of Melbourne

Predictive Coding is a theory of how the brain interprets its sensory inputs by building an internal model of the world based on the statistics of those inputs. The theory has been hypothesized to explain a wide range of observations including the hierarchical organization of (sensory) cortex, the architecture of cortical microcircuits, the structure of cortical receptive fields, single cell integration properties and synaptic plasticity. A central hypothesis of predictive coding is that the brain carefully controls which information from the sensory periphery is passed up through successive stages of the cortical hierarchy: "top-down" predictions are passed down the cortical hierarchy via descending connections, and only information that was not correctly predicted at higher cortical levels is allowed to pass further up the hierarchy. Using multi-electrode recordings from visual cortex of anaesthetised cats, together with reverse correlation techniques to recover the non-linear receptive field structures of single units, we compared receptive field structures in multiple cortical layers to assess whether they are consistent with predictive coding. The approach is uncovering a greater diversity of receptive field structures than revealed in previous studies.

A RODENT MODEL OF SENSORY DECISION-MAKING: NEURONAL CORRELATES OF SENSORY PRIORITISATION

Associate Professor Ehsan Arabzadeh[1,2]
[1]Australian National University, [2]ARC Centre of Excellence for Integrative Brain Function

A principal goal of systems neuroscience is to understand the computations that underlie information processing in neural circuits and to quantify the link between brain activity and the complex behaviour of organisms. Rodents use their vibrissae to explore the environment and to collect information about the location, shape, size and texture of objects around them. The rodent vibrissal-barrel cortex exhibits a fine columnar and laminar organisation and provides an ideal model for cortical processing due to its functional efficiency and well-studied circuitry. Here, I present an overview of the physiological properties of neurons in the vibrissal cortex, the behavioural modes of operation of the system, and how the neuronal codes in the sensory cortex account for the rat's detection and discrimination capacities in a sensory decision-making paradigm. I will then demonstrate how rats can optimise processing depending on the context in which sensory stimuli are encountered. This sensory prioritisation allows the animals to allocate limited quantities of attentional resources to the modality that is likely to provide the key information, and thus enhance the reliability and the speed with which they detect weak sensory stimuli. Electrophysiological recordings from primary vibrissal somatosensory cortex reveal the neuronal correlates of such sensory prioritisation.

Monday 5th December
Symposia 4: Motor Cortex Excitability in Health and Disease

SUBTYPE-SPECIFIC ALTERATION OF INHIBITORY CIRCUITS IN THE PRIMARY MOTOR CORTEX IN MOTOR NEURON DISEASE: A CELLULAR BASIS FOR CORTICAL PATHOPHYSIOLOGY

Clark R[1], Brizuela M[1], Blizzard C[1], King A[2], Young K[1], Dickson T[1]
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In amyotrophic lateral sclerosis (ALS), a loss of motor neuron function defines the disease, however, increasingly, cortical hyperexcitability is recognised as a prominent event, often proceeding motor neuron degeneration. While many factors may be attributed to this altered pathophysiology, a possible candidate, the interneuron has largely been overlooked.

In a systematic immunohistochemical study of interneuron subsets, we demonstrate region-specific pathological alteration in end-stage ALS cases and in $SOD1^{G93A}$ transgenic mice. Investigations are performed in motor and control somatosensory cortex from presymptomatic to end-stage disease.

We report two distinct interneuron populations are altered in the motor cortex and both exhibit presymptomatic, but contrasting pathology. In $SOD1^{G93A}$ mice, relative to controls, NPY^+ interneuron number was decreased (~17%) prior to motor symptom onset (8 weeks), but had increased by end-stage (~30%). In contrast, $calretinin^+$ interneurons had altered branch complexity at 8 weeks, with progressive reductions in cell number (~31%) from symptom onset (16 weeks). Interneurons were unaltered in the somatosensory cortex, suggesting that NPY^+ and $calretinin^+$ populations drive a motor-specific inhibitory phenotype early in disease. In human ALS brain tissue calretinin-interneuron pathology was recapitulated in a proportion of cases, and positively correlated with the extent of cortical motor neuron pathology in all cases.

$Calretinin^+$ GABAergic neurons play a crucial role in cortical disinhibition, by regulating other interneurons, whereas NPY^+ populations are coupled to circuit excitability. Therefore, their differential involvement is likely to alter the motor cortex inhibitory circuit at early stages of disease, contributing to pathophysiology and motor neuron vulnerability within this region.

MODULATING POST-STROKE NEURONAL EXCITABILITY IMPROVES MOTOR FUNCTION

Dr Andrew Clarkson[1,2], Dr Raghavendra Nagaraja[1], Mr Ahmed Has[2], Professor Mary Chebib[2]
[1]Department of Anatomy, University Of Otago, [2]Faculty of Pharmacy, The University of Sydney

Stroke induces a change in network activity in the brain that hinders recovery. Understanding the profile for how cortical plasticity occurs and is altered after a stroke is critical for fully determining when to start treatments and with which therapeutic compound to use. Functional recovery in peri-infarct tissue that surrounds the stroke involves changes in brain excitability that alters the way the brain represents motor and sensory function. We have previously shown that this is due to an elevation in tonic GABAA receptor current and by dampening this elevation in tonic GABA activity can lead to improved functional recovery. We report here that the level of inflammation and oedema can influence cortical excitability with large cortical oedema resulting in hypoexcitability and low oedema hyperexcitability. Interestingly, chronic and not acute administration of L655,708 is required to normalise cortical excitability and increase motor representational maps after stroke irrespective of the level of inflammation and oedema. We show that tonic inhibitory currents remain elevated for up to 42-days. However we report that the therapeutic window for administration of L655,708 is only up to 14-21-days post-stroke, indicating that the cause of tonic inhibitory currents after this time is most likely mediated by another GABAA receptor. Interestingly, administration of zolpidem, the α1-subunit-specific ligand is effective at improving functional recover after 14-days through an increase in tonic inhibition. These data indicate that brain excitability is ever changing after stroke and opens up different treatments window that requires different drug treatments to improve motor function.

NEURAL REPRESENTATIONS OF MOVEMENT IN THE MOTOR THALAMUS AND PRIMARY MOTOR CORTEX.

Authors: Joshua Dacre, Brian Premchand, Jussi Pardoe, Federico Claudi, Alastair Macdonald, Julia Schiemann, Alex Harston, Julian Ammer and **Ian Duguid**

Affiliation: *Centre for Integrative Physiology and Patrick Wild Centre, Edinburgh Medical School: Biomedical Sciences, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh, EH8 9XD, UK.*

The primary motor cortex (M1) plays a crucial role in the generation of volitional and sensory-driven motor behaviours. During movement preparation and execution long-range inputs from motor thalamus – which consists of several subcortical nuclei including the ventral anterior (VA), ventrolateral (VL) and antero-medial (AM) nuclei – are thought to drive complex spatiotemporal changes in the activity of output neurons in layer 5 of M1. Using the mouse motor system as an experimental

model, I will discuss our recent work that combines patch-clamp and extracellular recordings, 2-photon population imaging and 2D high-speed kinematic analysis of forelimb movement to unravel the mechanisms by which motor thalamic output shapes M1 activity and behaviour in mice trained to perform a cued object manipulation task.

CORTICAL HYPEREXCITABILITY: A PATHOGENIC PROCESS IN ALS/MND

Professor Steve (ostoja) Vucic[1]

[1]*University Of Sydney*

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and invariably fatal neurodegenerative disorder of motor neurons, with median survival of 3-5 years. Cortical hyprexcitability appears to be an early and intrinsic feature of both sporadic and familial ALS phenotypes, preceding lower motor neuron dysfunction and correlating with ensuing lower motor neuron degeneration. Importantly, cortical hyperexcitability could form the pathogenic basis of ALS, with corticomotoneuronal hyperexcitability mediating motor neuron degeneration via a transsynaptic glutamate-mediated excitoxic mechanism, the "dying forward" hypothesis. Support for cortical hyperexcitability as an important pathogenic mechanism in ALS has been provided by clinical, neurophysiological, molecular and genetic studies. Most notably the recent identification of the c9orf72 gene as an important genetic basis for both ALS and frontotemporal dementia, underscored the importance of cortical processes in ALS pathogenesis. Separately, cortical hyperexcitability appears to be an important diagnostic biomarker, significantly enhancing the diagnosis of ALS at early stages in the disease process. Consequently, the identification of cortical hyperexcitability as an important pathophysiological and diagnostic biomarker in ALS has provided insights provided novel diagnostic and therapeutic approaches, thereby providing hope for more effective management of ALS patients.

Monday 5th December
Symposia 5: Genetic dissection of hypothalamic neural networks

USING MOUSE GENETICS TO UNRAVEL THE CNS PATHWAYS CONTROLLING ENERGY BALANCE AND GLUCOSE HOMEOSTASIS
Joel Elmquist
University of Texas

OPTOGENETIC ANALYSIS OF THE HYPOTHALAMIC NEURAL NETWORK CONTROLLING FERTILITY
Herbison AE, Han S, Iremonger K, Kalil B, McLennan T, Piet R, Porteous R.
Centre for Neuroendocrinology and Department of Physiology, University of Otago School of Medical Sciences, Dunedin 9054, New Zealand

The gonadotropin-releasing hormone (GnRH) neurons are the final output cells of an hypothalamic neuronal network controlling fertility in all mammalian species. The GnRH neurons secrete GnRH into the pituitary portal system to drive pituitary release of luteinizing hormone (LH) in two modes; as short pulses or as a prolonged surge that lasts for many hours. The cellular mechanisms underlying the episodic pulse- and surge-like activities of the GnRH neurons are unknown. Two independent populations of neurons utilizing the neuropeptide kisspeptin are currently suspected of driving these different behaviours. We used AAV transduction in kisspeptin-Cre mice to target channelrhodopsin (ChR2) to each of the kisspeptin populations. Frequency-dependent optogenetic activation of kisspeptin neurons located in the hypothalamic arcuate nucleus was found to generate pulsatile LH secretion (Han et al., PNAS 112, 2015). The optogenetic activation of the other kisspeptin neuron population located in the preoptic area generated large increases in LH secretion reminiscent of the GnRH/LH surge. Brain slice ChR2 electrophysiology and GCaMP6 calcium imaging investigations are currently identifying the cellular pathways underlying the ability of the two kisspeptin neuron populations to drive pulse and surge modes of GnRH neuron activation.

CENTRAL NEURAL CONTROL OF BROWN AND BEIGE FAT – NEUROCHEMICAL CHARACTERISATION REVISITED
Professor Brian Oldfield[1]
[1]Monash University

Brown adipose tissue (BAT) has been shown to exist in adult human in amounts inversely proportional to fat mass and intriguingly it is recognized that brown-like or "beige" fat can be transformed from white fat under environmental conditions that involve activation of sympathetic neural inputs to fat. It is imperative to understand the neurochemical profile of the innervation of this transformed fat if it is to be recruited in a therapeutic setting. fat. A range of approaches have been applied to the definition of neural projections to brown and beige fat and to the neurochemical characterization of these pathways. These include the multiple injection, into the same animals, of different isoforms of neurotropic viruses which are distinguishable based on their expression of different fluorescent reporters as well as novel (Brainbow) viruses that change the profile of fluorescent reporter expression in the presence of Cre recombinase. Such virus-based tracing strategies, in conjunction with RNA seq and bioinformatics analyses, have allowed the identification of 1) a central re-organisation of descending pathways from the brain to fat depots coincident with the transformation of white fat to beige fat, 2) a transformation of the profile of gene expression in sympathetic ganglia innervating inguinal white fat, after browning of this fat pad, to a neurochemical signature that is more "brown-like", 3) a short list of candidate transmitters that may be specific for the control of beige fat and 4) the trajectory through the brainstem of pathways projecting to BAT depots in the rat.

POPULATION-SPECIFIC DELETION OF HYPOTHALAMIC PROLACTIN RECEPTORS IDENTIFIES A CRITICAL ROLE FOR MATERNAL BEHAVIOUR.

Rosie Brown

University of Otago

During pregnancy and lactation, a critical adaptation that occurs in the maternal brain is the establishment of appropriate behaviour to enable the mother to feed and nurture offspring. Prolactin is known to be involved in stimulating the onset of maternal behaviour, but the mechanism and the site of action is unknown. The medial preoptic area (MPOA) of the hypothalamus is important for maternal behaviour, and we have shown prolactin receptor expression and an increase in prolactin responsive neurons in this region during lactation. Therefore, we deleted prolactin receptors from the MPOA of adult female mice by injecting an adeno-associated virus expressing Cre-recombinase into the MPOA of prolactin receptor flox transgenic mice. Although fertility, length of gestation and parturition was unaffected in these mice, no litters survived beyond day 2 of lactation, due to a complete failure of maternal care. We have shown that prolactin acting in the MPOA plays an essential role in the initiation of maternal behaviours.

Monday 5th December
Symposia 6: Traumatic brain injury and the development of neurodegenerative diseases

ACUTE AND CHRONIC PATHOLOGIES OF HUMAN TBI: LINKS TO NEURODEGENERATIVE DISEASE

Dr. Victoria Johnson[1]
[1]*The University Of Pennsylvania*

In recent years the topic of traumatic brain injury (TBI) has received increasing public and media attention, yet it has long been a major health issue worldwide. Moreover, the link between TBI and dementia has generated considerable public concern following increased reporting of 'chronic traumatic encephalopathy' (CTE) associated with repetitive mild TBI in athletes. Initially called 'dementia pugilistica' and linked to professional boxing, neuropathological features of CTE have been proposed to include brain atrophy, cavum septum pellucidum, and amyloid-β, tau and TDP-43 pathologies. We have identified various similar chronic pathologies years after just a single moderate to severe TBI. However, the establishment and characterization of CTE as a neuropathologically or clinically distinct disease entity is in its infancy. In addition, the mechanisms that may contribute to neurodegeneration following TBI are largely unknown. Here we will explore the current understanding and controversies in the study of chronic neuropathologies post-TBI.

THE LONG-TERM EFFECTS, PATHOBIOLOGY, AND TREATMENT OF MILD TRAUMATIC BRAIN INJURIES

Sandy Shultz[1]
[1]*The University Of Melbourne*

Mild traumatic brain injury (mTBI) is a serious and common medical issue worldwide. Of particular concern are individuals who are at a high risk of suffering recurring mTBIs as these injuries have been linked to persisting mental health disabilities and neurodegenerative disease. However, the long-term consequences and related pathologies of mTBIs still remain poorly understood, and there are currently no interventions that are known to prevent their effects. This presentation will include findings from both patient and animal model studies, and aims to provide insight into the long-term effects, pathogenesis, and treatment of mTBIs. MRI studies in retired professional rugby league players with a history of mild TBI identified structural, microstructural, and metabolic brain abnormalities compared to age and education matched controls with no history of mTBI. Complementing these patient studies, animal model experiments found that repeated mTBIs can induce cumulative and progressive neurodegeneration, and implicated neuroinflammation, hyperphosphorylated tau, and oxidative stress as key pathophysiologies underlying the neurological consequences of mTBIs that can be targeted to mitigate their effects.

DELINEATING THE NEUROPROTECTIVE ROLE OF THE AMYLOID PRECURSOR PROTEIN IN TRAUMATIC BRAIN INJURY – A NOVEL THERAPEUTIC AGENT

Prof. Roberto Cappai[1], Ms. Stephanie Plummer[2], Ms. Chaitanya Inampudi[1], Dr. Frances Corrigan[2], Dr. Emma Thornton[2], Prof. Robert Vink[3], A.Prof Peter Crack[4], A.Prof Corinna Van Den Heuvel[2]
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Traumatic Brain Injury (TBI) can result in considerable neurological damage which occurs through a cascade of deleterious physiological events over subsequent days. The amyloid precursor protein (APP) is up-regulated following TBI. APP is a ubiquitously expressed, highly conserved, integral membrane glycoprotein that has been extensively studied due to its important association with Alzheimer's disease. Using a combination of animal and injury models, we found that administration of soluble APP (sAPP) into rodents post-injury significantly improved behavioural outcomes, and it reduced neuronal cell loss and glia activation. APP knockout mice were more susceptible to TBI. APP knockout mice were rescued from TBI by administration of sAPP. We localised the neuroprotective active site in sAPP to a 15 amino-acid sequence in the N-terminal growth factor region (residues 96-110). Our current studies are investigating the efficacy of APP96-110 via intravenous administration. A single intravenous dose of APP96-110 (0.05mg/kg), 30 minutes following moderate-severe diffuse impact acceleration injury, in rats, improved both motor and cognitive outcomes, and reduced axonal injury and microglia activation. We are examining the molecular basis for APP96-110's neuroprotective activity, which we believe correlates to its heparin binding activity, by testing APP96-110 mutants. These studies have demonstrated that APP and its upregulation following injury represents a protective reaction towards TBI, and that APP is part of a reparative / neuroprotective response.

AGEING WITH A TRAUMATIC BRAIN INJURY

Dr Jenna Ziebell[1,2,3], Dr Rachel Rowe[2,3], Dr Jessica Collins[1], Mr Jordan Harrison[2,3,5], Dr Matthew Law[2,3], Dr P. David Adelson[2,3,5], Dr Jonathan Lifshitz[2,3,4,5]
[1]*Wicking Dementia Research and Education Centre, University of Tasmania,* [2]*BARROW Neurological Institute at Phoenix Children's Hospital,* [3]*Department of Child Health, University of Arizona College of Medicine – Phoenix,* [4]*Phoenix Veteran Affairs Healthcare System,* [5]*Interdisciplinary Graduate Program in Neuroscience, Arizona State University*

Mounting evidence indicates that brain injury, even moderate injury, can initiate a lifetime of physical and mental health impairments. In fact, age-at-injury influences the extent to which the brain can repair itself after injury. To assess the extent of chronic behavioural and inflammatory responses to traumatic brain injury (TBI), a single cohort of male Sprague-Dawley rats was received at post-natal day (PND) 10. Rats were subjected to a single moderate midline fluid percussion injury at PND 17, PND 35, 2, 4, or 6 months; and compared to a control group of naïve rats. The entire cohort was assessed for motor function, anxiety-like behaviour, cognitive performance, and depressive behaviour throughout a 10-month ageing period. All tissue was collected at 10 months of age.

Motor and cognitive deficits occurred in rats injured at younger ages, but not in the older-injured rats. In contrast, rats injured during adulthood showed increased anxiety-like behaviour compared to naïve rats. Diffuse TBI did not result in chronic depression-like behaviours in any groups. Robust changes in the morphology of microglia were detected in all injured groups compared to naïve. Further investigation into microglia phenotype and morphology can determine their persistence in the chronic stages of injury.

Overall, brain injury may augment the ageing process, such that glial and neuronal alterations that impact neurological impairments and mental health. This interplay of age-at-injury and ageing with an injury are translationally important factors that influence behavioural performance. Understanding these factors can direct rehabilitative efforts and personalized medicine for TBI survivors.

Monday 5th December

Symposia 7: Cognitive Training & the Aged Brain: Mechanisms, Disease-Specific Efficacy and How to do it at Scale

GAMING FOR TRAINING: INCORPORATION OF VIDEO GAMING PRINCIPLES & PRACTICE INTO NEXT-GENERATION COGNITIVE TRAINING

Ms Verity Chadwick[1]
[1]*University Of Sydney*

Computerised cognitive training is a low cost, safe and effective method to overcome deficits in cognition associated with ageing. Research in older adults with and without cognitive impairment suggests that training must be engaging to maintain patient motivation and study adherence. Virtual reality, gaming consoles and one-on-one tutoring are more popular and effective than traditional at-home commercial cognitive training. Therefore, lessons from effective cognitive training must be used to create a scalable and engaging cognitive training platform for long-term use.

We are tackling current challenges in the NHMRC 'Maintain your Brain', which is the world's largest e-health platform of its kind, incorporating the active ingredients of effective training with the aim of delaying or preventing the onset of dementia. The training schedules are based on a novel and dynamic algorithm, which adapt training on participant's recent task performance to continuously customise both the difficulty and content of training. To ensure high motivation and study adherence, participants are encouraged to videoconference expert trainers when encountering difficulties or in need of support, and family members provide motivational rewards at training milestones for positive reinforcement. From the clinician's side, the platform will flag participants who are struggling on exercises or protocol adherence, such that an online trainer can be "called up" during training sessions to instruct, mentor and motivate.

Overall, next generation brain training aims to socialise the online brain training experience, connecting participants with like-minded peers, expert trainers and their own social network for long term adherence and sustained cognitive benefit.

UFOV TRAINING IMPROVES EVERYDAY FUNCTIONAL PERFORMANCE AND REDUCES RISK OF DEMENTIA

Jerri Edwards

Over the last thirty years, several cognitive training programs have been developed to improve specific fluid abilities. Although these training programs typically demonstrate improvement of the cognitive abilities targeted, transfer to broader cognitive function has been limited, which has led to debates about the value of such interventions. However, one particular approach, Useful Field of View (UFOV) training (a.k.a., cognitive speed of processing training), shows far transfers to improved everyday function including reduced risk of dementia. UFOV training is a process-based, computerized intervention involving perceptual practice of speeded visual attention tasks. Systematic review and meta analyses of this training technique will be presented, demonstrated both near transfer to improved speed of processing and attention, and far transfer to improved everyday function, well-being, and driving mobility. The effects of UFOV training endure longitudinally, and recent results show those randomized to training are 33% less likely to develop dementia over 10 years (HR 0.67, 95%CI 0.49-0.91, p=.012). Despite the ongoing debate regarding the potential efficacy of cognitive training, there is clear evidence that some cognitive interventions transfer more broadly to positively affect older adults' everyday functional performance and well-being. The potential underlying mechanisms of UFOV training will be discussed.

COGNITIVE RESPONSE PROFILES FOR COMPUTERISED COGNITIVE TRAINING ARE DISEASE-SPECIFIC

Dr Amit Lampit[1,2]
[1]*School of Psychology, University of Sydney,* [2]*Regenerative Neuroscience Group, Brain and Mind Centre, University of Sydney*

Clinical research on cognitive training is transforming from basic questions of efficacy into differential effects across populations and design factors. We systematically investigated cognitive and other clinical benefits of cognitive training in a series of meta-analyses encompassing nearly 7,000 participants across 102 randomised trials in healthy elders, mild cognitive impairment (MCI), dementia, Parkinson's disease and traumatic brain injury (TBI). Analyses of each population investigated overall and domain-specific benefits, moderators of effect and potential sources of bias.

Cognitive training was efficacious on overall cognition across all populations without evidence of publication bias or difference between active- and passive-controlled trials. Small to moderate effect sizes were also noted on individual domains and differed across populations. For example, memory benefits were noted in healthy elders and MCI, but not in Parkinson's and TBI. Conversely, significant effects on executive functions were noted only in the latter two populations. In MCI, a therapeutic benefit of training was corroborated by effect across clinical measures of global cognition and psychosocial functioning and lack of response in control groups. Across dementia trials, however, cognitive benefits were observed only across trials of immersive

technologies, suggesting that these methods are more stimulating and effective in people with overt dementia. Key predictors of training effects are supervised training, sessions longer than 30 minutes and up to three times per week.

Cognitive training researchers working on different populations should adopt methods from one another in older to design training regimens that effectively target key therapeutic indicators.

NEURAL MECHANISMS UNDERLYING THERAPEUTIC BENEFITS OF BRAIN TRAINING

Associate Professor Michael Valenzuela[1]
[1]*University Of Sydney, Brain And Mind Centre*

Brain training (or more formally computerised cognitive training) is effective on cognitive outcomes across many brain disorders, ranging from schizophrenia to Mild Cognitive Impairment (MCI) and Parkinson's disease. Yet the underlying neural mechanisms are largely unknown. In general, classes of mechanisms include disease modification, that is the amelioration or attenuation of classical neurodegeneration or pathology, or, compensation, whereby training produces changes that allow for more efficient use of extant functional networks or recruitment of newly reorganised networks to support brain function.

To date there is little support for disease modification. For example, there are no studies showing that brain training alters amyloid binding in ageing or MCI, slows medial temporal lobe atrophy in AD, or impacts on striatal degeneration in PD. On the other hand, there is accumulating evidence for the recruitment of new functional brain networks. For example, our own data shows that brain training in MCI enhances hippocampal-frontal lobe connectivity, a change that formally mediates mnemonic benefits in this group. Other examples of augmented brain training-related connectivity will be discussed and a general framework for thinking about neural mechanisms proposed.

Monday 5th December
Symposia 8: Pathophysiology of the Blood-Labyrinth Barrier

USE OF MRI TO ASSESS THE BLOOD-LABYRINTH BARRIER

Professor Peter Thorne[1], Dr Jerome Plumat[1], Dr Srdjan Vlajkovic[1], Dr Beau Pontre[1], Dr Ravindra Telang[1], Dr Michel Neeff[1]
[1]*University Of Auckland*

Inner ear tissues and fluids are separated from the vasculature by the Blood-Labyrinth Barrier (BLB). It is thought BLB changes may be involved in development or progression of inner ear disorders such as Ménière's Disease (MD).Changes in the BLB may also affect progression of an inflammatory response and recruitment of immune cells in response to injury. Isolation of the inner ear and its encapsulation in bone, presents considerable challenges to studying such disease processes and dynamics in the BLB in the intact animal and human inner ear. We have developed methods using Dynamic Contrast Enhanced (DCE) MRI to study inflammation and vascular permeability in the guinea-pig inner ear and then used these to investigate the BLB in animal models of inner ear injury and humans with MD. In the guinea-pig I, following inner ear challenge with bacterial lipopolysaccharide (LPS, 0.8mg/kg) introduced into the middle ear, we observed a significant ($P<0.0001$) 6-fold increase in vascular permeability (quantitative change in MR signal due to presence of gadolinium contrast agent) which recovered within 7-10 days. In humans a similar approach shows a substantial increase in patients with MD, although there was no correlation with the level of hearing loss or vestibular dysfunction. Modelling of the vascular permeability enabled calculation of the permeability constant (Ktrans), which increased in patients with MD. These findings show that the DCE-MRI is useful to study vascular permeability in the intact inner ear of animals and humans and that it can show BLB disruptions with disease and inflammation.

THE ENDOLYMPH-PERILYMPH BARRIER AND ENDOLYMPHATIC HYDROPS.

Brown DJ[1], Pastras CJ[1], Southwell CS[1]
1. The University of Sydney. Brain and Mind Centre. Sydney NSW

Endolymphatic hydrops is a swelling of the endolymphatic fluid compartment of the inner ear, and is thought to underlie the symptoms of Meniere's syndrome, such as sudden attacks of severe vertigo and fluctuating hearing sensitivity. Where endolymph is produced and absorbed, or how its volume is regulated, is currently unclear. Moreover, how hydrops causes the symptoms of Meniere's is also unknown. To study endolymph dynamics with hydrops, we have injected fluorescein isothiocyanate–dextran (FITC-dex) plus artificial endolymph into scala media of anaesthetized guinea pigs, with subsequent imaging of the inner ear using Light Sheet Fluorescence Microscopy (LSFM) as a means to obtain highly resolved 3D visualization of fluid movements. Our results demonstrate endolymph movement into the utricle, semicircular canals, and endolymphatic sac only when there is a 2-fold increase in endolymph volume. The remarkable uptake of the FITC-dex into the endolymphatic sac, including an absorption into the periductal channels surrounding the endolymphatic duct, highlights the functional role this structure plays in endolymph volume regulation. In other animals, we induced hydrops by injecting lipopolysaccharide into the endolymphatic compartment, with subsequent animal recovery. Here, the lipopolysaccharide causes excessive glycoprotein production in the endolymphatic duct, causing an apparent blockage. These results suggest endolymph is absorbed in the endolymphatic duct, but that antigen can cause this compartment to become blocked, resulting in hydrops of the endolymphatic compartment.

BLOOD-LABYRINTH BARRIER COMPROMISE WITH SYSTEMIC INFECTION

Dr. Keiko Hirose[1], Dr. Song Zhe Li[1], Mr. Jared Hartsock[1], Dr. Alec Salt[1]
[1]*Washington University In St. Louis*

Integrity of the blood-labyrinth barrier is believed to be critical to the normal function of the inner ear. However, a direct quantitative method to assess the integrity of the barrier has been a major limitation to exploring this concept. Our group has developed a rigorous, quantitative method to assess the integrity of the blood labyrinth barrier by injecting solute intravenously and measuring the concentration of the solute in the serum and in perilymph. We have found that in low level sepsis, induced by systemic lipopolysaccharide, the blood-labyrinth barrier is transiently disrupted. Furthermore, in the case of bacterial meningitis induced by Streptococcus pneumoniae, the blood labyrinth barrier is even further compromised, but it is reestablished rapidly and overshoots the baseline such that almost no solute enters the perilymph after recovery. This breach in the blood labyrinth barrier caused by meningitis is accompanied by a massive migration of leukocytes into the perilymph. Thus, we believe that the blood labyrinth barrier is highly dynamic and permits leukocyte entry into cochlear duct when bacteria are present, and facilitates cochlear entry of leukocytes that comprise the initial line of host defense. While in the past, this event of barrier breach has been perceived as detrimental, it appears to be critically important in clearing infection, and plays an important role in survival.

STRIAL BLOOD LABYRINTH BARRIER DAMAGE AND REGENERATION

Associate Professor Xiaorui Shi[1], M.D. PhD Xiaohan Wang[1], Dr Allan Kachelmeier[1]
[1]*Oregon Hearing Research Center, Department of Otolaryngology/Head & Neck Surgery*

To sustain hearing acuity, a normal function of blood-labyrinth-barrier (BLB) must be maintained in the ear. This is particularly true since generation of the endocochlear potential (EP), the essential driving force for hair cell transduction, is so metabolically demanding. Is angiogenesis key to restoring damaged strial vessel function? Angiogenesis in the ear has not been studied, nor has the role of angiogenesis in hearing been investigated. In this study, using an in vivo pericyte depletion animal model in association with a newly established ex vivo stial tissue based-model, we demonstrate for the first time that damaged vascular function (blood labyrinth barrier) can be restored by activating the vascular endothelial growth factor signal pathway. Using transgenic neural/glial antigen 2 (NG2) fluorescent reporter mice, we have shown the progenitors of “de novo” strial vessels are pre-existing ECs and converted perivascular NG2-derived cells. Most important, the pattern of the newly formed vessels resembles the natural ‘mesh pattern’ of in situ strial vessels, with both lumen and expression of tight junctions. Taken together, our data shows that damaged strial microvessels can be regenerated by reprogramming angiogenesis. The restoration of functional vasculature may be crucial for restoration of vascular dysfunction related hearing loss.

Monday 5th December
Symposia 9: Current research approaches in animal models relevant to schizophrenia

TOWARDS UNDERSTANDING THE BIOLOGICAL MECHANISM UNDERLYING ESTROGEN'S THERAPEUTIC EFFECT IN SCHIZOPHRENIA

Dr Andrea Gogos[1], Jeehae Sun[1], Madhara Udawela[1], Elizabeth Scarr[1], Brian Dean[1]
[1]Biological Psychiatry Division, Florey Institute Of Neuroscience And Mental Health

Growing evidence supports the notion that sex steroids, particularly estrogen, play an important role in psychiatric disorders. Our previous animal behaviour studies showed antipsychotic-like effects of estrogen, where chronic estrogen treatment prevented drug-induced deficits in prepulse inhibition. Estrogen mainly acts through estrogen responsive elements on gene promotors to change gene expression. The impact of estrogen on gene expression in the frontal cortex, a region particularly affected in psychiatric disorders, is unknown. This study aimed to identify gene expression changes caused by sex steroid hormone removal and estrogen treatment.
Adult female Sprague-Dawley rats were either intact (sham-operated), ovariectomised, or ovariectomised and treated with 17b-estradiol for 4 weeks. Frontal cortical RNA was extracted and hybridised to an Agilent SurePrint G3 Rat Gene Expression microarray. Using JMP Genomics, expression data was analyzed to identify genes with different expression profiles and Ingenuity Pathway Analysis was used to identify pathways.
Ovariectomy significantly altered the expression profiles of 145 genes, while estrogen treatment altered 332 genes. The altered gene expression profiles between the groups highlights that some, but not all, genes which are down-regulated by ovariectomy can be up-regulated by estrogen treatment. Further, the changes in expression caused by chronic estrogen treatment, may reflect the genes implicated in regulating the therapeutic effects of estrogen. These data suggest that the effects of estrogen on gene expression will have profound and wide-ranging effects in the frontal cortex, supporting the concept that sex steroids play a role on modulating pathways associated with schizophrenia.

INCREASED DA SYNTHESIS IN THE ADOLESCENT BRAIN. AN ANIMAL MODEL OF THE PRODROME IN SCHIZOPHRENIA

Prof Darryl Eyles, A Petty [1], X Cui [1], Eyles DW[1,2],
1. Queensland Brain Institute, The University of Queensland, Brisbane, QLD, Australia
2. Queensland Centre for Mental Health Research, Brisbane, QLD, Australia

My group's research focuses exclusively on the neurobiology behind the non-genetic risk factor epidemiology of severe mental illness such as schizophrenia and autism. In particular we attempt to understand how adverse factors such as developmental vitamin D deficiency or maternal immune activation (MIA) during the early (foetal) stages of brain development affect brain ontogeny to produce these disorders.

Using animal models for these risk factors we have shown that developing dopamine neurons are adversely affected in both models. We conclude that adverse developmental factors may operate via early convergent pathways related to the ontogeny of developing dopamine systems to predispose an individual to developing schizophrenia. We have now developed a new animal model based precisely on this assumption which we call "Elevated Dopamine in the Prodrome of Schizophrenia" (*EDiPs*) This model employs a virally delivered genetic construct to the substantia nigra of adolescent rats to directly reproduce an essential neurobiological feature in the prodrome of schizophrenia of increased dopamine activity in the striatum. In this symposium I will describe the nature of regional selectivity of this increase in dopamine along with initial behavioural and molecular phenotypes and describe how this model represents an ideal model in which to test agents directed at *preventing* the onset of schizophrenia.

MODELING CANNABIS EXPOSURE IN A VARIETY OF MOUSE MODELS FOR THE SCHIZOPHRENIA RISK FACTOR NEUREGULIN 1

David Lloyd[2,4,5], Juan Olaya[2,4,5], Doctor Carrie Heusner[3], Professor Micky Matsumoto[3], Prof Cyndi Shannon Weickert[2,4,5], **Associate Professor Tim Karl[1]**
[1]Western Sydney University, [2]Neuroscience Research Australia, [3]Astellas Research Institute of America LLC, [4]University of New South Wales, [5]Schizophrenia Research Institute

Objective
Neuregulin 1 (NRG1) is a risk factor for schizophrenia. In line with the Two-Hit hypothesis, the cumulative environmental risk factor cannabis induces more pronounced neuro-behavioural consequences in transmembrane domain Nrg1 mutant mice. However, NRG1 biology is complex and seven different isoform types are known. We recently validated a novel mouse model for Nrg1 type III isoform overexpression for schizophrenia. Here, we compare the behavioural effects of cannabis exposure (i.e. delta-9-tetrahydrocannabinol: THC) in various Nrg1 mouse models.
Methods
Adult Nrg1 type III transgenic as well as knockout mice and appropriate controls were exposed to different doses of acute or chronic THC. All mice were assessed for behaviours relevant to schizophrenia and cannabis including locomotion, anxiety, social domains, and sensorimotor gating.

Key Findings
Nrg1 type III knockout and control mice were susceptible to THC effects in a dose-dependent manner. THC reduced open field locomotion, increased anxiety-related behaviours, and also had a moderate effect on sensorimotor gating. Different to what has been found in other Nrg1 mouse models, Nrg1 type III knockout mice appeared not to be more sensitive to the behavioural effects of THC. These data will be compared with the impact THC had on Nrg1 type III transgenic mice.
Conclusion
Our findings show that it is highly likely that the reported Nrg1-cannabis interaction in the context of schizophrenia is dependent on the Nrg1 isoform type. This also suggests that mutant NRG1-cannabis interactions found in humans should be investigated in more detail thereby considering the particular NRG1 isoform types affected.

ANIMAL MODELS OF MATERNAL IMMUNE ACTIVATION AND RELEVANCE FOR SCHIZOPHRENIA

Harms L[1], Meehan C[1], Dunn A[1], Gray A[1], Tattoli R[1], Fulham R[1], Todd J[1], Schall U[1,2], Shannon Weickert C[2], Hodgson D[1], Michie P[1]

1. *Priority Research Centre for Brain and Mental Health Research, University of Newcastle, Callaghan, NSW, Australia*
1. *Schizophrenia Research Institute, Neuroscience Research Australia, Randwick, NSW, Australia*

Exposure to infection during gestation has been shown to increase risk for developing schizophrenia in offspring. Bacterial or viral mimetics have been administered to pregnant rodents to create animal models of 'maternal immune activation' (MIA) and have demonstrated that immune stimulation during gestation is associated with a range of schizophrenia-related changes to brain development and behaviour in rat and mouse offspring. To further investigate the impact of MIA on schizophrenia-related behaviour as well as electrophysiological 'biomarkers' for schizophrenia, we investigated the impact of MIA (via Poly (I:C) injection) at mid (Gestational day, GD, 10) and late (GD19) gestation rat offspring. Regardless of GD of exposure, rats exposed to MIA had reductions in prepulse inhibition. In addition, rats exposed to MIA on GD19, but not GD10, exhibited transient working memory impairments. While MIA-exposed rats did not exhibit changes in mismatch negativity or spontaneous oscillatory activity, stimulus-driven gamma oscillations were impaired in MIA rats, similar to patients with schizophrenia. These findings indicate that the MIA rat model is a highly relevant model for the investigation of the behavioural and neurobiological correlates of aberrant gamma oscillations.

Monday 5th December

Symposia 10: Vision in invertebrates: Decision making models, neural mechanisms, and quantitative behavior

THE EFFECTS OF SENSORY LIMITATIONS ON DECISION-MAKING IN FIDDLER CRABS

Dr Jan Hemmi[1], Ms Anna-Lee Jessop[1], Ms Monika Siekelova[1], Dr. Yuri Ogawa[1]
[1]*University of Western Australia*

Despite their small brain size, fiddler crabs are autonomous, versatile and robust. Catching them is exceedingly difficult and we have previously shown that they can also learn to ignore a potential threat. In order to understand the decision making process, we need an accurate description of the crabs' sensory input.
We have developed a range of behavioural and ElectroRetinoGram (ERG) techniques that allow us to accurately and quickly measure the spatial, spectral and temporal visual sensitivities of animals. This includes a full spatial contrast sensitivity function in both the vertical and horizontal direction. Detailed characterisation of the visual capabilities of the fiddler crab, our model species, shows that the sensory information available to individuals, during predation events, is substantially coarser than expected. They have acuity of less than 1 cycle per degree, yet their eyes have a (fast) critical flicker fusion frequency above 100 Hz. They also have the ability to see colours and UV light. Work on the characterisation of Fiddler Crab polarization sensitivity is still in progress.
In the light of this limited sensory input, fiddler crab behavioural accomplishments are outstanding. By deciphering the sensory limitations of animals and therefore, knowing the sensory and contextual information that is available during predation events, the importance of the decision making rules that underlie behavioural decision making process is exposed, allowing us to understand how relevant (escape) decisions are made.

HOW HONEY BEES LEARN ABSTRACT CONCEPTS

Dr Andrew Barron[1], Dr Alex Cope[2], Dr Eleni Vasilaki[2], Dorian Minors[1], Prof. James Marshall[2]
[1]*Macquarie University,* [2]*University of Sheffield*

Perhaps the most remarkable demonstration of honey bee cognitive ability is their capacity to solve a delayed match-to-sample task. In this task, to get a sugar reward bees must choose a stimulus that matches an exemplar they have been shown previously. Bees' ability to solve this task has been interpreted as evidence that they can learn the abstract concepts of 'same' and 'different'. In vertebrates this is considered a higher cognitive function and a property of processing within the cortex. Bees do not have a cortex, and their brain is 6,000 times smaller than the smallest brained primate that can solve this task. How then do they do it? Here we present a computational model constrained by the known structure and functions of the honey bee brain that can learn the delayed match-to-sample task. In the model the task can be solved if activation of a subset of neurons in the learning pathway of the mushroom body of the bee brain is strongly influenced by synaptic facilitation rendering this population more likely to fire in response to a stimulus experienced twice in succession. This can support the phenomenon of learning to respond to sameness and difference without any neuron in the system responding to the abstract concept of sameness or difference. This model might explain how honey bees can solve a higher cognitive task with a simple brain, and may also be the mechanism of similar abstract learning in larger brained animals.

POLARISATION VISION – MORE THAN RUNNING BACKWARDS WITH POO

Prof Justin Marshall[1]
[1]*University Of Queensland*

Polarization vision in terrestrial species has been implicated famously in navigating to and from food sources, a variety of polaro-taxes, dung-ball rolling and more recently in possible mate choice in butterflies. Its purpose in marine animals is less well understood, may include navigation in fish, but recent evidence suggests polarization signalling in some cephalopods and many stomatopods. Mate choice or territorial conflict seems to drive the evolution of this communication strategy but a 400million year old arms-race between the ocean's top invertebrate predators may have played a part. As with colour, as soon as a signal exists, potential strategies of camouflage and deception as well as conspicuousness must be considered. A long-held hypothesis is that polarization vision in water is used to break the mirror camouflage of silvery fish. However, direct in situ evidence that silvery fish are more visible to polarization vision than they are to radiance vision is poor or lacking. Here we use in situ polarization imagery to quantify the visibility of species of fish, cephalopod and stomatopod (crustaceans) and review possible neural mechanisms and optical modifications underlying polarization sensitivity. Receptor optics and nanofabrication of retinal elements from the eyes of stomatopods are providing bio-inspiration in three areas: satellite design, data storage in computing and early cancer detection.

VISUAL TARGET DETECTION AND IDENTIFICATION DECISIONS IN JUMPING SPIDERS

Dr Ximena Nelson[1], Yinnon Dolev
[1]*University Of Canterbury*

For over a century it has been known that jumping spiders (Salticidae) use vision to a level unprecedented in other groups of spiders, and the visual system and associated visual behaviour of salticids has, since this time, been of considerable interest to researchers. Like other spiders, salticids have eyes that fall into two anatomically and ontogenetically distinct categories: primary and secondary. Classic work on the salticid visual system has focused primarily on the unique characteristics of their primary eyes. Often using behaviour as a 'bioassay' to probe the underlying characteristics of salticid vision, we have also examined the information-processing role of the secondary eyes. I will discuss some of the decision-making processes, involving both primary and secondary eyes, regarding object classification and salience in salticids.

Monday 5th December
Oral Session 1: Injury and Repair I

NEURONAL PROGENITOR TRANSPLANTATION TO PROMOTE RESPIRATORY RECOVERY FOLLOWING CERVICAL SPINAL CORD INJURY

Victoria Spruance[1], Lyandysha Zholudeva[1], Kristiina Negron[1], Paul Wisniowski[1], Tatiana Bezdudnaya[1], Michael Lane[1]
[1]*Drexel University*

Spinal cord injuries (SCI) at the cervical level result in devastating functional consequences, including respiratory dysfunction. This is largely due to the disruption of phrenic pathways, which control the diaphragm. Though spontaneous, functional recovery of respiratory systems has been demonstrated in laboratory and clinical settings, this recovery – or 'neuroplasticity' – is limited and life-threatening deficits remain. Previous work has suggested that pre-phrenic interneurons are involved in post-injury plasticity mechanisms, suggesting this neuronal population represents a potential target for therapeutic strategies. The present work investigates if the transplantation of developing spinal cord tissue, inherently rich in interneuronal progenitors, could provide an additional substrate for neuronal plasticity and facilitate the formation of novel relays that will restore input to the diaphragm. Following a lateralized C3/4 contusion injury, Sprague Dawley Rats received transplants of E14 fetal spinal cord suspension and were allowed to recover for one month or one year before undergoing tracing and terminal electrophysiology (diaphragm EMG). Anatomical analysis of pseudorabies virus (retrograde, transynaptic tracer) reveals significant graft-host integration with the phrenic circuit at one-month post transplantation. However, this connectivity appears to be lost over time and is virtually absent at one-year post-transplantation. Functional assessments reveal variable recovery at both time points, though an overall improvement of function is still evident when compared to vehicle treated animals at one year post-transplantation. Ongoing studies are examining ways to optimize recovery and sustain long-term connectivity.

REALIZING THE POTENTIAL OF ADULT HUMAN SKIN-DERIVED SCHWANN CELLS FOR TREATING NERVOUS SYSTEM INJURY

Dr Jo Stratton[1], Prof Rajiv Midha[1,2], Ass Prof Jeff Biernaskie[1,3]
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Nervous system injury causes significant long-term disability. With this in mind, we believe that supplementing the injured nervous system with autologous Schwann cells (SCs), a glia cell type that can be harvested directly from the skin of injured patients, and that has potential to rejuvenate the injury environment, will improve functional outcomes. Here we describe the purification and characterization of SCs from adult human skin (SkSCs) of 4 male donors. Within 2 weeks of culturing adherent mixed skin cells in SC media, colonies of bipolar shaped cells were sporadically detectable. Within 2-4 weeks of growth, we selected these colonies using cloning cylinders, and then re-plated these colonies. By 5 weeks, we obtained 3-5 million purified SCs. Using a battery of Schwan cell lineage-specific markers, we demonstrate that SkSCs are phenotypically indistinguishable from nerve-derived SCs (nSCs). Namely, a battery of SC associated genes (SOX10, SOX9, EGR1, PAX3, SOX2, POU3F1, S100B and YY1) were highly expressed in both nSCs and SkSC cultures compared to dermal fibroblast cultures. Using immunocytochemistry we demonstrated that the percentage of cells in nSC and SkSC cultures expressing Sox10 were 89.3±6.3% and 77.3±6.2%, respectively (P=0.2). Following transplantation into nerve injury in immune-deficient mice, the majority of transplanted SCs maintained Sox10 immunoreactivity in both nSCs and SkSC transplant conditions, and also expressed the promyelinating factor, POU3F1, in 60.85±2.21% and 50.85±1.87% of cells respectively. In addition, subsets of these cells were associated with myelin aligned on neurofilament+ axons. Such findings suggest that SkSCs should be considered for clinical application.

CHARACTERISING THE TEMPORAL PROFILE OF CEREBRAL OEDEMA AND INTRACRANIAL PRESSURE FOLLOWING STROKE IN AN OVINE MODEL

Ms Annabel Sorby-Adams[1], Dr Renée Turner[1], Dr Anna Leonard[1], Dr Emma Thornton[1], Professor Robert Vink[2]
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Cerebral oedema and elevated intracranial pressure (ICP) are the leading cause of death in the first week following stroke. The deleterious effect of space-occupying swelling and raised ICP leads to secondary neurological deterioration and irreversible brain tissue damage. Despite this, current treatments are limited and fail to address the underlying mechanisms of swelling, highlighting the need for targeted treatments. When screening novel agents it is essential to use clinically-relevant models to improve the likelihood of successful clinical translation. As such, we sought to determine the temporal profile of cerebral oedema and elevated ICP following transient stroke in our novel clinically-relevant Ovine model. Merino-sheep (14M;20F) were anaesthetised-and-subject to 2hrs middle-cerebral-artery-occlusion (MCAo) with reperfusion or sham surgery. ICP was measured for a duration of 4hrs prior to the terminal time-point (1d, 2d, 3d, 4d, 5d, 6d, 7d post-stroke), followed by MRI to determine infarct volume infarct (T2) and cerebral oedema (FLAIR). ICP and MRI scans were normal in sham animals. Following stroke, ICP rose gradually over time and by 6d was significantly elevated above sham ($p<0.0001$). Profound cerebral oedema was observed as early as 2d post-stroke and continued to evolve out to 6d days, in keeping with the increasing ICP. These findings suggest that novel therapeutic agents targeting cerebral oedema and elevated ICP will likely be effective in reducing

these complications when administered prior to 6d post-stroke. As such, our future studies will focus on examining the efficacy of an NK1 tachykinin receptor antagonist at 5d post-stroke.

DISCOVERING AND STUDYING NOVEL MOLECULES THAT REGULATE AXONAL DEGENERATION

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Maintenance of neuronal integrity is essential for the preservation of correct neuronal function. The disproportionate length of the axon, and its highly specialized function, makes it extremely vulnerable to damage and maintenance defects that can result in axonal degeneration. Axonal degeneration is an active process and a key early pathological hallmark of several neurodegenerative diseases; it often precedes the death of the neuronal cell body and is a critical determinant of disease development and progression. However, a full understanding of the molecular mechanisms and genetic causes of axonal degeneration are lacking. Using the powerful genetic tools available in C. elegans, and focusing on a specific subset of sensory neurons, we have isolated a novel mutant strain with enhanced axonal degeneration. Using classical genetic mapping, combined with deep sequencing, we have identified the mutated gene that causes axonal degeneration. This conserved molecule functions non cell-autonomously in the epidermis of the animal, in which the axon is embedded, to protect it from degeneration induced by genetic injury. Here we will present the characterization of this conserved molecule, and its previously unknown functional role in protecting the axon from degenerating.

PERICYTE CONSTRICTION IS ASSOCIATED WITH NO-REFLOW FOLLOWING ISCHAEMIC STROKE

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Approximately 26% of stroke patients receiving recanalisation therapy do not achieve microvascular reperfusion, known as "no-reflow". Capillary pericytes actively control capillary diameter. We tested whether no-reflow occurs in our animal model of stroke and if this is associated with pericyte constriction of capillaries. Two cohorts of adult male rats were subjected to 90 minutes of middle cerebral artery occlusion (MCAO) followed by 60-90 minutes of reperfusion. CBF was monitored by laser Doppler flowmetry throughout. A novel gel-filling method was used to determine capillary diameter (FITC-albumin perfusion), pericyte location (Alexa 647-isolectin B_4 labelling from which pericytes can be identified) and pericyte death (propidium iodide labelling). In cohort one (Wistar) after 90 minutes MCAO, CBF sharply increased upon retraction of the intraluminal filament. However, the average level of CBF throughout the first 60 minutes of recanalisation was significantly (p<0.0001) lower than baseline (reduced by 27.5%±3.1%, n=44). In cohort two (Sprague-Dawley) at 90 minutes reperfusion, CBF was reduced by 16.8%±14.0% (n=6) while capillary diameter was reduced in the ipsilateral striatum (by 21.3%±1.2%) and cortex (by 11.1%±1.1%) compared to controls (p<0.001, n=4). This constriction was associated significantly (p=0.035, n=4) with pericyte location. Only a small amount of pericyte death (8%) occurred at this timepoint compared to 70% following 24h reperfusion. Thus, there is substantial no-reflow during the first 60-90 minutes following stroke. This is associated with pericyte constriction of capillaries but not pericyte death, which occurs later. Pericyte constriction and death may have important implications for reperfusion therapy for ischaemic stroke patients.

Monday 5th December
Oral Session 2: The Synapse

SINGLE MOLECULE IMAGING OF SYNTAXIN-1A REVEALS CHANGE IN LATERAL MOBILITY ASSOCIATED WITH NEUROTRANSMITTER RELEASE IN VIVO

Mr Adekunle Bademosi[1], Dr Elsa Lauwers[2], Dr Pranesh Padmanabhan[3], Mr Lorenzo Odierna[4], Miss Ye Jin Chai[1], Dr Andreas Papadopoulos[1], Prof Geoffrey Goodhill[5], Prof Patrik Vertstreken[2], Assoc. Prof Bruno van Swinderen[3], Prof. Frederic Meunier[1]

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Syntaxin1A is a crucial protein that mediates synaptic transmission. Together with its cognate partners – SNAP-25 and VAMP2 – it forms the SNARE complex necessary for vesicle fusion. Syntaxin1A molecules have been shown to be organized in nano-clusters in neuro-secretory cells as well as in membrane sheets. However, how molecules of Syntaxin1A enter and exit these nano-clusters by lateral diffusion and how stimulation affects their dynamic equilibrium in pre-synapse in vivo in unknown. To image single molecules of Syntaxin1A in live synapses, we generated a Drosophila line constitutively expressing fluorescently tagged syntaxin1A (syx1A-mEos2) to carry out single particle tracking Photoactivated Localization Microscopy (sptPALM) on live Drosophila larval neuromuscular junction (NMJ), using slightly oblique Total Internal Reflection Florescence (TIRF) microscopy. We examined the change in Syntaxin1A mobility and micro-domain organisation elicited by increased pre-synaptic activity, using both optogenetic and thermogenetic tools. Here, we show that in Drosophila larvae motor nerve terminals, Syntaxin1A is also organized in nanoclusters. The overall mobility of Syntaxin1A molecules was lower in live resting motor nerve terminals, while opto- and thermo-genetic stimulation of neurotransmitter release led to an increase in syx1A mobility. Importantly, this change at the motor terminal was also promoted by concomitant expression of tetanus toxin-light chain, suggesting that syntaxin1A molecules are released from the confinement of nano-clusters following disassembly of the SNARE complex. Our results suggest that the relative immobility of Syntaxin1A molecules within synapses is indicative of a high level of primed vesicles in live motor nerve terminals.

GENETIC INTERACTION OF DISEASE-RELEVANT GENES MODULATES SELECTIVE COMPONENTS OF COGNITION IN TOUCHSCREEN ASSAYS

Dr Jess Nithianantharajah[1,2], Dr N.H Komiyama[2], Prof L.M Saksida[3], Prof T.J Bussey[3], Prof S.G.N. Grant[2]

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Cognitive deficits are a core feature of most neurological and psychiatric disorders, however unravelling the genetic basis of cognitive disorders is complex due to the involvement and interaction of multiple genes, which manifest in overlapping cognitive impairments. Human genetic studies have elucidated that many of the mutations implicated in cognitive disorders converge upon genes associated with the synapse - the connection between neurons that form the most fundamental information-processing units in the nervous system. Little is known about the genetic basis of distinct aspects of higher cognitive functions such as complex forms of learning and memory, attention and executive functions. Moreover, there is negligible evidence exploring the involvement of epistasis or non-additive gene interactions in the context of cognitive functions. Towards this, using mice with mutations in Dlg2 and Magi2, two key synaptic scaffold genes implicated in cognitive disorders, we examined the role of these genes and gene interactions on different aspects of cognition. Exploiting the emerging technology of the touchscreen assays, a useful behavioural tool for modelling higher cognitive functions in rodents, we observe evidence for complex genetic interactions whereby Dlg2 x Magi2 double mutants either show a neutral phenotype or a genetic suppression or enhancement in selective cognitive functions. These findings provide novel evidence for gene interactions underlying cognition. This work will be informative towards elucidating how mutations in multiple susceptibility genes give rise to distinct and overlapping cognitive phenotypes, and influence disease susceptibility.

FUNCTIONAL DEFICIT OF IQSEC2 WITH MISSENSE MUTATIONS DISRUPTS NORMAL DENDRITIC SPINE MORPHOGENESIS

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There is considerable genetic heterogeneity associated with intellectual disability (ID), autism and epilepsy. Our laboratory has been involved in identifying genetic causes of ID, focusing on genes of the X-chromosome including the IQ motif and SEC7 domain containing Protein 2 (IQSEC2) gene. The disease spectrum due to mutations in IQSEC2 includes ID though to early onset seizure phenotypes. The pathogenesis underpinning these mutations is not known. Here we investigate the role of IQSEC2 on the morphology of dendritic spines. A lentiviral shRNA approach achieved a 57% knock-down of Iqsec2 expression in primary hippocampal cell cultures from mice, modeling partial loss-of-function mutations. Investigating gross morphological parameters

after eight days of in vitro culture (8DIV) identified a ~ 32% reduction in axon length, and 27% increase in the number and 31% increase in complexity of dendrites protruding from the cell body. Development of dendritic spine structures at 15DIV show an increase of 34% in the number of protrusions per dendritic segment compared to control with the proportion of immature filopodia to mature spines similar across all treatments. In contrast, overexpression of IQSEC2 WT leads to neurons that are more compact and display simpler dendritic branching than controls. The morphological disturbances due to knock-down of Iqsec2 are recapitulated in neurons from mice with Iqsec2 knocked out by CRISPR/Cas9 generated in our laboratory. These observations provide evidence of dosage sensitivity for this gene that normally escapes X-inactivation in females and links these disturbances in expression with alterations in the morphology of developing neurons.

DENDRITIC PROCESSING OF ODOUR INFORMATION IN THE MOUSE PIRIFORM CORTEX IN VIVO

Dr Malinda Tantirigama[1], Prof John Bekkers[1]
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The piriform cortex (PC) is the first cortical destination of odour information. The PC receives afferent olfactory bulb input exclusively in layer 1a onto the distal dendrites of principal cells, which have their somas anatomically segregated in layers 2 and 3. Integration of synaptic inputs at the dendrites is the first stage of cortical odour processing. However, previous work has focused on the output at the somas, and the dynamics of the input in the dendrites has been ignored. To address this, we labelled dendrites in the anterior PC with the calcium sensor GCaMP6f and imaged dendritic activity using in vivo 2-photon microscopy. We found that the distal dendrites of principal cells were spontaneously active, and odours excited a unique set of dendritic segments consistently across multiple trials (trial 1 vs. 10, p=0.32). Both spontaneous and odour-evoked activity was significantly reduced in the presence of the NMDA channel blocker MK-801 (control vs. MK-801, p<0.001), suggesting that the observed transients are due to NMDA-receptor-dependent calcium spikes. To identify whether these calcium spikes are triggered locally in individual dendritic branches of the same cell (i.e. branch-specific) or triggered globally across the dendritic tree, we used sparse GCaMP6f labelling of a few identified neurons. Both spontaneous and odour-evoked calcium spikes were observed globally in all identified sibling branches, and exhibited similar amplitudes and time courses. These findings suggest that global calcium spikes induced in the dendritic tree of principal cells interact with bulbar synaptic input, thereby enriching the dendritic processing of olfactory information.

PACSIN1 REGULATES THE DYNAMICS OF AMPA RECEPTOR TRAFFICKING

Dr Jocelyn Widagdo[1], Dr Huaqiang Fang[2], Ms Se Eun Jang[1], **Dr Victor Anggono[1]**
[1]Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, [2]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine

Dynamic trafficking of AMPA receptors (AMPARs) into and out of synapses plays an important role in synaptic plasticity. We previously reported that the protein kinase C and casein kinase II substrate in neurons (PACSIN) forms a complex with AMPARs through its interaction with the protein interacting with C-kinase 1 (PICK1) to regulate NMDA receptor (NMDAR)-induced AMPAR endocytosis and cerebellar long-term depression (Anggono et al., Proc. Natl. Acad. Sci. USA, 2013). However, the molecular mechanism by which PACSIN regulates the dynamics of AMPAR trafficking remains unclear. Using a pH-sensitive green fluorescent protein, pHluorin, tagged to the extracellular domain of the GluA2 subunit of AMPARs, we demonstrate dual roles for PACSIN1 in controlling the internalization and recycling of GluA2 after NMDAR activation. Structure and function analysis reveals a requirement for the PACSIN1 F-BAR and SH3 domains in controlling these NMDAR-dependent processes. Interestingly, the variable region, which binds to PICK1, is not essential for NMDAR-dependent GluA2 internalization and is required only for the correct recycling of AMPARs. These results indicate that PACSIN is a versatile membrane deformation protein that links the endocytic and recycling machineries essential for dynamic AMPAR trafficking in neurons.

IMPAIRED RETRIEVAL OF SYNAPTOBREVIN TO SYNAPTIC VESICLES CAUSES A PROGRESSIVE REDUCTION IN EXOCYTOSIS

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Synaptophysin is an integral synaptic vesicle (SV) protein which is responsible for facilitating the retrieval of the essential v-SNARE synaptobrevin II (sybII) back to SVs during endocytosis. Here, we use synaptophysin knockout hippocampal neurons to investigate the consequence of impaired sybII retrieval on the efficiency of exocytosis. Synaptophysin knockout (KO) hippocampal neurons were transfected with vGLUT-pHluorin and mCerulean empty vector (KO) or synaptophysin-mCerulean (rescue), and were subjected to 4 repeated trains of 300 action potentials (10Hz), and changes in pHluorin fluorescence monitored. We find that upon repeated stimulation there is a progressive reduction in evoked exocytosis in synaptophysin KO neurons (p<0.05), whilst the efficiency of exocytosis is maintained in neurons rescued with synaptophysin. This is due to a reduction in sybII retrieval back to synaptic vesicles, and importantly, can be rescued by increasing the basal load of sybII on SVs by transfecting neurons with exogenous sybII. These findings demonstrate that perturbed sybII retrieval has knock-on consequences for exocytic efficiency, and suggests that the fidelity of neurotransmission may be compromised in systems with defective sybII trafficking. This may provide the molecular basis for the cognitive impairments that are seen in both synaptophysin knockout mice, and in individuals with X-linked intellectual disability who harbour mutations in synaptophysin.

Monday 5th December
Oral Session 3: Neurodevelopmental Disease

SPATIAL AND TEMPORAL FEATURES OF CALLOSAL AXON INNERVATION, EXUBERANCE AND ELIMINATION DURING MOUSE DEVELOPMENT INVOLVE ACTIVITY-DEPENDENT AND -INDEPENDENT EVENTS

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[1]The University Of Queensland, Queensland Brain Institute, [2]The University of Queensland, The School of Biomedical Sciences

The corpus callosum is a large fibre tract that connects the two hemispheres of the brain, facilitating their communication. However, despite the importance of this structure in mediating communication between the two cortical hemispheres, many aspects of its anatomy, organisation and development remain unclear. A particular aspect of callosal development that is poorly understood is how callosal axons locate and innervate their targets in the contralateral cortex, after crossing the midline. Recently we demonstrated that two major contralateral callosal projections that arise from mouse L2/3 primary somatosensory cortical neurons are differentially affected by manipulations of neuronal activity (Neuron, 2014, 82(6): 1289-1298). However, the precise organisation, sequence of development and temporal dependence upon sensory input that these projections undergo remain unclear. Here, using *in utero* electroporation in mice, we investigated these questions and first found that these projections have distinctive anatomical organisations and arise from separate neuronal populations. By analysing the mean axonal innervation over different developmental stages, we found that these projections enter the cortex in a dorsal-to-ventral and region-specific order. Furthermore, we identify two periods of region- and layer-specific developmental exuberance that correspond to initial callosal axon innervation and subsequent arborisation. Early sensory deprivation affects only the latter of these events. Taken together, this work constitutes the first systematic, quantitative characterisation of the organisation and development of the full range of callosal projections from a single cortical area in mouse. These results also provide an experimental model for the investigation of neurodevelopmental disorders of interhemispheric connectivity in the future.

DISEASE-ASSOCIATED MUTATIONS TO RP58 ALTER ITS CAPACITY TO PROMOTE CELL MIGRATION AND NEURITE OUTGROWTH WITHIN THE DEVELOPING CEREBRAL CORTEX

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The development of the cerebral cortex requires the activities of DNA-binding transcription factors which control neuronal production, cell migration, and the differentiation of appropriate numbers of cells which integrate into functional networks. Of these factors, the zinc-finger transcriptional repressor known as Rp58 has been shown to be a key regulator of neuronal maturation and migration in the developing cortex, and genetic association studies have revealed a link between mutations to RP58 (also known as ZNF238) and brain developmental disorder. In this study, we studied two substitution variants to RP58 (N461S and R495G) which were detected as heterozygous missense variants in two unrelated patients diagnosed with intellectual disability and brain growth disorder. Using in utero electroporation experiments in mice embryos, we showed that, both substitution mutations disrupted the neuronal migration function for Rp58. Particularly, cells expressing the N461S variant displayed enhanced migration within the cortical plate whereas R495G rescued cells failed to reach the cortical plate. In addition, we found that these RP58 variants influenced the neurite outgrowth of immature neurons within the embryonic cerebral cortex. These results demonstrate that disease-associated mutations of RP58 disrupt its capacity to regulate neuronal migration and neuritogenesis during the development of the cerebral cortex.

MUTATIONS IN DCC CAUSE AGENESIS OF THE CORPUS CALLOSUM AND MIRROR MOVEMENTS ASSOCIATED WITH A FAILURE OF CORTICOSPINAL AXONAL DECUSSATION

Ashley Marsh[1,2], Delphine Heron[3], Timothy Edwards[4], Charles Galea[5], Angélique Quartier[6], Amélie Piton[6], Melanie Bahlo[7], Richard Leventer[8,9], Linda Richards[4], Christel Depienne[6], Paul Lockhart[1,2]
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The corpus callosum (CC) is the main cerebral commissure in placental mammals with a key role in the communication between the brain hemispheres. Agenesis of the corpus callosum (ACC) is the complete or partial absence of the CC and is one of the most frequent brain malformations in humans. ACC is genetically and clinically heterogeneous, therefore prognostication is difficult due to widely variable neurodevelopmental outcomes. We performed genetic studies in 4 multigenerational families and 70 sporadic ACC cases and identified mutations in the gene encoding the Netrin receptor DCC (*DCC*) in 9 unrelated families. Affected individuals presented with dominant isolated ACC with or without mirror movements (MM), in the absence of intellectual disability. MRI tractography demonstrated a failure of corticospinal (CS) axonal decussation in 7 affected individuals

(from 2 families), all of whom displayed MM with or without ACC. Sex bias in phenotype expression was observed for truncating but not missense mutations. *DCC* haploinsufficiency in males significantly correlated with MM while females only presented with ACC. *DCC* expression in human neural stem cells was measured using RNAseq and RT-qPCR and we identified a significant dose-dependent upregulation by testosterone treatment. Our results show that *DCC* mutations are associated with isolated ACC and a relatively mild phenotype with a favourable cognitive outcome. We provide evidence that MM in *DCC*-mutant individuals results from failure of CS axonal decussation. Finally, our data indicates that distinct molecular mechanisms may contribute to disease in haploinsufficient individuals, perhaps related to testosterone levels.

AUTISM ASSOCIATED MIR-873 KO AFFECTS NEURONAL DIFFERENTIATION

DR Jing Lu[1], Sarah Williams[2], **Michelle Watts[1]**, **Charles Claudianos[1]**
[1]Monash Institute of Cognitive and Clinical Neuroscience, Monash University, [2] Diamantina Institute, University of Queensland

Autism Spectrum disorders (ASD) are highly heritable disorders with significant genetic heterogeneity. Non-coding RNAs, such as microRNAs (miRNAs), are thought to play key roles in development of autism and other mental health disorders. However, the exact regulatory mechanics of these miRNAs remain unknown. To this end, we conducted whole genome (exome) sequencing of 50 Australian families with ASD and identified a number of novel single nucleotide mutations in miRNA genes. Of particular interest is a unique A/T point mutation observed in the conserved 'seed' region of miR-873 (gene ID: 100126316) found in an idiopathic case of ASD. This mutation was predicted to affect target site interaction and regulation of key neurodevelopmental genes. Target genes were subsequently validated by RNA pull-down sequence analysis using biotinylated miRNA probes. We identified over 3000 mRNA targets of miR-873, among the most overrepresented targets were autism risk genes, SHANK3 and SYNGAP1. Quantitative PCR confirmed the down regulation of SHANK3, SYNGAP1 and ARID1B in SH-SY5Y cells with the same condition. The downregulated expression of SHANK3, ARID1B and NLGN2 were also validated using a dual-luciferase reporter system. Furthermore, transforming primary cultured neurons by addition of miR-873 mimic, and mutant probes resulted observable changes in neuronal morphology (Sholl-analysis) and that CRISPR/CAS9 miR-873 KO caused SH-SY5Y cells to spontaneously differentiate into neuronal-like cells. In summary, our results confirm a unique point mutation in a microRNA found in a family with autism regulates key ASD risk genes and potentially combines with other coding and non-coding DNA variations to affect neurodevelopment.

ELUCIDATION OF INTERNEURON DEFICIT IN THE TWO MOST COMMON ARX MUTATIONS CAUSATIVE TO INTELLECTUAL DISABILITY IN CHILDREN

Dr Kristie Lee[1,2], Miss Kelsey Ireland[1], Miss Megan Bleeze[1], Miss Tessa Mattiske[1,2], A/Prof Cheryl Shoubridge[1,2]
[1]The University Of Adelaide, [2]Robinson Research Institute, University of Adelaide

The *Aristaless-related homeobox gene (ARX)* is indispensable for interneuron development. Patients with ARX polyalanine expansion mutations of the first two tracts (namely PA1 and PA2) suffer from intellectual disability of varying severity, with seizures a frequent comorbidity particularly in PA1 patients. The impact of PA1 and PA2 mutations on the development of the brain is unknown, hindering the search for therapeutic interventions.
Here we report disturbances to interneuron development due to ARX PA1 and PA2 mutations modelled in mice. There was a consistent ~30% reduction of calbindin positive interneurons within the rostral cortex of newborn mice ($p \leq 0.05$) for both mutant strains compared to wildtype. We found no evidence of precursor cell loss, overall loss of cell density, or disrupted differentiation that underlies the calbindin cell loss. Interestingly, PA1 cells demonstrated subtle migration retardation in ex vivo assay with similar but not significant changes in migration in neurons from PA2 mice. Our data indicates that PA1 is a stronger hypomorphic mutation due to consistent and marked reduction in mutant protein expression contributing to the more severe phenotype presentation. We contend that the loss of cortical calbindin interneurons occurs through a lag in precursor cell migration. This disturbance to interneuron development likely provides the cellular basis underpinning aspects of PA1 and PA2 clinical presentations. In addition, our study demonstrates that expansion of two distinct ARX polyalanine tracts lead to similar cellular outcomes, indicating a common pathogenic mechanism, which may infer disease mechanism caused by similar mutations in other genes.

THE NEURODEVELOPMENTAL AND BEHAVIOURAL IMPACT OF AUTISM-RISK GENES IN ZEBRAFISH

Dr Lena Constantin[1], Miss Jamie Wyatt[1], Miss Neha Hebbani[1], Mrs Sarah Piper[1], Dr Charlotte Lupton[2], A/Prof. Suresh Jesuthasan[2], A/Prof. Ethan Scott[1]
[1]University Of Queensland, [2]Institute of Molecular and Cell Biology

For two decades, autism spectrum disorder (ASD) has been associated with histoanatomical abnormalities in the number and size of Purkinje cells in the cerebellum. Yet still, the precise role of the cerebellum and Purkinje cells are poorly understood in ASD. Clinical evidence supports a pathological role for the cerebellum in ASD. This includes observations that syndromic patients co-diagnosed with ASD exclusively display histoanatomical abnormalities in the cerebellum, and that cerebellar pathology and cerebellar-associated motor impairments precede the diagnosis of ASD. Using clustered regularly interspaced

short palindromic repeats (CRISPRs), we generated seven lines of zebrafish with disruptions in conserved ASD risk-genes that were endogenously expressed in the developing cerebellum. We next coupled zebrafish behaviour to Purkinje cell biology by assessing the development of Purkinje cells in vivo, using genetically-encoded calcium indicators to track Purkinje cell activity, and then relating these mechanistic findings to a variety of behavioural assays for baseline locomotion, motor coordination, motor learning during environmental manipulation, and social interaction. We provide proof-of-principle that zebrafish are an effective model for investigating how developmental and functional abnormalities in Purkinje cells contribute to the etiology of ASD.

Monday 5th December
Oral Session 4: Reward

UNDERSTANDING ESCALATION OF METHAMPHETAMINE INTAKE IN ADOLESCENT RATS

Miss Sophia Luikinga[1,2], Dr. Heather Madsen[1], Miss Isabel Zbukvic[1,2], Prof. Andrew Lawrence[1,2], Dr Jee Hyun Kim[1,2]
[1]Florey Institute of Neuroscience and Mental Health, [2]University of Melbourne

Methamphetamine (Meth) abuse is a growing problem in Australia, with the rise in its purity as a main concern. Additionally, children as young as 10 years are admitted to hospital on Meth in the country. Therefore, we used the intravenous self-administration paradigm to compare Meth abuse-related behaviour in adolescent (postnatal day 35) and adult (postnatal day 70) rats. Rats were trained to lever press for Meth at a dose of 0.03 mg/kg/infusion. It was found that both adults and adolescents acquired in a similar manner however, after acquisition, adolescent rats escalated their intake when the dose was increased (0.1mg/kg/infusion, $p<0.05$). To test whether this escalation was due to pre-exposure to Meth or due to a particular affinity for 0.1mg/kg/infusion, a new group of rats acquired at 0.1mg/kg/infusion, and then were tested on 0.3mg/kg/infusion. Surprisingly, both age groups acquired in a similar manner and again an escalation of intake was seen in the adolescent group on the high dose ($p<0.05$). To investigate the underlying mechanisms of this increased intake in adolescents, we performed genome-wide transcriptome analysis on dorsal striatal tissue after 11 days of acquisition on the 0.03mg/kg dose. Interestingly, several genes involved in apoptosis and linked to neurodevelopmental disorders such as HN1 and TOP3b are differentially expressed after Meth self-admin in adolescent compared to adult rats ($p<0.001$). Taken together, Meth consumption during adolescence leads to differential expression of genes compared to adulthood, which may be related to escalation of Meth intake.

HUNGER-SENSING AGRP NEURONS MODULATE STRESS AND ANXIETY BEHAVIOUR IN RESPONSE TO RESTRAINT STRESS

Ms Rachel Clarke[1], Ms Moyra Lemus[1], Mr Alex Reichenbach[1], Dr Romana Stark[1], Dr Stuart Mazzone[1], Dr Sarah Lockie[1], Associate Professor Zane Andrews[1]
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Agouti related peptide (AgRP) expressing neurons in the hypothalamic arcuate nucleus (ARC) are activated during starvation, rapidly driving hunger and food intake in order to ensure survival. However, in the absence of food availability AgRP neurons drive adaptive non-feeding behaviours such as reducing anxiety and increasing compulsive and repetitive behaviour. In this study, we tested the hypothesis that AgRP neurons regulate the hypothalamic-pituitary-adrenal stress axis to reduce anxiety-like behaviour in response to acute restraint stress.

To investigate this we used excitatory hM3Dq 'designer receptors exclusively activated by designer drugs' (DREADDs) to remotely stimulate AgRP neurons. Male, 8-10 week old AgRP-cre mice were injected with rAAV-hSyn-DIO-hM3D(Gq)-mCherry bilaterally into the ARC. Both acute and chronic activation of AgRP neurons (CNO; 1mg/kg; i.p.) 10 minutes or 3 hours before a 15-minute restraint stress, respectively, significantly increased plasma corticosterone concentrations ($p<0.05$), with no treatment difference in blood glucose levels. Anxiety behaviours following chronic AgRP activation and acute stress were measured using open field (OF) and light-dark box (LDB) tests. Interestingly, there was a mild reduction in anxiety behaviour observed in the LDB test ($p<0.05$). Cre-dependent anterograde viral tracing using the herpes simplex virus 1 H129 strain revealed that AgRP neurons influence neural circuits regulating stress. Our results suggest that hunger-sensing AgRP neurons regulate a physiological response to stressors and limit anxiety-like behaviour. We argue this adaptive response limits anxiety in the face of acute stress to promote food-seeking behaviour.

RXFP-3 ANTAGONISM IN THE LATERAL HYPOTHALAMUS INCREASES ALCOHOL SELF-ADMINISTRATION

Dr Christina Perry[1,2], Dr Kastman Hanna[1,2], Ms Sarah Ch'ng[1,2], Ms Liubov Lee-Kardashyan[1], Ms Leigh Walker[1,2], Dr Craig Smith[1,2], Professor Andrew Gundlach[1,2], Professor Andrew Lawrence[1,2]
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Alcohol is the most widely abused drug worldwide, and the search for novel and effective therapeutic targets to treat alcoholism is an ongoing task. One potential target is RXFP3, the cognate receptor for the neuropeptide relaxin-3. Recently we showed that RXFP3 is necessary for alcohol seeking, since central antagonism reduced alcohol self-administration and relapse-like behaviour in alcohol-preferring rats. Relaxin-3 is predominantly expressed in the brainstem, in neurons that project rostrally including to the lateral hypothalamus (LH), which is heavily implicated in alcohol consumption and relapse. We sought, therefore, to examine the effect of RXFP3 antagonism in the LH. Alcohol-preferring P rats were trained to administer 10% ethanol for a minimum of 30 days. The selective antagonist R3(B1-22)R was then infused bilaterally into the LH prior to a standard self-administration session. In contrast to the effect of a central (icv) or intra-BNST infusion, rats showed increased alcohol consumption following administration of R3(B1-22)R compared to vehicle ($p < 0.05$). To better understand this unexpected result, we subsequently sought to characterise RXFP3 positive neurons in the hypothalamus using a transgenic reporter mouse expressing YFP protein on RXFP3 neurons. RXFP3 are not expressed on orexin or MCH neurons, but a subset of LH RXFP3 positive neurons project directly to the ventral tegmental area. These findings have implications for understanding how relaxin-3 acts

centrally to regulate alcohol-seeking and alcohol consumption, and ongoing studies will elucidate the mechanism for this effect, which somewhat parallels observations with the opioid receptor antagonist naltrexone.

CRITICAL ROLE OF NPY SYSTEM IN THE COORDINATED CONTROL OF ACTIVITY AND ENERGY HOMEOSTASIS

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Biological processes are centrally coordinated to ensure homeostasis. In anorexia nervosa (AN), extremely low body weight is often associated with paradoxical hyperactivity, representing the failure of the brain to adjust activity to energy storage. One neurotransmitter critically involved in the regulation of energy homeostasis, physical activity and food-motivated behaviour is neuropeptide Y (NPY). Here we investigated the role of NPY system in the coordinated control of activity and energy metabolism using an activity-based anorexia paradigm combining food restriction with unlimited running wheel access. Wild-type (WT) and NPY-/- mice were randomly assigned to either the home-cage (HC) or running-wheel cage (RW) group. After a 7-day ad lib baseline period, mice were restricted for food access to the 1st hour of the dark-phase for 10 days (RF). WT mice in both HC and RW groups showed rapid weight loss during RF without significant difference between the two groups. In contrast, the weight loss in NPY-/- mice during RF was significantly greater in RW compared to the HC group with an accelerated weight loss in the RW group at the second half of the RF period. Importantly, NPY-/- mice showed significant and persistent increase in wheel-running activity during the whole RF period, whereas WT mice only showed initial increase then declined to the level below baseline. These results suggest NPY signaling plays a critical role in adjusting motor activity in response to feeding and energy homeostatic disturbances, which may have important implication in the hyperactivity observed in AN patients.

CONTROL OF BEHAVIOUR THROUGH TRANSCRIPTIONAL REGULATION OF SATIETY

Dr Vaida Juozaityte, Mr David Pladevall Morera, Dr Agnieszka Podolska, Mr Ryan Sheridan, Dr Brent Neumann, **Associate Professor Roger Pocock[1]**
[1]Monash University

Animal behavior is shaped through interplay between genes, the environment and previous experience. We previously published that the ETS-5 transcription factor, ortholog of mammalian FEV/Pet1, is required to specify the carbon dioxide-sensing function of the BAG neurons. More recently, we identified a function for ETS-5 in the regulation of behavioral quiescence of well-fed animals.

Nutritional status has a major influence on Caenorhabditis elegans behavior. When foraging, food availability controls behavioral state switching between active (roaming) and sedentary (dwelling) states. When provided with high-quality food, however, C. elegans become sated and enter quiescence. Our data suggest that ETS-5 controls these behavioral states by setting the internal 'satiety quotient' through fat regulation. Acting from sensory neurons, ETS-5 functions in a complex network with serotonergic and neuropeptide signaling pathways to control food-regulated behavioral state switching. Finally, we found that genetic and chemical control of quiescence through fat storage is reversible such that it may aid the development of appetite-controlling interventions.

IMPACT OF HIGH SUCROSE DIETS ON BEHAVIOURAL PATTERN SEPARATION AND NEUROPROLIFERATION

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Rodent models have shown that high sucrose diets can reduce hippocampal neurogenesis, which is required for minimizing interference between memories, a process that involves "pattern separation". We provided rats in the sucrose group with two hours daily access to a 10% sucrose (w/v) solution for 28 days and then assessed their performance on either the "Trial-Unique Non-matching to Location" task (TUNL), conducted in a touchscreen chamber, or a spatial exploration task "Spontaneous Location Recognition" (SLR) as a measure of behavioral pattern separation. These tasks permit manipulation of the pattern separation load by exposing rats to a spatial arrangement where touchscreen stimuli or objects were either maximally separated (low pattern separation load), or were close together (high pattern separation load). In the TUNL task, rats first respond to a sample location and are then rewarded if they respond to a novel rather than the sample location. Sucrose consuming rats performed the non-match discrimination correctly when there was a large spatial separation between sample and the new locations, but were significantly impaired compared to controls when the spatial separation on the touchscreen was smaller ($P<0.05$). In the SLR task, sucrose consuming rats discriminated between objects in novel and familiar locations when there was a large spatial separation between the objects, but differed significantly to control animals when the spatial separation was smaller ($P<0.002$). Doublecortin and proliferating cell nuclear antigen immunoreactivity in the dentate gyrus of sucrose-consuming rats were significantly reduced relative to controls. Thus, sucrose consumption impaired behavioral pattern separation performance and reduced measures of hippocampal neuroproliferation.

Oral Session 5: PNS

ANATOMICAL AND FUNCTIONAL EVIDENCE THAT THE PARATRIGEMINAL NUCLEUS IS INVOLVED IN LARYNGEAL SENSORY NEURON REGULATION OF RESPIRATION

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Objective: Some vagal airway sensory neurons project to the medullary paratrigeminal nucleus (Pa5). We therefore investigated the anatomical connectivity of the Pa5 and its involvement in evoked respiratory responses.
Method: Conventional retrograde and anterograde neuronal tracing and novel herpes viral transynaptic tracing were employed to determine the afferent and efferent connectivity of the Pa5. Additionally, neurotransmission in the Pa5 was modified using pharmacological agents or targeted neuronal toxins to assess the role of the Pa5 in respiratory reflexes in anaesthetised guinea-pigs evoked by electrical stimulation of the larynx, and cough behaviours in conscious guinea pigs induced by inhaled airway irritants.
Key findings: The Pa5 was exclusively innervated by jugular ganglia afferents. Pa5 output neurons terminated extensively throughout the ventrolateral medulla and innervated ascending circuits projecting to somatosensory processing regions of the thalamus. Pharmacological inhibition of the Pa5 prevented the respiratory slowing associated with laryngeal stimulation (Emax = 34 ± 4.57 versus 18 ± 2.34 breaths per minute in vehicle and muscimol treated animals, respectively; $P<0.05$; n= 6/group), and reduced cough behaviours in response to inhaled bradykinin (total behaviour score = 173.4 ± 38.81 versus 95.01 ± 9.53 seconds in control and Pa5 lesioned animals, respectively: $P<0.05$, n=12/group).
Conclusion: These data suggest that the Pa5 integrates jugular ganglia airway sensory information important for the reflex control of breathing and the conscious perception of airway irritations. Better understanding of this putative airway somatosensory system may help identify therapeutic targets to alleviate respiratory discomfort in disease.

OSSEOINTEGRATED NEURAL INTERFACE (ONI): A NOVEL MODEL FOR INTEGRATING PERIPHERAL NERVES WITH ROBOTIC PROSTHETICS

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Current methods for controlling state of the art prosthetic limbs rely on myoelectric signals from soft tissue interfaces subject to motion artifact and muscle signal cross talk, ultimately preventing widespread clinical application. This study was undertaken as a proof of principle for creating an Osseointegrated Neuronal Interface (ONI) for controlling prosthetic devices. Re-directing terminal nerve endings into the medullary cavity of long bones protects the nerve from mechanical and electrical stimuli, and surrounds the damaged nerve end with the regenerative bone marrow stem cell niche. This unique environment presents the perfect in vivo bioreactor for the potential interface between severed nerves and electronic prosthetic devices. This study describes the development of the ONI model.

Methods: transfemoral amputations were performed in rabbits, whereby the sciatic nerve was re-directed into the medullary cavity through a corticotomy. The terminal end of the nerve was integrated with a PDMS mock electrode inside the bone. Animals were explored at 5 weeks via electrophysiology and histology.

Results: Gross examination demonstrates nerves are healthy and stable within bone, with no signs of neuroma formation. The ONI nerve contains smaller myelinated axons than the control and is correlated with reduced conduction velocity compared to the contralateral limb. Conclusions: Terminal ends of amputated nerves are functional following being re-directed into the medullary cavity of the femur at 5 weeks. This result acts as proof of principal for the ONI model and its ability to house functional prosthetic interfaces. Work is currently underway to test various electrodes in this model.

OPTOGENETICS DEMONSTRATES FUNCTIONAL INNERVATION OF COLONIC MUSCLE BY NEURONS DERIVED FROM TRANSPLANTED PROGENITORS

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The enteric nervous system (ENS) plays an essential role in gut motility. Diseases of the ENS result in bowel motility disorders that are some of the most challenging clinical conditions to manage. Cell therapy offers the potential to treat gastrointestinal motility disorders caused by enteric neuropathies. We have previously shown that following transplantation into the colon of recipient mice, enteric neural proliferate, migrate and differentiate into a variety of neurochemical types of neurons. However, it was unclear whether graft-derived neurons integrate into the circuitry of the recipient and directly regulate gut motility. We have used optogenetic and electrophysiological approaches to examine whether transplanted enteric neural progenitors generate neurons that functionally innervate the colon. Neural progenitors expressing the light-sensitive ion channel, channelrhodopsin, were isolated from fetal or postnatal bowel and transplanted into the colon of postnatal mice. The responses of recipient colonic smooth muscle cells to selective (light) stimulation of graft-derived neurons were examined. Light stimulation of graft-derived cells resulted in excitatory and inhibitory junction potentials, the electrical events underlying contraction and relaxation respectively, in colonic circular muscle cells. The pharmacological properties of the junction potentials evoked by stimulation of graft-derived cells were similar to those elicited by stimulation of endogenous excitatory and inhibitory motor neurons. Interneurons were also generated from graft-derived cells, but their pharmacological properties varied with the age of the donors from which enteric neural progenitors were obtained. Our data demonstrate that transplanted progenitors generate different functional classes of enteric neurons involved in the control of gut motility.

INNERVATION CHANGES IN THE MURINE VAGINA FOLLOWING INFLAMMATION

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Vulvodynia is a prevalent chronic pain disorder in which the vagina shows hyperinnervation. The aetiology and types of nerves contributing to hyperinnervation are unknown. We compared the organisation of vaginal nerve fibres in young nulliparous and older multiparous mice (n=6/group), and tested the hypothesis that mild chronic inflammation induces hyperinnervation. Nerve fibres were identified by immunoreactivity (IR) for the pan-neuronal marker PGP9.5 and subpopulations were identified by IR for substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), neuronal nitric oxide synthase (nNOS) and neuropeptide tyrosine (NPY). Inflammation was induced by injection of complete Freund's adjuvant (CFA, 5 µl) in the distal vaginal wall and changes were assessed at one or two weeks (n=7/group). Control mice received saline (n=4/group).
PGP9.5-IR and SP-, CGRP-, VIP-IR fibres were most abundant in the lamina propria whereas NPY-IR fibres were mainly restricted to the adventitia. Compared to the distal vagina, the proximal vagina received more fibres ($p < 0.01$) whereas neurochemically distinct types of intraepithelial fibres were more abundant distally. Age/parity and inflammation changed the innervation pattern. Older, multipara mice showed changes in the distal vagina including more nonpeptidergic intraepithelial fibres, more NPY-IR fibres and more blood vessels. Inflammation induced morphological signs of oedema, macrophage infiltration (CD-68 labelling) and hyperinnervation involving CGRP-, SP- and VIP-IR fibres at day 7 ($p<0.0001$) and CGRP-IR fibres at day 14, but no change in behaviour.
Conclusion: This novel mouse model of CFA-induced vaginal inflammation is accompanied by hyperinnervation involving multiple, neurochemically distinct classes of neurons.

OPTICAL EXAMINATION OF NEURONAL PROJECTIONS AND FUNCTION DURING DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

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[1]*University Of Leuven*

Introduction: The wall of the gastrointestinal tract is made up of many concentric layers of different cell types innervated by enteric neurons, whose cell bodies are located in the myenteric and submucous plexus. Communication with target cells is vital for gastrointestinal function, however, there is currently little known about the development of neuronal projections. In this study, we investigated the extension of enteric neurites into the mucosal villi during embryonic development using immunohistochemistry and live calcium imaging from Wnt1-Cre;R26R-GCaMP3 mice, where all enteric neurons express the genetically-encoded Ca2+ indicator, GCaMP3. Results: Tuj1-immunoreactive neurites were first observed projecting out of the myenteric plexus to the epithelial cell layer lining the gut lumen at embryonic day (E)14.5, even prior to villi formation. To examine whether these neurites are capable of transmitting information to the plexus, the tips of individual villi were stimulated by through a small focal electrode (50µm diameter). Electrical stimulation triggered increases in intracellular Ca2+ in myenteric neurons from E15.5 onwards. The basolateral release of 5-HT from enterochromaffin cells was mimicked by applying local spritz injections (20 psi) of 5-HT (10 µM) into the tips of individual villi using glass micropipettes (tip diameter = 10-20 µm). 5-HT

injections triggered Ca2+ transients in myenteric neurons from P0 onwards. Conclusions: Enteric neurons project out of the myenteric plexus early during ENS development, innervating the gastrointestinal mucosa even prior to villus formation. Some of these neurites are already able to conduct electrical information at E15.5 and responses to 5-HT develop before birth.

SCHWANN CELL CHOLESTEROL SYNTHESIS IS REGULATED BY THE NEUROTROPHIN BDNF

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[1]University of Melbourne, [2]Queensland Brain Institute

Aims: Normal development of the mature peripheral nervous system is dependent upon the neurotrophin family of growth factors. We have identified that the neurotrophin BDNF exerts complex influences upon peripheral nervous system myelination. Here we show that the precursor form of BDNF, proBDNF, exerts a specific influence on Schwann cells to increase cholesterol synthesis and promote peripheral nervous system myelination.

Results: We adopted an unbiased approach to assess the influence that mature BDNF and its precursor form proBDNF exerted upon the Schwann cell transcriptome in vitro. The data identify that mature BDNF exerts little effect, whereas proBDNF significantly upregulated multiple genes in the cholesterol biosynthesis pathway. This is a physiological response, as quantitative analysis revealed that proBDNF significantly increased total cholesterol levels in Schwann cell cultures. Cholesterol is a major constituent of myelin, so we investigated whether proBDNF also influenced Schwann cell myelination. Our data show that proBDNF significantly promoted Schwann cell myelination in vitro. This result was verified following knockdown of p75NTR in Schwann cells, which abolished both the upregulation of cellular cholesterol and promotion of myelination. We have generated a mouse with conditional deletion of p75NTR in Schwann cells, and are currently assessing its peripheral myelin.

Conclusions: Our results suggest that proBDNF exerts a key role in regulating the availability of cholesterol for incorporation into peripheral nervous system myelin, and that p75NTR plays a critical role in this process.

Monday 5th December
Oral Session 6: Injury and Repair II

A COMBINATION OF CALCIUM CHANNEL INHIBITORS FACILITATES TISSUE SPARING AND SIGNIFICANT HIND LIMB FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY

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[1]Experimental and Regenerative Neurosciences, The University of Western Australia, [2]School of Animal Biology, The University of Western Australia, [3]School of Anatomy, Physiology and Human Biology, The University of Western Australia, [4]Monash University

Following injury to the central nervous system, cells beyond the initial injury site undergo secondary degeneration, with Ca2+ overload exacerbating further loss of neurons, compact myelin and function. Reducing the extent of secondary degeneration following spinal cord injury (SCI) is likely to result in greater functional recovery, but treatment options have thus far been limited. A combination of the Ca2+ channel inhibitors lomerizine, YM872 and oxATP, to inhibit voltage gated Ca2+ channels, Ca2+ permeable AMPA receptors and purinergic P2X7 receptors respectively, effectively limits secondary degeneration following partial optic nerve transection. Here we investigated the effects of this combinatorial therapeutic strategy in a clinically relevant model of SCI.
Fischer rats were subjected to a moderate (150kD) contusive SCI at thoracic level T10 using an Infinite Horizons impactor. Lomerizine was administered orally, twice daily, and YM872 and oxATP were administered by continuous infusion via osmotic mini-pump. Behavioural analyses were conducted from day 0 to 14, post injury. Basso, Beattie, and Bresnahan functional scale analysis revealed that treatment with all three inhibitors in combination significantly improved functional recovery of the hind limb as early as 3 days post injury, with beneficial effects persisting to 14 days post injury ($p \leq 0.05$). Functional improvement observed with the combinatorial treatment was associated with significantly decreased cyst size and increased tissue sparing at 14 days ($p \leq 0.05$). The combination of Lomerizine, YM872 and oxATP shows promise for control of secondary degeneration following SCI and investigation of long term outcomes is warranted.

REDUCTION OF THE NEUROPROTECTIVE TRANSCRIPTION FACTOR NPAS4 RESULTS IN INCREASED NEURONAL NECROSIS, INFLAMMATION AND BRAIN LESION SIZE FOLLOWING ISCHAEMIA

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Stroke is the second leading cause of death and the most frequent cause of adult disability. Neuronal Per-Arnt-Sim domain protein 4 (Npas4) is an activity-dependent transcription factor whose expression is induced by various brain insults, including cerebral ischaemia. Although previous studies have demonstrated that Npas4 plays a critical role in protecting neurons against neurodegenerative insults, the neuroprotective effect of Npas4 in response to ischaemic brain injury remains unknown. In this study, we used a loss-of-function approach to examine the neuroprotective potential of Npas4 in the context of ischaemic damage. Using oxygen and glucose deprivation, we demonstrated that the knockdown of Npas4 in mouse cortical neurons resulted in increased susceptibility to cell death. The protective effect of Npas4 was further investigated in vivo using a photochemically-induced stroke model in mice. We found a significantly larger lesion size and increased neurodegeneration in Npas4 knockout mice as compared to wild-type mice. Moreover, we also showed that ablation of Npas4 caused an increase in activated astrocytes and microglia, pro-inflammatory cytokines interleukin-6 and tumour necrosis factor alpha levels and a switch from apoptotic to necrotic cell death. Taken together, these data suggest that Npas4 plays a neuroprotective role in ischaemic stroke by limiting progressive neurodegeneration and neuroinflammation.

AXON-SPECIFIC DELETION OF NOGO RECEPTOR 1 (NGR1) PRESERVES AXONAL TRANSPORT LIMITING DEMYELINATION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

Mr Speros Thomas[1], Dr. Jae Young Lee[4], Ms Peimun Aui[1], Ms Min Joung Kim[1], Professor Alan Harvey[3], Dr Stephen Strittmatter[2], Dr Steven Petratos[1]
[1]Monash University, [2]Yale University, [3]University of Western Australia, [4]Toolgen

We have previously shown that deletion of the ngr1 allele limits the severity of experimental autoimmune encephalomyelitis (EAE) by preserving central nervous system (CNS) axons. However, whether this is governed by the preservation of intact myelin, or through limiting the molecular drivers of axonal degeneration is yet to be defined. In optic nerves, we show that Cre-loxP-mediated axon-specific deletion of ngr1 in ngr1flx/flx mice limited axonal degeneration, whereas re-expression of ngr1 in ngr1-/- mice potentiated axonal degeneration during EAE. As a corollary, myelin integrity was preserved under conditional deletion and significant demyelination observed in the ngr1-/- optic nerves where ngr1 was re-inroduced. Moreover, the interaction between the axonal motor protein, kinesin-1 (KIF5) and collapsin response mediator protein 2 (CRMP2) was unchanged upon conditional deletion of ngr1 whereas this was reduced when ngr1 was re-expressed in the ngr1-/- optic nerves. Our data suggest that NgR1 governs axonal degeneration in the context of inflammatory-mediated demyelination through the phosphorylation

of CRMP2 by stalling axonal vesicular transport. Moreover, the axon-specific deletion of ngr1 preserves axonal transport mechanisms, blunting the induction of inflammatory demyelination and limiting the severity of EAE.

ARCHITECTURAL TRANSCRIPTION FACTORS CONTROL NEURAL STEM CELL QUIESCENCE AND ACTIVATION DURING HOMEOSTASIS AND REGENERATION

Dr Jan Kaslin[1]
[1]*Australian Regenerative Medicine Institute*

Zebrafish display a robust potential to regenerate brain or spinal cord tissue after injury. One of the key mechanisms enabling the regeneration is the remarkable ability of zebrafish to activate quiescent stem cells for tissue repair. Dormant stem cells are an important cell reservoir and defects in the regulation of stem cell quiescence lead to impairments of in their function and distort the balance of stem cell population. Quiescent stem cells undergo several sequential activation steps prior to entry into cell-cycle but it is poorly understood how this process is controlled. During juvenile stages a large proportion of zebrafish neural stem cells in the brain are actively proliferating while in the adult zebrafish the majority of neural stem cells are quiescent Furthermore, injury significantly activates dormant neural stem cells in the adult. We used these differential conditions to define factors regulating neural stem cell activation and identified an architectural transcription factor with DNA and chromatin binding propensities whose expression correlated with stem cell activation during homeostasis and after injury. Genetic gain and loss of function experiments using TALEN technology and heat inducible expression resulted in reduced neural stem cell proliferation, altered organism size and impaired regenerative capability suggesting impaired stem cell function. RNA-seq, CLIP-Seq and CHIP-seq experiments in WT and mutant fish showed altered energy metabolism, translation and expression of growth factor signaling. Taken together, we have identified a novel neural stem cell regulator that primes quiescent stem cells towards activation.

PLASTICITY BEYOND THE CORTEX: CHANGES IN SPINAL CORD INTERNEURONS AFTER CORTICAL ISCHEMIC STROKE

Dr Michelle M Rank[1], Dr Neil Spratt[2], Prof Robin Callister[2], Prof Robert J Callister[2]
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Although it is recognised that plasticity within surviving cortical areas is crucial for sensorimotor recovery after stroke our understanding of plasticity outside the brain, such as in the spinal cord, is in its infancy. Anatomical and molecular changes in the spinal cord are known to occur during stroke recovery. However, little is known about the functional plasticity of spinal cord neurons after a stroke in the brain. Here we use a photothrombotic model of ischemic stroke and whole-cell patch clamp electrophysiology to assess changes in cervical spinal cord neurons involved in forelimb motor circuits. Adult male mice (C57Bl/6; ~P63) received a photothrombotic stroke in the sensorimotor cortex (0.2mm anterior, 2mm lateral to Bregma) and were allowed to recover. Age matched mice served as controls. After recovery, mice were sacrificed and horizontal spinal cord slices (C2-T1, 250µm thick) were prepared for whole-cell patch-clamp electrophysiology. Intrinsic properties of deep dorsal horn (DDH) interneurons were characterised at 7 and 28d during stroke recovery. DDH neurons showed significant changes in several important intrinsic membrane properties including rheobase current, input resistance and action potential (AP) amplitude, beginning at 7d and persisting 28d after stroke. AP discharge, and the voltage-activated subthreshold currents underlying AP discharge, were also significantly reorganised after stroke. These data demonstrate that long-term changes in the intrinsic excitability of spinal cord neurons occur after a cortical ischemic stroke. Uncovering these specific mechanisms underlying spinal cord plasticity after stroke is important in the identification of novel therapeutic targets to enhance functional recovery after stroke.

PROFILING REMOTE SECONDARY DAMAGE AND INFLAMMATION FOLLOWING CNS TRAUMA

Dr Nicole Smith[1,2], Miss Charlotte Bailey[1], Miss Nicole Hortin[1], Miss Carole Bartlett[1], Professor Swaminathan Iyer[2], Professor Sarah Dunlop[1], Associate Professor Melinda Fitzgerald[1]
[1]*Experimental and Regenerative Neurosciences, School of Animal Biology, The University of Western Australia,* [2]*School of Chemistry and Biochemistry, The University of Western Australia*

Inflammation following CNS trauma has beneficial and detrimental effects both at the primary injury and in adjacent regions vulnerable to secondary damage. Clinical studies suggest a link between damage remote from the primary injury and symptomatology ("diaschisis"), yet relatively few studies have investigated inflammation in regions remote from the primary injury. Using an in vivo partial optic nerve transection model in rat we demonstrate a previously unknown transient opening of the blood brain barrier (BBB) remote from the optic nerve injury, in the brain, indicated by intense Evans Blue fluorescence along the visual pathways and a disruption in the tight junction protein Caveolin-1 (Scientific Reports 6, 2295, 2016). This change in BBB permeability enabled intravenously administered polymeric nanospheres (200 nm) to enter the brain and the primary injury site. Multiplex cytokine analysis of homogenized brains showed an increase in pro-inflammatory cytokines TNFα ($p<0.0001$) and IL-2 ($p<0.01$) at 1 day post injury, while the anti-inflammatory cytokine IL-10 was biphasic, being significantly elevated at 1 day ($p<0.001$), decreasing to control levels at 3 days and peaking again at 7 days ($p<0.05$) post injury. Inflammatory cell phenotypic analysis of the left midbrain region, containing projections from the injured right optic nerve, was conducted using flow cytometry. Activated microglia and infiltrating leukocytes were present at 1, 3 and 7 days post injury, with no

significant differences relative to control ($p>0.05$). A more complete understanding of remote secondary damage and inflammation will inform strategies aimed at preventing damage and aiding post-trauma recovery.

Monday 5th December
Oral Session 7: Glia

HIGHLY EFFICIENT CONDITIONAL ABLATION OF OLIGODENDROCYTE PROGENITOR CELLS (NG2 GLIA) INDUCES ANXIETY-LIKE BEHAVIOUR IN ADULT MICE

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Oligodendrocyte progenitor cells (OPCs), also known as NG2-glia, are mitotically active cells known primarily for their role in producing myelin-forming oligodendrocytes in the central nervous system (CNS). Additional roles of OPCs in adult brain physiology, particularly the modulation of neural processing, have been suggested but the underlying mechanisms remain elusive. Attempts to investigate the function of OPCs by targeted cell ablation in the adult CNS have been limited by methodological challenges resulting in only partial and transient OPC ablation. To overcome these limitations, we have developed a novel transgenic mouse model of conditional OPC ablation. By crossing Pdgfra-CreERT2 mice with a Cre-conditional cell ablation line called Sox10-DTA mice, tamoxifen-mediated Cre recombination resulted in both the deletion of GFP cassette in the recombined PDGFR+ cells and the expression of a suicide gene (diphtheria toxin fragment A, DTA), which rendered OPCs selectively sensitive to DTA-mediated apoptosis. In combination with intracisternal infusion of the antimitotic drug cytosine-D-arabinofuranoside (AraC), tamoxifen-administered Pdgfra-CreERT2: Sox10-DTA mice exhibited complete OPC depletion throughout the entire brain for up to 10 days post AraC infusion. To determine the behavioural consequences of conditional OPC ablation, we assessed OPC-deficient animals using the elevated plus maze and saccharin preference tests during 10 days after AraC withdrawal. Both behavioural tests revealed that OPC-deficient mice exhibited significantly more anxiety-like features than non-ablated littermate controls. The precise mechanisms underlying the homeostatic regulation of neuronal function by OPCs are currently under investigation.

OVERCOMING MONOCARBOXYLATE TRANSPORTER 8 (MCT8)-DEFICIENCY TO PROMOTE HUMAN OLIGODENDROCYTE DIFFERENTIATION AND MYELINATION

Miss Min Joung (Erica) Kim[1], Dr Jae Young Lee[2], Dr Devy Deliyanti[3], Dr Michael Azari[4], Dr Fernando Rossello[5], Professor Ed Stanley[6,7], Professor Andrew Elefanty[6,7,8], Professor Jennifer Wilkinson-Berka[3], Dr Steven Petratos[1]
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Cell membrane thyroid hormone (TH) transport is primarily mediated by the monocarboxylate transporter 8 (MCT8). Human mutations of the gene, slc16a2, result in the X-linked-inherited psychomotor retardation and hypomyelination disorder, Allan-Herndon-Dudley syndrome (AHDS). We posited that abrogating MCT8-dependent TH transport limits oligodendrogenesis and myelination. We show that human oligodendrocytes (OL), derived from the Nkx2.1-GFP human embryonic stem cell (hESC) reporter line, express MCT8. Moreover, treatment of these cultures with DITPA (an MCT8-independent TH analog), up-regulates transcription factors specific to OL differentiation and myelin gene expression. DITPA treatment promotes hESC-derived OL myelination of retinal ganglion axons in co-culture. Pharmacological and genetic blockade of MCT8 induces significant OL apoptosis, impairing myelination. DITPA treatment reverses OL apoptosis mediated by slc16a2 down-regulation and promotes myelination. Our results highlight the potential role of MCT8 in TH transport for human OL development and may implicate DITPA as a promising treatment for developmentally-regulated myelination in AHDS.

REGULATION OF ASTROCYTIC GLUTAMINE RELEASE BY INTRACELLULAR SODIUM

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Astrocytes located adjacent to synapses in the central nervous system are thought to release glutamine, which may be sequestered by presynaptic neurons for the production of glutamate as part of a glutamate-glutamine cycle. One possible mechanism for the release of astrocytic glutamine is via membrane transporters of the system N (SN) family, which transport a neutral amino acid, co-transport Na+ and counter-transport H+. We have previously shown that activation of excitatory amino acid transporters (EAATs) on astrocytes causes rapid release of glutamine and we hypothesise that the rise of [Na+]i that accompanies EAAT activation by glutamate stimulates glutamine efflux via SN.

To investigate this we recorded SN activity in situ, from whole-cell voltage-clamped astrocytes in rat brainstem slices. SN activity was assessed by glutamine application while measuring the resulting intracellular pH and [Na+] changes using the fluorescent dyes HPTS or SBFI included in the patch pipette. We show that activation of EAATs, ionotropic glutamate receptors or system A

amino acid transporters all produce an increase of [Na+]i in astrocytes, and each of these independent manipulations causes an efflux of glutamine from astrocytes via system N transporters.

These results demonstrate a functional coupling between astrocytic glutamate influx and glutamine efflux, mediated by intracellular Na+ levels. This provides a mechanism by which the glutamate-glutamine cycle may be regulated by activity in neighbouring synapses, which is advantageous for the maintenance sustained neurotransmission in a defined location.

OLIGODENDROGLIAL PROLIFERATION DYNAMICS ARE REGULATED BY CANONICAL WNT/β-CATENIN SIGNALLING DURING DEVELOPMENT

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Excessive canonical Wnt signalling is inhibitory to oligodendrogilal migration and differentiation. To more comprehensively understand the role of canonical Wnt signalling in the oligodendroglial lineage, we conditionally deleted the downstream Wnt mediator β-catenin at the onset of oligodendroglial specification. We show that blocking Wnt signalling leads to precious cell cycle exit, with a greater proportion of oligodendrocyte precursor cells (OPCs) observed in G0 early in postnatal development. However, later in development the loss of β-catenin significantly increases the time it takes for OPCs to divide in vivo. Our preliminary data indicates that this is likely due to an OPC specific decrease in Cyclin D1, a Wnt regulated target important for controlling the length of the G1 phase of the cell cycle and the choice to remain in a proliferative sate. Interestingly, immunohistochemical assessment of differentiation and myelination indicates robust myelination in the β-catenin conditional knockouts. In fact, our preliminary qualitative assessments of semi thin sections from the cervical spinal cord, indicates thicker myelin in conditional knockout animals during development. Finally, we did not observe any evidence for an OPC migration deficit and report that β-catenin conditional knockouts live past 1.5 years. Excessive Wnt signalling is common to a range of myelin disorders including MS. Collectively, we show that inhibiting Wnt signalling in oligodendroglia is a potential means to encourage cell cycle exit and differentiation with no obvious detrimental long-term effects. This work highlights that further investigation into the Wnt signalling pathway may reveal novel therapeutic targets relevant to myelin disorders.

INHIBITION OF MICROGLIAL ACTIVATION VIA BRAIN INFUSION OF MINOCYCLINE PREVENTS DIABETIC CARDIOVASCULAR COMPLICATIONS IN STZ–INDUCED DIABETIC RATS

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[1]School of Health and Biomedical Sciences, RMIT University, [2]Centre of Cardiovascular Research and Education in Therapeutics, Department of Epidemiology and Preventive Medicine, Monash University

Our previous studies indicate that microglia are activated in cardiovascular autonomic and endocrine control regions including the paraventricular nucleus (PVN) in rats with streptozotocin (STZ)-induced diabetes. Dysfunction in these brain regions may contribute to diabetic cardiovascular and renal complications. We therefore investigated whether inhibition of microglial activation could prevent diabetic complications. Sprague Dawley rats were made diabetic via STZ injection (iv). One week later, an osmotic minipump was implanted to deliver minocycline (5 μg/h) or saline into the lateral ventricle (icv infusion). At 6 weeks following STZ, rats were anaesthetized and cardiac echocardiography and direct left ventricular pressure measurements performed before collecting tissues for analysis. When compared to control rats given saline icv, rats treated with STZ alone showed significantly increased plasma osmolarity, haematocrit, haemoglobin concentration and significant left ventricular dysfunction based on multiple echocardiographic and haemodynamic parameters (LVIDs, LVIDd, E/A ratio, EDP, dP/dtmax). STZ-treated rats given minocycline icv showed no statistically significant differences from control in any parameter measured (n=6-8 per group, $P< 0.05$, 1 way ANOVA). Immunohistochemistry in the PVN of STZ-treated rats showed significantly increase neuronal activation (c-fos) and microglial activation (ox42) when compared with controls rats. In rats treated with STZ and minocycline, microglial activation and increased neuronal activation were not seen. Minocycline had no significant effect on blood glucose levels. We conclude that the complications seen in STZ diabetic rats at 6 weeks were not caused by direct effects of high glucose on organs, but due to dysfunction in brain regions controlling these organs.

ACTIVATION OF INFANT ASTROGLIOSIS PATHWAY SUPPRESSES GLIAL SCARRING IN ADULTHOOD FOLLOWING A NEOCORTICAL STROKE

Dr. Leon Teo[1], Dr. Jihane Homman-Ludiye[1], A/Prof James Bourne[1]
[1]Australian Regenerative Medicine Institute

Glial scarring in the neocortex after injury (e.g. ischemic stroke) is a major impediment to neuroregeneration, leading to permanent neurological impairments. However, glial scarring in the infant is less severe compared to adults, allowing greater potential for functional recovery. EphA4 is a major modulator of astrogliosis after CNS injuries; however, the ephrin ligands involved in the injured infant and adult brain remains unknown.
A clinically translatable primate (marmoset monkey; n=24) model of ischemic stroke in the infant and adult neocortex, possessing identical astrogliotic time-course with humans, was utilized in this study.
We reveal that the NHP brain undergoes age-dependent Eph/ephrin mediated astrogliotic responses after stroke. Specifically: the post-stroke infant brain upregulates ephrin-A1, which induces astrocyte repulsion, reduced proliferation and reactivity

resulting in a small, discrete scar. Conversely, in Adults, upregulation of ephrin-A2/-A5 induces astrocyte attraction, increased proliferation and reactivity resulting in dense, widespread scarring. Ephrin-A1 treatment successfully suppressed ephrin-A2/-A5-induced astrocyte reactivity in vitro. Intracortical infusion of ephrin-A1-fc (0.1mg/kg/day; 7 days) post-stroke (prior to the peak of astrogliosis) successfully suppressed astrocyte reactivity resulting in ~50% ($p<0.05$) reduction in glial scar volume and density, reduced CSPG deposition and significantly reduced secondary astrocyte recruitment ($p<0.05$) in vivo. Most importantly, ephrin-A1-fc treatment improved functional sparing of neural circuitry and neuronal survival after stroke compared to untreated controls.

This study demonstrates that infant and adult primate brains regulate astrogliosis through separate Eph/ephrin pathways. Furthermore, reintroduction of ephrin-A1 in the adult neocortex successfully attenuated astrogliosis, resulting in more discrete scarring, thereby promoting cellular and functional recovery.

Monday 5th December
Oral Session 8: Neurodevelopment

A PAN-MAMMALIAN INTERHEMISPHERIC PROGRAM PREDATES THE EVOLUTION OF THE CORPUS CALLOSUM

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Communication between both cerebral hemispheres is essential for everyday sensory-motor and cognitive functions. In placental mammals this is achieved by the corpus callosum, the largest tract of the human brain. Non-placental mammals, such as monotremes and marsupials, lack a corpus callosum and instead the cortical hemispheres interconnect through the anterior commissure. In placentals, callosal development involves a Satb2+/Ctip2- genetic program, a spatial topography of axons based on the position of their cell bodies, and co-existence of homotopic, heterotopic and hyper-connected interhemispheric circuits. Whether any of these traits are directly linked to the origin of the corpus callosum in the placental lineage, or instead represent conserved features that arose earlier in mammalian evolution, remains unknown. Here we show that the main molecular, developmental, cytoarchitectural and connectivity features of interhemispheric circuits arose before the evolution of the corpus callosum. We found that interhemispheric isocortical connections coursing through the anterior commissure of monotremes and marsupials share a spatial axonal topography with the corpus callosum. Commissural axons arise from Satb2+/Ctip2- neurons in marsupials, and establish homotopic and heterotopic connections, as well as hyperconnected hubs at the medial (cingulate-retrosplenial) and temporal (claustro-insular) margins of the neocortex. Additional features of cortical layer distribution, gene expression and projection fates are similarly conserved in cortico-fugal projections of mammals with and without a corpus callosum. Thus, our results suggest an ancient origin and conservation of a mammalian isocortical connectome that predates the origin of the corpus callosum by at least 40 million years.

DISRUPTED NEOGENIN SIGNALLING RESULTS IN POSTNATAL HYDROCEPHALUS

Conor O'Leary, Natalie Lee, Amanda White and Helen Cooper
Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

Hydrocephalus is a prevalent neurodevelopmental defect characterised by enlargement of the intracerebral ventricles due to an increase in cerebrospinal fluid volume. Non-communicating hydrocephalus is often caused by obstruction of the Aqueduct of Sylvius, whereas communicating hydrocephalus is attributed to impaired reabsorption of cerebrospinal fluid. Defects in the ependymal cell (EC) layer contribute to the aetiology of both forms. ECs, multiciliated epithelial cells, line the ventricular walls and possess adherens junctions (AJs), the sites of cell to cell adhesion which allow ECs to form a cohesive epithelium. Loss of AJs results in EC denudation leading to hydrocephalus. However, the molecular pathways directing EC junctional assembly are poorly understood.

We have recently demonstrated that the netrin/RGM receptor, Neogenin, is essential for AJ assembly. Intriguingly, postnatal Neogenin loss-of-function mice exhibit pronounced hydrocephalus. Preliminary analysis revealed that the EC layer in these mutants is disrupted. ECs are derived from Radial Glial Cells (RGCs), the multipotent neural stem cells within the embryonic ventricular zone. We now show that Neogenin is essential for AJ assembly between RGCs in the developing mouse cortex. Using in utero electroporation to acutely disrupt Neogenin signalling in the E14.5 cortex we find that there is a significant increase in RGCs that have lost AJs compared to control samples (0.5 vs 30, p=0.0024, n=4). These data support the hypothesis that Neogenin is required to maintain the junctional integrity between RGCs in the embryonic cortex as well as within the EC layer in the postnatal brain to ensure a functional ventricular system.

RNA-SEQ OF FACS PURIFIED SYMPATHETIC NEURONAL PROGENITOR CELLS REVEALS THE MOLECULAR TRAITS OF CELL FATE DECISIONS

Mr Wing Hei Chan[1], Dr E. Michelle Southard-Smith[2], Professor Heather Young[1], Dr Matthew Wakefield[3], Dr Anderson Colin[1]
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Peripheral sympathetic neurons and adrenal medullary chromaffin cells originate from a common sympathoadrenal (SA) progenitor cell. However, the transcription factors and signalling mechanisms underlying lineage separation during embryonic development are not fully understood. The present study investigates the whole transcriptome of purified embryonic murine sympathoadrenal cells to understand the process of dynamic cell fate decisions and the signaling mechanism that underlie the diversification. The neuroblasts and chromaffin precursor cells were isolated from the E12.5 TH-IRES-Cre;ROSA26-EYFP transgenic mouse by fluorescence-activated cell sorting (FACS) based on differences in EYFP between the two cell types. RNA-Seq analysis was performed on an Illumina® HiSeq system using non-strand specific single-end sequencing. Bioinformatics and statistics analysis were done by using the Galaxy tool. Droplet digital PCR was used to confirm the differentially expressed genes. By using the differences in tyrosine hydroxylase associated EYFP expression, we successfully isolated the neuroblasts from the

differentiating chromaffin cells transgenic embryonic mouse. RNA-Seq analysis on the isolated cells revealed more than 5,000 differentially expressed genes during lineage segregation. Among genes that were significantly differentially expressed were Foxq1, Fzd10 and Dll4 which are involved in Wnt and Notch signalling. This study gives insight into the cellular processes during differentiation of sympathoadrenal cells in to neurons and chromaffin cells.

MUTATIONS TO GENES ENCODING COMPONENTS OF THE MICROTUBULE ASSEMBLY PATHWAY UNDERLIE BRAIN GROWTH AND EARLY ONSET NEURODEGENERATION

Associate Professor Julian Heng[1], Ms Hayley Cullen[1], Professor Nicholas Cowan[2], Doctor David Keays[3]
[1]The Harry Perkins Institute of Medical Research, [2]Department of Biochemistry & Molecular Pharmacology, NYU Langone Medical Centre, [3]Institute of Molecular Pathology

The development of the cerebral cortex relies on a step-wise process of neurogenesis, cell migration and terminal differentiation as neurons are added to the growing fetal brain. These developmental functions are underpinned by the activities of the microtubule cytoskeleton within fetal brain cells which coordinate cell division, neurite outgrowth and directional movement, as well as dendritic branching. Failures in the production of neurons as well as their assembly can lead to early onset mental conditions such as intellectual disability and epilepsy. In this presentation, we highlight our recent studies which identify causative mutations to genes encoding components of the microtubule assembly pathway that underlie primary microcephaly (a condition resulting from a failure in brain growth) as well as secondary microcephaly (a condition postnatal slowing or arrest of head circumference growth). We discuss how our studies define the roles for these genes in neural circuit assembly, and how we have applied our discoveries to improve the genetic diagnosis of human brain developmental disorder.

THE ROLE OF THE INFERIOR PULVINAR IN THE EARLY DEVELOPMENT OF VISUALLY-GUIDED ACTIONS

Dylan Fox[1]
[1]Australian Regenerative Medicine Institute

The pulvinar is the largest thalamic nucleus in primates (including humans) and yet defining its function has been enigmatic due to its complex array of multi-modal connections. Of particular interest, the medial division of the inferior pulvinar (PIm) receives direct retinal input during development and relays visual information to the middle temporal area (MT) pertaining to motion processing. This direct connection bypasses the primary visual cortex (V1), indicating a potential role in the early development of visually-guided behaviours. While this connection has been established in nonhuman primates, its role in the development of visual function has yet to be explored.
All procedures culminated in training four adult marmosets (two animals received left unilateral lesions to PIm at PD14) to perform a series of visually-guided tasks designed to assess their control over accurately locating and retrieving static and moving objects in their environment. The kinematics of their reaching and grasping behaviours were recorded for offline analysis.
Grasping profiles observed in the marmoset showed salient resemblances to humans and other nonhuman primates. Pulvinar lesion animals revealed a marked increase in their grip aperture, coupled with faster, yet inaccurate, attempts. Marmosets also displayed the capacity to scale their grip mid-flight according to the size of the object.
The current study firstly shows that marmosets are akin to other human and nonhuman primates in terms of their flexible online control during reach-to-grasp actions. Furthermore, the results reveal a critical role of the inferior pulvinar for the appropriate development and functioning of visuomotor networks.

EXPOSURE TO THE ANTIBIOTIC VANCOMYCIN MODIFIES DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM IN EARLY POSTNATAL MICE

Petra Unterweger[1], Lin Hung[1], Pavitha Parathan[1], Dr Tor Savidge[2], Prof Joel Bornstein[1], **Dr Jaime Foong[1]**
[1]University Of Melbourne, [2]Baylor College of Medicine

The trillions of microorganisms living in the adult bowel (microbiota) can influence the firing of enteric neurons, gut motility and function. Colonization and establishment of microbiota in the gut primarily occurs after birth and significant development of the enteric nervous system (ENS) still occurs postnatally. While it is likely that disruption of microbiota by external factors such as antibiotics after birth affects ENS development, this remains unproven. We examined whether antibiotics ingested during early postnatal stages affect ENS development. Mouse pups were fed from birth with a daily dose of vancomycin (83.3 mg/kg/day) or water and their duodenums and colons were examined at postnatal day 10/11. Vancomycin-treatment significantly shifted microbial composition with higher relative abundances of Firmicutes replacing Bacteroidetes and Proteobacteria. Using video imaging, we found a significant increase in colonic motility of vancomycin-fed pups compared to their water-fed littermates, whereas no effects were observed in the duodenum (n=12; colon $P<0.05$; duodenum $P=0.9$). The density of myenteric neurons marked by the pan-neuronal marker Hu, and proportion of neurons that expressed neuronal nitric oxide synthase (nNOS) and calbindin were examined immunohistochemically. Vancomycin-fed pups had a significantly lower myenteric neuron density, lower proportion of nNOS and a larger proportion of calbindin-expressing neurons in the colon (n=8-9, $P<0.01$ and $P<0.05$ respectively). No effects on neuron density or proportions were observed in the duodenum (n=8-9). In

conclusion, neonatal exposure to vancomycin alters development of enteric neurons and motility patterns in the colon in response to aberrant microbial colonization after birth.

Monday 5th December
Oral Session 9: Neurobiology of Behaviour

CHRONIC RXFP3 ACTIVATION IN VENTRAL HIPPOCAMPUS AND MEDIAL AMYGDALA MODULATES FEAR, ANXIETY AND SOCIAL BEHAVIOUR IN RATS

Miss Valeria Rytova[1,2], Dr Despina Electra Ganella[1,2], Dr Ross A.D. Bathgate[1,2,3], Dr Sherie Ma[1,2], Dr Andrew Gundlach[1,2,4]
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The neuropeptide, relaxin-3, preferentially activates the Gi/o-protein-coupled receptor, RXFP3. Relaxin-3/GABA neurons constitute a conserved ascending neural network in mammalian brain, enriched in limbic areas involved in stress, arousal and emotion-related behaviours, such as hypothalamus, amygdala, ventral hippocampus (vHipp), septum and prefrontal cortex. We have previously observed differential effects of acute RXFP3 activation on conditioned fear in rats, which were dependent on the site of administration and presumed action. In this study we characterized the effects of chronic RXFP3 activation in key limbic regions on 'affective' behaviours, including anxiety and social avoidance. Adeno-associated viral vectors driving local secretion of a RXFP3 agonist, R3/I5, were bilaterally injected into the vHipp or medial amygdala (MeA) of adult male, Sprague-Dawley rats (7-10/group). Chronic vHipp RXFP3 activation decreased time and distance travelled in the open arms of the elevated plus maze (EPM) and the aversive light zone of the light-dark box (LDB); and decreased social interaction with a conspecific stranger, compared to control (all $p<0.05$). Conversely, RXFP3 activation in the MeA increased time and distance travelled in EPM open arms and the centre of a large open field (LOF). We are currently assessing the effect of chronic RXFP3 activation on relevant neurochemical indices in both regions. Our data suggest 'topographic' effects of RXFP3 signalling on fear and innate anxiety, related to precise site(s) and timing of endogenous and exogenous relaxin-3 peptide actions. These and ongoing studies will provide a better understanding of the neurochemical basis of anxiety-related disorders, with potential for identifying novel therapeutic targets.

INTERACTION OF GLUCOCORTICOID STRESS HORMONE WITH THE BDNF VAL66MET GENOTYPE TO REGULATE PREPULSE INHIBITION OF ACOUSTIC STARTLE, AN ENDOPHENOTYPE OF SCHIZOPHRENIA

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Reduced expression of Brain-Derived Neurotrophic Factor (BDNF) has been implicated in the pathophysiology of schizophrenia. The BDNF Val66Met polymorphism, which results in deficient activity-dependent secretion of BDNF, has been reported to mediate stress sensitization and clinical features of schizophrenia, although clinical studies have been inconsistent (Notaras et al., 2015).
We studied the effect of this polymorphism on Prepulse Inhibiton (PPI), a translational model of sensorimotor gating which is disrupted in schizophrenia. We utilized hBDNFVal66Met mice genetically modified to carry a humanized BDNF transcript expressing the Val66Met polymorphism and studied the effect of chronic corticosterone (CORT) exposure in these animals at 6-9 weeks of age as a model of chronic, young-adult stress. PPI was assessed at 100msec and 30msec inter-stimulus intervals (ISI) at baseline and after acute injection of apomorphine or MK-801.
PPI at the commonly used 100msec ISI, irrespective of CORT treatment, was reduced in the hBDNFVal/Met heterozygote mice, but not hBDNFMet/Met mice compared to hBDNFVal/Val mice. At the 30msec ISI, CORT treatment selectively disrupted PPI of hBDNFVal/Met mice only. Chronic CORT reduced startle reactivity of hBDNFVal/Val male mice by 51%. However, ANCOVA suggested that this was independent of the effect of CORT and hBDNFVal66Met genotype on PPI. The differential effects of apomorphine and MK-801 in this model will be presented.

We provide the first robust evidence of a distinct BDNF 'heterozygote disadvantage' phenotype using the sensorimotor gating endophenotype of schizophrenia. A history of stress hormone exposure affects PPI and startle depending on BDNF Val66Met genotype.

EXTINCTION GENERATES OUTCOME-SPECIFIC CONDITIONED INHIBITION MEDIATED BY INFRALIMBIC CORTEX AND MODULATED BY DORSAL HIPPOCAMPUS

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[1]University Of New South Wales

Although it is widely-accepted that extinction generates some form of inhibitory learning, direct evidence for this claim has been lacking and the nature of the associative changes induced by extinction have, therefore, remained a matter of debate. In the current experiments we used a novel behavioral approach that we recently developed and that provides a direct measure of conditioned inhibition to compare the influence of extinguished and unextinguished cues on choice between goal-directed

actions. Using this approach, we provide direct evidence that extinction generates outcome-specific conditioned inhibition. Furthermore, we demonstrate that this inhibitory learning is controlled by infralimbic cortex (IL); inactivation of the IL using M4 DREADDs abolished the outcome-specific inhibition and rendered the cue excitatory. Importantly, we found that context modulated this specific inhibition: Outside its extinction context the cue was excitatory and functioned as a specific predictor of its previously associated outcome, biasing choice towards actions earning the same outcome. In its extinction context, however, the cue acted as a specific inhibitor and biased choice towards actions earning different outcomes. The modulation of these excitatory and inhibitory memories was mediated by dorsal hippocampus (HPC) suggesting that the HPC and IL act in concert to control the influence of conditioned inhibitors on choice. Furthermore, these findings demonstrate for the first time that extinction turns a cue into a net inhibitor that can influence choice via counterfactual action-outcome associations.

NEUROTROPHIN SIGNALLING REGULATES CHOLINERGIC CONTROL OF FEAR EXTINCTION

Zoran Boskovic[1,2], Michael Milne[1,2], Lei Qian[1], Alice McGovern[2], Marion Turnbull[1,2], Stuart Mazzone[2], **Elizabeth J Coulson[1,2]**
[1]Queensland Brain Institute, [2]School of Biomedical Sciences, The University of Queensland, Brisbane, Australia

The cholinergic basal forebrain (cBF) neurons, which play important roles in higher cortical function, express the p75 neurotrophin receptor (p75NTR) throughout life, but the function of p75NTR in the adult cBF neurons is unclear. Using a novel conditional knock-out line (ChAT-cre p75in/in) we demonstrated that the absence of p75NTR induces a lasting increase in cBF cell number and cholinergic innervation to the cortex resulting in alterations to idiothetic navigation. This suggested that p75NTR normally mediates pruning of axonal arbours of the cBF neurons. We have also found that ChAT-cre p75in/in animals also show an inability to consolidate fear extinction memories and the opposite behaviour is observed of mice in which the cBF neurons are lesioned. The reduced extinction memory in ChAT-cre p75in/in was accompanied by down-regulated activity of prefrontal cortex neurons innervated by the cBF that are known to play a critical role in fear extinction and an increase in cholinergic innervation. Furthermore, fear extinction consolidation is rescued when p75NTR is acutely re-expressed in ChAT-cre p75in/in neurons projecting to the prefrontal cortex. We postulate that this phenotype is observed due to a lack of structural modulation of cBF projection neurons in the absence of p75NTR, which in turn affects cortical neuron precise connectivity and thus information flow. These findings indicate a novel role for p75NTR-mediated cBF cellular plasticity in the regulation of a cognitive function that requires previously undocumented cholinergically-dependant processes.

TACTILE INTENSITY CODED WITHIN AFFERENT BURST ACTIVITY

Mr Alexei Brown[1], Dr Sarah McIntyre[2,3], Dr Ingvars Birznieks[1,2], Dr Richard Martin Vickery[1,2]
[1]School of Medical Sciences, UNSW Australia, [2]Neuroscience Research Australia, [3]MARCS Institute for Brain, Behaviour and Development, Western Sydney University

Emerging evidence suggests that neural information can be encoded in the temporal features of spiking activity. We elicited specified temporal patterns of activity in tactile afferents and demonstrated how those temporal features profoundly shaped human perception. This enabled us to address whether a temporal or rate code is used to encode vibrotactile frequency and to examine intensity coding. Ten subjects aged 18 - 50 gave written consent for participating in the experiment which was approved by the UNSW HREC (11074). Our electrotactile stimuli were organised into bursts of impulses repeated at 21 Hz, delivered to the digital nerve. The bursts consisted of 2 to 5 impulses, ms apart. This stimulation felt like vibration, and we determined the perceived frequency using the method of constant stimuli with a 2-interval forced choice paradigm. When we increased the mean impulse rate by increasing the number of impulses per burst, this did not increase perceived frequency, but instead produced a small decrease (ANOVA, n=10, $p<0.001$). In a separate magnitude estimation experiment, we determined that the additional impulses per burst contributed to an increase in apparent intensity. Going from 2-impulse bursts to 5-impulse bursts resulted in an increase in perceived intensity of 79% (95% confidence interval 42-116%). Our study demonstrates that conscious perception of stimuli can be determined purely by temporal features of spike trains without contribution from population factors or the mean rate of neural activity.

NEURODEVELOPMENTAL CORRELATES OF FEAR EXTINCTION: AN FMRI STUDY

Dr Despina Ganella[1], Ms Eleni Ganella[2], Associate Professor Sarah Whittle[2], Dr Jee Hyun Kim[1]
[1]Behavioural Neuroscience Division, Florey Institute of Neuroscience And Mental Health, [2]Melbourne Neuropsychiatry Centre, Department of Psychiatry, University of Melbourne

The increased prevalence of anxiety disorders during adolescence is thought to be due to fear extinction deficits involving immature ventromedial prefrontal cortical (vmPFC) function, which has been demonstrated in preclinical rodent studies. We investigated the neural correlates of fear extinction learning and recall using functional magnetic resonance imaging (fMRI) in adolescent and adult humans.
Healthy adults (n=15, aged 25-35) and healthy adolescents (n=9, aged 14-16) underwent fMRI using a novel fear-learning paradigm involving the pairing of a neutral face (CS) with a fear-face plus scream (US). The paradigm involved four phases: (A) Conditioning, where one of two neutral faces (CS+) was paired with an aversive sound (scream, US) on 100% of trials. The other

neutral face was a control stimulus (CS-) that was never paired with the US, (B) Extinction, CSs were presented without the US, (C) Reinstatement, two CS+ US pairings, and (D) Extinction recall, where both CSs were again presented without the US.
fMRI analysis using SPM12 revealed that adults demonstrated the expected vmPFC activation during extinction (CS+>CS-); however adolescents showed decreased vmPFC and increased rostral/dorsal anterior cingulate cortex activity during extinction learning and recall, compared to adults. Male adolescents showed reduced vmPFC activity during extinction learning compared with females. When tested for fear recall, however, female adolescents showed reduced vmPFC activity compared to males, suggesting that vmPFC deficits may not appear until after the initial extinction learning phase in female adolescents. These findings have implications for understanding risk factors and developing novel treatment strategies for adolescent anxiety.

Monday 5th December
Oral Session 10: Motor function and disease

DOSE DEPENDANT EFFECT OF EPOTHILONE D ON DISEASE PHENOTYPE IN THE SOD1G93A MOUSE MODEL OF ALS

Mr Jayden Clark[1], Miss Elise Yeaman[1], Dr Catherine Blizzard[1], Dr Jyoti Chuckowree[1], Associate Professor Tracey Dickson[1]
[1]*Menzies Institute For Medical Research*

Amyotrophic Lateral Sclerosis (ALS) is characterised by the degeneration of motor neurons (MNs), their axonal processes and neuromuscular junctions (NMJs). Therefore, stabilisation of the axon has been proposed as an attractive target for ALS therapeutics. We have utilised the microtubule stabiliser EpothiloneD (EpoD) in the mSOD1G93A mouse model of ALS. Mice were treated with either 1mg/kg EpoD, 2mg/kg EpoD or vehicle control from 50 days of age until endpoint. Behavioural evaluation consisted of rotarod performance and grip strength testing. Immunohistochemical evaluation of pathological changes was also established in mSOD1G93A mice at 10 weeks, 20 weeks and endpoint. High dose treatment resulted in initially improved motor function ($p<0.05$), however, a toxic effect on motor function occurred later in the disease, followed by a decrease in survival ($p<0.05$). Low dose treatment improved all aspects of motor function ($p<0.05$), but failed to alter survival ($p>0.05$). Moreover, EpoD significantly increased MN and axonal survival at the early stages of treatment ($p<0.01$); however, this was not paralleled with improvements in motor function. We posit that EpoD is a dose dependent disease-modifying agent that has both positive and negative effects on the ALS phenotype. These results highlight the heterogeneity of ALS, with a combination and varied doses of therapeutics most likely being needed to positively modify or alleviate the disease phenotype.

MAJOR CONTRIBUTION OF TRPC3 ION CHANNELS TO CALCIUM LOADING IN MOUSE CEREBELLAR PURKINJE NEURONS

Miss Jasneet Parmar[1], Dr Amanda J Craig[1], Prof Matthias Klugmann[1], Dr Georg Von Jonquieres[1], Prof Lutz Birnbaumer[2], A.Prof Andrew J Moorhouse[3], Dr John M Power[1], Prof Gary D Housley[1]
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Coupled to the mGluR1, TRPC3 ion channels mediate the slow excitatory post-synaptic current in cerebellar Purkinje neurons (PNs), but not the associated synaptically - evoked dendritic Ca2+ transients (Hartmann et al. 2008; Neuron 59, 392–398). Here we tested the hypothesis that sustained mGluR activation causes TRPC3-mediated Ca2+ entry in PNs. Using a UNSW Animal Care and Ethics Committee approved protocol, a genetically encoded GCaMP5G Ca2+ reporter packaged into an adeno-associated virus vector with a CMV enhancer/chicken beta-actin promotor was injected into the cerebellum of cold-anaesthetised TRPC3+/+ and TRPC3-/- mouse pups (129/SvEv) at post- natal day 3. At 5 – 8 weeks, cerebellar brain slices were imaged for GCaMP5G fluorescence during superfusion with the mGluR1 agonist (S)-3,5-dihydroxyphenylglycine (DHPG,100 µM in CSF, room temperature, 10 min). The mean pixel intensity of individual PN somata before, during and after DHPG was quantified using ImageJ (NIH). This showed that the Ca2+ responses, characterised by an initial peak lasting ~ 1 minute, followed by a reduced plateau phase that resolved with washout, was reduced in the TRPC3-/- PNs ($P < 0.05$; ANOVA; average peak TRPC3-/- PN $\Delta F/F = 0.47 \pm 0.12$ vs TRPC3+/+ $\Delta F/F = 1.15 \pm 0.43$; plateau TRPC3-/- PN $= 0.01 \pm 0.02$ vs TRPC3+/+ $= 0.35 \pm 0.05$ (3 slices from 2 TRPC3+/+ and 5 slices from 3 TRPC3-/-; range 3 to 14 PNs per slice). These data suggest that TRPC3 channel - mediated Ca2+ loading in PNs may be a factor in the vulnerability of the cerebellum to ischaemic injury.

DISTINCT ROLES FOR DSCAM2 ISOFORMS AT THE DROSOPHILA NEUROMUSCULAR JUNCTION

Mr Lorenzo Odierna[1], Dr Grace Shin[2], A/Prof Nick Lavidis[1], A/Prof Peter Noakes[1], Dr Sean Millard[1]
[1]*School Of Biomedical Sciences, The University Of Queensland,* [2]*Department of Neuroscience, Columbia University Medical Centre*

Drosophila Down syndrome cell adhesion molecule 2 (Dscam2) is a multifunctional cell surface protein involved in wiring the brain. Cell-specific alternative splicing of Dscam2 generates two proteins that mediate isoform-specific homophilic binding. Neurons in close contact with one another that express distinct isoforms exhibit morphological defects when engineered to express the same isoform, but how removing cell-specific isoform expression affects neurophysiology has not been investigated. Here, we demonstrate that larval motor neurons express isoform B of Dscam2, whereas interneurons in the ventral nerve cord express isoform A. Eliminating cell-specific isoform expression by knocking a single isoform into the endogenous Dscam2 locus causes severe locomotion defects in larvae. Consistent with these behavioural phenotypes, electrophysiological recordings at the neuromuscular junction revealed defects in spontaneous neurotransmitter release in flies that express a single isoform. These findings are in agreement with previous studies demonstrating similar morphological phenotypes in animals expressing either single isoform. Evoked responses, however, were different between the two isoforms. In larvae expressing only isoform A, there was a significant increase in the amplitude of evoked potentials relative to both controls and animals only expressing isoform B. Interestingly, Dscam2 null animals exhibited a similar phenotype suggesting that the increase in evoked release represents a Dscam2 loss-of-function phenotype. These data imply that isoform A and isoform B perform different functions in motor neurons, a surprising result considering that the two isoforms differ by only a single extracellular immunoglobulin domain. Whether these phenotypes are dependent or independent on homophilic binding will be discussed.

TARGETED NON-VIRAL GENE DELIVERY TO MOTOR NEURONS IN-VIVO: DOSING and TIME COURSE

Dr Mary-Louise Rogers[1], Dr Roshan Vasani[1], Dt Cher-Lynn Soh[1], Dr Kevin Smith[1], Dr Bradley Turner[2]
[1]Department of Human Physiology, Centre for Neuroscience, Flinders University, [2]The Florey Institute of Neuroscience and Mental Health, University of Melbourne

Background: Targeted gene therapy to motor neurons including delivery of growth factors genes has potential as a treatment for Motor Neuron Disease (MND). We have developed non-viral gene delivery system called immunogenes1 and shown delivery of reporter genes to motor neurons from the circulation in neonatal mice using an antibody to p75NTR (MLR2) conjugated to a PEGylated-polyethylenimine (PEI-PEG12)2.
Objectives: To determine immunogene dosing and length of gene expression in-vivo in neonatal mice.
Methods. MLR2-PEI-PEG12-pVIVO2-GFP was constructed as previously reported2. Neonatal C57BL/6 (B6) mice were injected intraperitoneally with different doses of immunogene (n=6-9 mice) and after euthanising, the percent of spinal motor neurons that expressed GFP and ChAT determined. Expression was also determined 72, 96, 144, 168, and 240-hours after delivery (n=3-9 mice). Delivery of Insulin-like Growth Factor (IGF-1) and Glial Derived Growth Factor (GDNF) to motor neurons were also determined (n=3-5 mice).
Results. 26.2±0.6% (n=9) of motor neurons in neonatal mice (n=7) identified by ChAT also expressed GFP 72h after intraperitoneal injection of MLR2-PEI-PEG12 (150µg) carrying pVIVO2 (116µg). Double the dose increased this to 48.7±1.1% (n=8). Expression persisted in 42.1±2 of the motor neurons for 7-day, declining to 10%±4% at 10-days. GDNF and IGF-1 delivery was shown.
Conclusion. Proof of concept for immunogenes has been demonstrated in neonatal mice, such that near 50% of motor neurons can be transfected from the circulation and transfection persists 7 days. Further work will be needed to determine if this can be translated to effective growth factor therapy for mice with MND.

A TRANSGENIC ZEBRAFISH MODEL OF SPINOCEREBELLAR ATAXIA-3 DEVELOPS SIGNS OF THE DISEASE EVEN WHEN TRANSGENIC EXPRESSION IS RESTRICTED TO MOTOR NEURONS

Ms. Maxinne Watchon[1,2,3], Ms. Kristy Yuan[3], Mr Nick Mackovski[2], Professor Garth Nicholson[2,3], Dr Nicholas Cole[3], **Dr Angela Laird**[3]
[1]Concord Clinical School, Sydney Medical School, University of Sydney, [2]ANZAC Research Institute, Concord Repatriation Hospital, [3]Department of Biomedical Science, Faculty of Medicine and Health Sciences, Macquarie University

The neurodegenerative disease spinocerebellar ataxin-3 (SCA-3), also known as Machado Joseph disease, is the most common form of hereditary spinocerebellar ataxia. It affects neurons of the brain and spinal cord, disrupting muscle control, leading to a loss of co-ordination, imbalance and paralysis. SCA-3 is caused by expansion of a trinucleotide (CAG) repeat region within the ATXN3 gene, encoding a long polyglutamine (polyQ) region within the ataxin-3 protein. We have successfully established the first transgenic zebrafish model of SCA-3, expressing human ataxin-3 protein containing either 19Q (wild-type) or 84Q (SCA-3). Characterisation of the transgenic SCA-3 zebrafish has revealed disease phenotypes similar to those found in human SCA-3 patients, including the presence of ataxin-3 neuropathology, decreased survival and the formation of ataxin-3 protein fragments. Monitoring the swimming behaviour of the transgenic zebrafish revealed that expression of human ataxin-3 84Q in all neurons results in significant motor impairment early in development (6 days post fertilization, dpf), throughout aging and at adult stages (1 year old). Interestingly, expression of ataxin-3 84Q within just motor neurons also results in significant motor impairment. This is the first evidence that expression of ataxin-3 with expanded polyQ only within motor neurons is enough to produce significant motor impairment, suggesting the importance of motor neurons in the disease. The finding of relevant disease phenotypes in our transgenic SCA-3 zebrafish suggests that these zebrafish are a useful model for studying SCA-3 and testing possible disease treatments.

REMOTE PHOTOBIOMODULATION IS NEUROPROTECTIVE AGAINST MPTP INSULT

Dan Johnstone[1,2], Ji Yeon Kim[1,2], Boaz Kim[1,2], Varshika Ganeshan[1,2], Cecile Moro[3], Alim-Louis Benabid[3], John Mitrofanis[1,4], Jonathan Stone[1,2]
[1]Bosch Institute, [2]Discipline of Physiology, [3]Clinatec, [4]Discipline of Anatomy & Histology

Photobiomodulation (PBM) – the irradiation of tissue with low-intensity near-infrared light (600–1,100nm) – exhibits strong neuroprotective properties in rodent models of neurodegenerative disease. However the clinical utility of PBM is limited by a lack of light penetration across the human skull. Attempting to overcome this barrier to translation, we investigated whether PBM targeted at peripheral tissues ("remote PBM") also provides protection of the brain, in animals exposed to the parkinsonian neurotoxin MPTP.
Applying PBM (670nm, 50mW/cm2, 180s/day) to the body of either Balb/c or C57BL/6 mice during MPTP insult (50mg/kg over 24h) mitigated loss of functional dopaminergic neurons in the substantia nigra pars compacta (SNc) by 50% ($p<0.05$). Furthermore, pre-conditioning with remote PBM (90s/day) for 10 days prior to MPTP insult maintained SNc dopaminergic cell

numbers ($p<0.05$) and striatal neuronal activity ($p<0.0001$) at healthy control levels. In a pilot study of MPTP-treated macaques receiving remote PBM to either the lower leg or abdomen (670nm, 50mW/cm2, 180s/day), monkeys treated with remote PBM had fewer clinical signs than untreated monkeys (average score 2-20 vs 31) and more midbrain dopaminergic cells (20-50%). Collectively, these data indicate that remote PBM offers neuroprotection against MPTP insult, substantiating the viability of remote PBM as a treatment modality for overcoming tissue penetration issues associated with transcranial delivery of light therapy. The discovery of neuroprotection by remote PBM provides impetus for a detailed exploration of putative molecular and cellular mediators underlying this phenomenon and an investigation of its potential utility for other neurodegenerative diseases.

Plenary 2 – Eccles Plenary Lecturer – Jeffrey Rosenfeld (Monash University)

Bionic vision and the future of the brain-machine interface.

Neuroscientists are learning to decipher and mimic the complex patterns of electrical activity in the animal and human brain. These signals may be used to drive robotic arms and exoskeletons, provide sensory feedback to the brain or peripheral nerves from the limbs of paralysed individuals and from prosthetic limbs, generate visual percepts for blind individuals, mimic memory input into the rat hippocampus and to detect the early onset of epileptic seizures. The prime use of this technology will initially be used to help the disabled. However, there will be pressure in the future for bionic enhancement of normal cognitive, sensory or motor function using these brain/machine interfaces. This technology will generate ethical challenges in relation to affordability, unfair advantage, military applications, privacy and informed consent.

I will describe bionic vision devices in more detail as this is my own area of research. The sites for electrical stimulation for bionic vision devices are the retina, optic nerve, lateral geniculate body, and the occipital cortex. There are many groups developing retinal prostheses and two commercial devices are currently available (Argus II and Alpha IMS). However, there are only a small number of groups developing a cortical implant. It is known that stimulation of the human visual cortex produces phosphenes. Our electrode carrier is a ceramic tile (8x8x2.5mm) containing a computer chip, wireless coil, and 43x2.5mm microelectrodes. Stimulation parameters have been explored in the rat brain, and bio-compatibility and effects of chronic electrical stimulation in sheep. All the animals have normal vision. An advanced pocket-sized vision processor has been developed using robotic vision transformative reality algorithms. These convert the photographic images from a small digital camera on a glasses frame into pixelated patterns which represent the relevant shapes and contours in the environment and then convert these patterns to electrical impulses transmitted wirelessly across the scalp and skull to the tiles. The aim is that the blind recipient would be able to navigate in the environment, identify objects and possibly read large print. The first human implantation is planned for late 2016. This interdisciplinary project is an excellent example of convergence science.

Tuesday 6th December

Plenary 3 – The ANS Plenary Lecturer

AXONAL FUSION: AN ALTERNATIVE MECHANISM TO REPAIR INJURED AXONS.

Massimo A. Hilliard.

Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

Understanding the molecular mechanisms that regulate axonal regeneration is essential for the development of effective therapies for nerve injuries. Despite substantial knowledge being gained into how axonal re-growth is initiated, our understanding of the mechanisms needed to achieve target reconnection remains very poor. In several species, reconnection of severed axons can occur through a process of axonal fusion, whereby the proximal regrowing fragment recognises and re-establishes membrane and cytoplasmic continuity with its own separated distal fragment, preventing it from undergoing degeneration. This represents a highly efficient way to re-establish connection between an injured neuron and its target tissue. Using the nematode *C. elegans* as a model system, we have characterised the axonal fusion process at the molecular level. We discovered that the recognition between the regrowing axon and its separated axonal fragment proceeds using the same conserved molecular elements previously shown to mediate the recognition of apoptotic cells by neighbouring phagocytes. Furthermore, we demonstrated that the re-establishment of membrane and cytoplasmic continuity between the two axonal fragments is achieved through the regulated expression and localisation within the damaged neuron of fusogens, molecules known to mediate developmentally regulated cell-cell fusion in most eukaryotes. These discoveries pave the way for the development of novel strategies for the treatment of nerve injury

Tuesday 6th December
Symposia 11: The Next Generation of Brain Machine Interfaces

VOLUNTARY MOVEMENT AND SPINAL CIRCUITS

Kazuhiko SEKI
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What is the function of spinal neural circuit for controlling voluntary movements? Motivating hypothesis here is that spinal neural circuits have unique contributions to both descending and ascending regulation of voluntary movement, and do not simply act as peripheral relays. Within the CNS, the spinal cord is well positioned to modulate the activity of effectors and sensory organs. Modulation of somatosensory signals at the first relay could have a profound influence on sensory processing in the brainstem and cortex, and modulation of descending motor commands at the last relay critically affects movement execution. With newly developed techniques, we can now study these important roles in non-human primates. In this lecture, I would like to give a summary of our recent observation on the function of these neural circuits, from gating sensory input, shaping muscle synergies, and application of these observation for BMI that aim to restore finger and arm movements.

DEVELOPMENT OF A HIGH-DIMENSIONAL BRAIN MACHINE INTERFACE

Dr Yan Wong

Over the last few decades neural prosthetics such as the cochlear implants and deep brain stimulators have greatly improved the lives of tens of thousands of patients. In recent years, there has been a push to translate this early success into new therapeutic devices to help those suffering from a broader range of sensory and motor deficits. One such device is the brain machine interface targeted towards upper limb amputees. Current state-of-the-art brain machine interface devices suffer limitations due to an inability to extract enough information from neural signals as well as instability in the neural signals under examination.
We propose that these devices could be improved through the utilization of movement synergies as well as the incorporation of the local field potential into decoding algorithms. We have developed a novel test system that allows recording from multiple depths of the frontal motor cortices simultaneously with all the movements of the arm and hand in non-human primates. In these subjects, we have successfully decoded high-dimensional upper limb reach and grasp movements both offline and online.

STENTRODE: MINIMALLY INVASIVE ENDOVASCULAR STENT-ELECTRODE ARRAY FOR HIGH-FIDELITY, CHRONIC RECORDINGS OF CORTICAL NEURAL ACTIVITY

Nicholas L Opie[1,2], Thomas J Oxley[1,2], Sam E John[1,3], Gil S Rind[1,2], S M Ronayne[1,2], Clive N May[2], David B Grayden[3], Terence J O'Brien[1]
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[2]*Florey Institute of Neuroscience and Mental Health*
[3]*NeuroEngineering Laboratory, Department of Electrical and Electronic Engineering, The University of Melbourne*

High fidelity intercranial electrode arrays for recording and stimulating brain activity have facilitated major advances in the treatment of neurological conditions over the last decade. However, traditional arrays require direct implantation into the brain via craniotomy, a risky procedure with a high chance of serious complication. We have developed the Stentrode, a self-expanding electrode array that can be delivered to the cortex via blood vessels, mitigating risks associated with craniotomy. Through proof-of -concept research, we have demonstrated the capability of the Stentrode to record high-fidelity, movement related neural information from the motor cortex in a cohort of animals for longer than six months. As electrodes are delivered to delivered to the cortical regions of interest through a small (<1.00mm internal diameter) catheter and self-expand to conform to the vessel wall on deployment, cortical vessels remained patent, and no sign of vascular occlusion was observed. Endothelialisation occurred within the first eight days which anchored the electrodes in place and enhanced signal quality, which was comparable to quality achieved with invasive subdural and epidural arrays. This technology has the potential to benefit people with paralysis, enabling direct, safe and high quality control of wheelchairs, exoskeletons and computers. We aim to conduct a first-in-human trial in 2018.

ENCODING AND DECODING OF 3D REACHES FROM NEURAL POPULATIONS IN THE POSTERIOR PARIETAL CORTEX OF MACAQUES

Dr Konstantinos Chatzidimitrakis

Accurate performance of arm movements depends on the integrity of a network of areas located in the parietal cortex, which integrate visual information about the location of objects in space with visual and proprioceptive information about hand position, to guide arm movements. Although every arm movement happens in 3D space, it has become traditional among

researchers to make a distinction between the direction of a movement and its depth. Psychophysical studies suggest that the direction and depth of reaching movements are likely to be specified independently, however there is no physiological evidence of this segregation. Here, we present work that addresses this gap of knowledge and we provide with a comprehensive model of how movement-related areas contribute to different phases of complex 3D movements. Neural activity was recorded from parietal cortex in two Macaca fascicularis monkeys while they fixated and reached towards visual targets located at different distances and directions from the body. We found two major categories of cells: a) neurons that encoded both distance and direction information, and b) cells encoding only one type of spatial information. Information about direction was stable throughout the task phases, whereas information about distance became prominent during the hand-movement. Furthermore, we show that both target location and hand movement related information in 3D space can be decoded from neural populations in parietal cortex. Our work suggests that parietal cortex can supply 3D signals during natural hand movements and thus would be useful for developing a new generation of neural prostheses.

Tuesday 6th December
Symposia 12: Spinal cord injury: from the bench to the bedside

MODULATION OF CONNEXIN43 HEMICHANNELS FOLLOWING MILD SPINAL CORD INJURY

Dr Catherine Gorrie[1], Mr Yilin Mao[1], Mr Ryan Tonkin[2], Ms Tara Nguyen[1], Dr Simon O'Carroll[3], Professor Louise Nicholson[3], Professor Colin Green[3], Dr Gila Moalem-Taylor[2]
[1]University Of Technology Sydney, [2]University of New South Wales, [3]University of Auckland

Connexin43 (Cx43) is one of the main gap junction proteins found in astrocytes in the central nervous system. In the rodent, levels of Cx43 protein increase within hours of spinal cord injury. Peptide5 (P5), a mimetic peptide against the gap junction protein Cx43 has been tested in vitro and in vivo, in a mild spinal cord contusion injury in a rat model. It modulates hemi-channel opening at low concentrations, is effective in reducing tissue damage in spinal cord explants and results in less tissue injury, less cellular disruption and increased locomotor recovery when applied locally to a spinal cord lesion using an intrathecal catheter. P5 has now also been delivered systemically to target the injured spinal tissue with the rats being assessed for up to 6 weeks post spinal cord injury. In the P5 treated rats there were improvements in hindlimb locomotor function and reductions in mechanical allodynia at the level of injury compared to scrambled peptide treated controls. Immunohistochemistry was performed on tissue samples at 8 hours, 1 week, 2 weeks and 6 weeks post injury and showed that P5 treatment reduced lesion size, reduced astrocytic (GFAP) response, and decreased macrophage and microglial (ED1/IBA1) responses while protecting neurons (NeuN). There were no off target effects seen in these animals. These results suggest that acute systemic administration of Peptide5 has a positive effect in ameliorating the effects of spinal cord injury and could readily be applied in a clinical setting.

APPLICATION OF BRAIN-COMPUTER INTERFACE FOR THE RESTORATION OF WALKING AFTER SPINAL CORD INJURY

An Do
University of California

There are currently no proven methods that can restore walking and lower extremity sensation after spinal cord injury (SCI). Brain-computer interfaces (BCIs) may be one novel technology that can be used to "restore" brain-controlled ambulation and lower extremity sensation after SCI. It can be envisioned that such a BCI will record and decode brain motor area signals underlying walking intention, and send commands to a lower extremity prosthesis to actuate walking. Sensors worn on the lower extremities can detect leg movements, which will be used to stimulate the sensory cortex to elicit artificial sensation of the lower extremities while walking. The presentation will review the current technology in BCI-controlled walking, the neurophysiological basis of such a system, and the ongoing technology development and scientific research in the area. It will discuss such BCI systems in context of complementary and alternative approaches that are being investigated to restore walking after SCI. The clinical translation pathway for this technology from bench research to clinical application will also be reviewed. Finally, it will also explore the potential significance and clinical impact that such BCI systems can have on the rehabilitation of SCI, as well as other neurological conditions (e.g. stroke or traumatic brain injury).

INTRAVENOUS IMMUNOGLOBULIN (IVIG) AS AN IMMUNOMODULATORY THERAPY FOR ACUTE SPINAL CORD INJURY

Dr. Faith H. Brennan[1], Dr. Nyoman D. Kurniawan[2], Dr. Adrian Zuercher[3], Dr. Fabian Kaesermann[3], Assoc. Prof. Thiruma V. Arumugam[4], Dr. Milan Basta[5], **Dr. Marc J. Ruitenberg[1,6,7]**
[1]School of Biomedical Sciences, The University of Queensland, [2]Centre for Advanced Imaging, The University of Queensland, [3]Research, CSL Behring, [4]Yong Loo Lin School of Medicine, National University of Singapore, [5]BioVisions Inc., [6]Queensland Brain Institute, The University of Queensland, [7]Trauma, Critical Care and Recovery, Brisbane Diamantina Health Partners

Traumatic spinal cord injury (SCI) elicits immediate neural cell death, axonal damage and disruption of the blood-spinal cord barrier, allowing circulating immune cells and blood proteins into the spinal parenchyma. The acute inflammatory response to SCI involves robust activation of the complement system, which contributes to secondary injury and impairs neurological recovery. This study aimed to determine whether intravenous immunoglobulin (IVIg), an FDA-approved immunomodulatory treatment, can attenuate complement activation products and improve recovery from SCI. We addressed these questions by using functional testing, non-invasive imaging, and detailed post-mortem analysis to assess IVIg's therapeutic efficacy in a mouse model of severe contusive SCI. Our results show that IVIg therapy at clinically relevant doses of 0.5-2 g/kg significantly improves functional and histopathogical outcomes from SCI, conferring protection against lesion enlargement, demyelination, and axonal degeneration. The benefits of IVIg were also detectable through non-invasive diffusion tensor imaging (DTI), with IVIg treatment counteracting the progressive SCI-induced increase in radial diffusivity (RD) in white matter. Diffusion indices significantly correlated with the functional performance of individual mice and accurately predicted the degree of myelin preservation. Further experiments revealed that IVIg therapy reduced complement activation and presence of phagocytically active macrophages at the lesion site, providing insight into its likely mechanisms of action. Our findings highlight the potential of using IVIg as an immunomodulatory treatment for SCI, and the value of DTI to assess tissue damage and screen for the efficacy of candidate intervention strategies in preclinical models of SCI, both quantitatively and non-invasively.

IMMEDIATE COOLING AND EMERGENCY DECOMPRESSION (ICED) FOR ACUTE SPINAL CORD INJURY: FEASIBILITY STUDIES

Professor Sarah Dunlop[1], Dr Camila Battistuzzo[2], Dr Peter Batchelor[2], Professor Brian Freeman[3]
[1]The University Of Western Australia, [2]The University of Melbourne, [3]University of Adelaide

Early decompression may improve outcomes after spinal cord injury (SCI), but is difficult to achieve. Also, clinical trials evaluating acute interventions such as hypothermia are challenging because of the lack of rapid neurological assessment. We determined time to decompression and delays in isolated cervical SCI cases in Australia and New Zealand. Data were extracted from medical records of 192 patients aged 15-70 years with C3-T1 traumatic SCI between 2010 and 2013 from each participating surgical centre (n=8). The median time from accident scene to decompression was 21h, with fastest times associated with closed reduction (6h). A significant decrease in time to decompression occurred from 2010 (31h) to 2013 (19h, $p = 0.008$). Patients undergoing direct surgical hospital admission had a significantly lower time to decompression compared to those undergoing pre-surgical hospital admission (12h vs. 26h, $p < 0.0001$). We also developed and retrospectively evaluated a brief assessment of neurological function (SPEED: Spinal Emergency Evaluation of Deficits) in 118 patients using foot motor and sensory function to evaluate injury severity and C3 dermatome sensation and handgrip strength to indicate injury level. 94% of patients with no foot movement were initially diagnosed as motor complete (American Spinal Injury Association Impairment Scale A/B) and all with foot movement as motor incomplete (AIS C/D). SPEED scores showed good correlation ($r_s=0.79$) and agreement (Kappa=0.85) with full acute hospital neurological assessments. Cervical cases had no or weak handgrip while thoracolumbar cases had strong handgrip. Both studies suggest that a full trial involving hypothermia is warranted.

Tuesday 6th December
Symposia 13: Protectors or destroyers? Decoding the function of microglia in ageing and disease

WHAT IS THE ROLE OF MICROGLIA IN ALZHEIMER'S DISEASE?

Dr Katherine Southam[1], Mr Benjamin Webster[1], Dr Adele Vincent[1], Professor David Small[1]
[1]*University Of Tasmania*

Alzheimer's disease (AD) results in memory deficits and cognitive impairment that has been shown to be due to a loss of synapses rather than an overall loss of neurons. Although a small percentage of AD cases have a clear genetic link, the underlying pathogenesis remains unknown for the majority of those affected. Recent studies indicate that immune dysfunction may be involved in early disease. Microglial involvement in AD may be through a number of different mechanisms. The presence of inflammatory microglia around amyloid plaques raises the possibility that Aβ induces inflammation resulting in widespread neuronal damage. Others suggest that inflammation is symptomatic feature of late disease, occurring in response to widespread cellular damage. A second option is that microglial dysfunction reduces the phagocytic capacity of the cells. Microglial phagocytosis is required for synaptic pruning, an important regulatory process that continues throughout life. Our results show that microglia prune synapse in co-culture with hippocampal neurons, providing a model to study synapse loss in AD. Microglial phagocytosis is considered an important pathway for clearing Aβ from the brain. Reducing Aβ uptake by microglia in mice results in rapid accumulation of Aβ in brain regions that are known to be affected early in AD in humans. Indeed, many of the risk genes that have been identified for AD are involved in reduced microglial phagocytosis. We will discuss current possibilities for microglial involvement in AD and how these questions may be answered using in vitro models.

ROLE OF GLIA IN ULTRASOUND-MEDIATED CLEARANCE OF PROTEIN AGGREGATES

Leinenga G[1], Nisbet R[1], Hatch RJ[1], Bodea L-G[1], **Götz J[1].**
[1]*Clem Jones Centre for Ageing Dementia Research (CJCADR), Queensland Brain Institute (QBI), The University of Queensland, Brisbane (St Lucia Campus), Australia*

Treatment strategies for neurological diseases such as Alzheimer's disease and frontotemporal dementia are hampered by the fact that the blood-brain barrier (BBB) establishes an efficient barrier for therapeutic agents. We have recently established that scanning ultrasound (SUS) is an efficient method to remove amyloid-beta from brains of transgenic APP mutant APP23 mice and to restore memory functions to wild-type levels (Leinenga and Götz, Science Transl Med 2015; Leinenga et al., Nat Rev Neurol 2016). This was achieved in the absence of overt damage. Importantly, we found a critical role for microglia in mediating the therapeutic effect of SUS. We since extended SUS (i) to transgenic mice with a prominent tau pathology characteristic of Alzheimer's disease and some forms of frontotemporal dementia to assess efficacy, and (ii) to wild-type mice to assess safety. Together, our findings suggest that SUS is a safe method that benefits diseases with protein aggregates more generally, whether the pathology is intra- or extracellular.

ROLE OF MICROGLIA IN ALZHEIMER'S DISEASE PATHOGENESIS

Professor Wolfgang "Jake" Streit

Although etiology and pathogenesis of late-onset Alzheimer's disease (LOAD) remain unknown, it is now increasingly recognized that, in addition to plaques and tangles, microglial dysfunction plays a critical role in disease development. Conventional thinking has portrayed microglia as out-of-control, hyperfunctional immune effector cells that upon activation by amyloid-beta protein (Aβ) produce neurotoxic inflammatory mediators that cause subsequent neurodegeneration. This has provided a link between Aβ deposition and neurofibrillary degeneration (NFD) in support of the amyloid cascade hypothesis. However, numerous findings argue against this dominant theory, such as for example the failure of non-steroidal anti-inflammatory drugs to delay disease progression, the finding that NFD occurs early in life before Aβ deposition even begins, as well as the fact that Aβ overexpression in transgenic mouse models fails to produce NFD. These facts support the idea of a non-causal relationship between Aβ deposits and NFD, i.e. that Aβ is merely an accompaniment of the disease process, and thus a form of microglial malfunction different from neurotoxic activation. Our work in the human AD brain has shown that regions showing extensive NFD also reveal senescent microglial degeneration, evident as structural abnormalities termed dystrophy, suggesting a link between functional failure of microglia and neurodegeneration. We hypothesize that microglial senescence occurs primarily due to an aging-related increase in oxidative stress rendering the cells less capable of providing neuroprotection and thus allowing NFD to occur and progress over time. We believe that slowly progressive failure of the brain's immune system is what causes LOAD.

BUILDERS OR DESTROYERS: THE ROLE OF MICROGLIA IN STROKE-INDUCED SECONDARY NEURODEGENERATION

Associate Professor Frederick Walker[1]
[1]*University Of Newcastle, [2]Hunter Medical Research Institute, [3]School of Biomedical Science and Pharmacy, [4]Priority Research Centre in Stroke and Traumatic Brain Injury*

Microgliosis was long considered to be problematic in the context of brain repair following stroke, more recent evidence, however, has suggested a far more neuroprotective role. With these shifting concepts as background, our research team has been interested in exploring the contribution of microglia to a process linked to stroke, known as secondary neurodegeneration (SND). This process involves the progressive death of brain regions connected to the original infarcted territories. Using a controllable photothrombotic model of stroke, we have identified that SND-linked neuroinflammatory disturbances spread out from the injury site, as indicated by; enhanced pro-inflammatory signalling, neuronal loss, and enhanced deposition of higher molecular weight oligomers of Amyloid beta. Most recently we have utilised in-vivo slice based imaging to identify that microglial functionality at sites of SND is severely disrupted. We have now established that when microglial mediated repair responses are suppressed (by psychological stress) the severity of tissue loss at sites of SND is greatly exacerbated. Together these results suggest that microglial responses at sites of SND are likely to be neuroprotective and if suppressed lead to adverse repair outcomes.

Tuesday 6th December
Symposia 14: Ion channels in pain and epilepsy-genetics, function and toxins

INSIGHTS INTO CELLULAR MECHANISMS IN GENETIC EPILEPSY AND IMPLICATIONS FOR TREATMENT

A/Prof Christopher Reid[1]
[1]*Florey Institute*

Epilepsy is a devastating disease with up to 30% of patients not adequately treated with current anti-epileptic drugs. Historically, we have chosen therapy based on the syndrome suffered by a patient. A rapid rise in our knowledge of the genetics causes of epilepsy gives hope that we can deliver precision medicine in this devastating disease. In this symposium I raise the question- "do you treat the syndrome or do you treat the mutation?" I will discuss two models of Dravet syndrome caused by different genes. Insight from rodent models based on the two genes suggests two very distinct cellular mechanisms underlie excitability. This suggests that different therapeutic strategies may be required to adequately treat patients. I will discuss these including ideas about how to directly target the channel deficit caused by a genetic lesion.

MOLECULAR GENETICS OF MIGRAINE

Professor Lyn R. Griffiths[1]
[1]*Qut, Institute Of Health And Biomedical Innovation*

Migraine is a severe neurological disorder that affects ~ 12% of the population. The disorder has a significant genetic component showing high levels of familial aggregation. A number of genes involved in a rare and severe sub-type of migraine, termed familial hemiplegic migraine (FHM) have been identified. Mutations in the CACNA1A, ATP1A2 and SCN1A genes cause the three types of Familial Hemiplegic Migraine, FHM1, FHM2 and FHM3, respectively. The three FHM types share similar symptoms including hemiparesis and severe migraine with aura, according to criteria specified by the International Headache Society. FHM is difficult to diagnose and has significant symptomatic overlap with several other disorders such as the stroke-like disorder CADASIL, Episodic Ataxia and Spinocerebellar Ataxia. At least one of the three genes that cause FHM can also cause epilepsy. The SCN1A gene harbours mutations that cause FHM3 as well as Dravet Syndrome, a common childhood epilepsy. For the more common forms of migraine, neurotransmitter pathways have been the main focus of studies investigating the molecular mechanisms of the disorder but vascular and hormonal disturbances also occur in migraineurs, as highlighted by alterations in cerebral blood flow and hormonal triggers of migraine. This presentation will focus on migraine gene studies and the translational outcomes of this research, including the development and use of a next generation sequencing diagnostic to identify severe migraine and related cerebrovascular, ataxia and epilepsy disorders, as well as the results of clinical trials that have shown promising results for a gene targeted nutraceutical therapeutic for migraine.

MODULATION OF NEURONAL CALCIUM CHANNELS VIA GABA(B) RECEPTOR ACTIVATION BY ANALGESIC CONOTOXINS

Professor David J. Adams[1]
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Marine cone snails have developed a distinctive repertoire of small, disulphide-bonded peptides (conotoxins) as part of highly evolved venoms used for prey capture and defence. These peptides target a wide range of voltage- and ligand-gated ion channels, transporters and receptors with exquisite selectivity making them an invaluable source of ligands for studying the role and properties of these targets in normal and diseased states. A number of these peptides have shown efficacy in vivo as inhibitors of voltage-gated neuronal calcium (Cav) channels and are in preclinical development for the treatment of chronic and neuropathic pain. In this context, I will discuss the discovery and development of a class of analgesic conotoxins that modulate Cav channels in sensory neurons via a G protein-coupled receptor mechanism. Our findings identify GABAB receptor-mediated inhibition of Cav2.2 and Cav2.3 as targets in pain pathways for these and novel 'designer' conotoxins which exhibit oral activity in rat models of neuropathic pain. The involvement of intracellular signalling pathways was investigated using specific inhibitors of GABAB receptor-G protein coupling. Inhibition of Cav2.3 occurs via voltage-independent G protein signalling mechanisms which is in marked contrast with the primarily voltage-dependent modulation of Cav2.2 channels. These results suggest GABAB receptors control specific members of the Cav2 channel family via differential signalling pathways.

DEVELOPING NEW EXPERIMENTAL MODELS BASED ON GENETIC DISCOVERIES IN THE EPILEPSIES

Holger Lerche
Centre for Integrative Neuroscience, Germany

Tuesday 6th December
Symposia 15: Development of the Enteric Nervous System: Past, Present & Future

MASSIVE DIFFERENTIAL CLONAL EXPANSION IN ENTERIC NEURAL PROGENITOR CELLS: LUCK OR WHAT?

Donald Newgreen[1], Dongcheng Zhang[1], Bevan Cheeseman[2,4], Benjamin Binder[3], Kerry Landman[2]
1. Murdoch Childrens Research Institute, Parkville, 2. Department of Mathematics and Statistics, University of Melbourne, 3. School of Mathematical Sciences, University of Adelaide. 4. Center of Systems Biology, Dresden, Germany.

The enteric nervous system (ENS) cell population increases dramatically during development, which raises the question of clonal expansion of ENS progenitor cells. We have constructed cellular automaton (CA) models of ENS progenitor cell-agent migration encoding stochastic ENS agent movement and stochastically placed but logistically limited ENS agent proliferation. From this emerged *in silico* a stereotyped pattern of ENS agent proliferation and field (i.e. gut) occupation at the agent-population level as in normal development. This also revealed extraordinary variability in numbers of daughter-agents (spanning 4 orders of magnitude) and in their geographical distributions, which was dominated by a few giant or "superstar" clones, with clonal heterogeneity decreasing with distance along the field (i.e. representing the ENS of the distal colon). This occurred without any clonal advantage built into the model. We then performed biological co-grafting experiments with one labelled ENS progenitor cell plus large (thousands of cells) and smaller (hundred cells) populations of unlabelled but otherwise comparable precursor cells set up to colonise aneural gut: this showed identical outcomes to the model. Thus in simulations and in experiments, a few "superstar" ENS progenitor cells in a colonising population can show massively disproportionate quantitative clonal contributions to the final population without any innate proliferative or migratory advantage. We suggest that ENS formation involves great inequalities in clonal contributions, and stochastic events may be under-appreciated as contributors to this. This in turn has implications for the prevalence and importance of somatic mutations in such a system, especially at the distal colon.

THE IMPACT OF RET GAIN-OF-FUNCTION MUTATION ON DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

Hideki Enomoto

RET receptor tyrosine kinase mediates signaling of the GDNF family ligands and regulates development of the enteric nervous system (ENS) by orchestrating cell migration, proliferation, survival and differentiation of ENS precursors. Various types of mutation of the RET gene have been identified in patients of Hirschsprung disease (HSCR) and neuroendocrine tumors. To date, the pathogenetic mechanisms underlying HSCR have been accounted for almost exclusively by loss-of-function effects of the given RET mutants. However, it has remained unexplored whether gain-of-function RET mutants do not have any adverse effects on ENS development. To address this issue, we generated mice engineered to express cDNA of RET51C618F, a mutation identified in neuroendocrine tumor patients, under the Ret promoter. Histochemical analysis of the ENS revealed an increase in the density of the ENS meshwork in both RetRET51C618F/+ and RetRET51C618F/RET51C618F mice, which is consistent with a gain-of-function effect of RET51C618F. Surprisingly, RetRET51C618F/- mice displayed severe intestinal aganglionosis, which stood in sharp contrast to RetRET51/- mice (control) that displayed no ENS deficit. In RetRET51C618F/- embryos, migration of ENS precursors was delayed, which was associated with abnormal downregulation of Sox10 in ENS precursors at the migratory wavefront, revealing that premature neuronal differentiation causes impaired migration in these mutant embryos. The data collectively suggest that proliferation and differentiation of ENS precursors are exquisitely regulated by both the activation levels and total amount of RET. Decreased amount of RET with its high activity confers susceptibility to intestinal aganglionosis, which provides novel insight into the pathogenesis of HSCR.

ENTERIC NEURONAL CONTROL OF ENTERIC NEUROGENESIS AND INTESTINAL MUCOSAL GROWTH

Michael D. Gershon
Columbia University, College of Physicians and Surgeons

The enteric nervous system (ENS) is the largest and most complex division of the peripheral nervous system and is uniquely able to function without input from the CNS. Enteric neurons are born (undergo terminal mitosis) in a reproducible order in which early-born mature neurons coexist with and innervate still-dividing precursors. Serotonergic and cholinergic neurons are born first, while neurons that contain tyrosine hydroxylase (TH), γ-aminobutyric acid (GABA), or calcitonin gene related peptide (CGRP) are born much later. This observation led us to test the hypothesis that the activity of early-born neurons can, through their neurotransmitters, 5-HT and/or acetylcholine (ACh), affect the development of later-born neurons. Supporting of this idea, we showed that 5-HT, through 5-HT_4 receptors, promotes development of TH-, GABA-, and CGRP-containing neurons, that these phenotypes are deficient, and the ENS is hypoplastic when tryptophan hydroxylase 2 (TPH2) is deleted and mice thus lack neuronal 5-HT. The late-born neurons are also deficient and the ENS is hypoplastic in animals that carry an autism-associated human variant of the serotonin transporter (SERT; SERT Ala56), which is hyperfunctional and clears 5-HT from its receptors too rapidly. In contrast, mice that lack SERT (SERTKO) or which are exposed during development to a SERT inhibitor have a hyperplastic ENS and excessive numbers of late-born neurons. Recent data, obtained with mice that under- or overexpress the presynaptic choline transporter, suggest that ACh functions like 5-HT, with excessive ACh signaling leading to hyperplasia and inadequate signaling to hypoplasia. Because serotonergic and cholinergic neurons are thus essential for ENS development, defects in their signaling during ontogeny lead, not only to ENS hypo-or hyperplasia, but also to dysmotilities and abnormally

regulated mucosal growth that are readily demonstrated in animals that mature with these defects. We thus postulate that abnormalities that arise due to errant serotonergic or cholinergic signaling in ontogeny, possibly due to environmental perturbations, may contribute to functional gastrointestinal disease in adults. Although TPH2-derived 5-HT is more important than that from TPH1 in ENS formation under basal conditions, TPH1-derived 5-HT from mucosal enterochromaffin (EC) cells might disturb ENS neurogenesis and/or function if it reaches the neuronal compartment. Studies in mice in which SERT expression is selectively ablated in the mucosa with a villin-Cre driver demonstrate that mucosal SERT activity is essential to prevent 5-HT from overflowing from the mucosa to disturb neurogenesis and/or neuronal function. Insults that up- or downregulate SERT will thus cause abnormal ENS formation and that persists as adult dysfunction.

ROLE OF KIF1 BINDING PROTEIN AND INTRACELLULAR SIGNALLING PATHWAYS IN THE DEVELOPMENT OF GUT INNERVATION

Dr Sonja McKeown[1], Dr Caroline Hirst[1], Annette Bergner[1], Huynh Nguyen, Dr Lincon Stamp[1], **Professor Heather Young[1]**
[1]*University Of Melbourne*

Enteric neural crest-derived cells (ENCCs) migrate along the developing gut and give rise to the enteric nervous system. We examined the roles of Kif1 binding protein (KBP) and intracellular signalling pathways in the development of the gut innervation. Patients with Goldberg Shprintzen megacolon syndrome have microcephaly and Hirschsprung disease, which is an absence of neurons in the distal bowel. We examined the effects of loss of KBP in mice using CRISPR/Cas9 technology. There was a significant delay in the migration of ENCCs along the gut of E12.5 Kbp null mutant mice, although the entire gut was colonized by ENCCs. We examined if there were interactions between RET and KBP. The number of vagal nerve fibres in the stomach of Ret-/-;Kbp-/- mutants was reduced compared to Ret-/- single mutants. In addition to defects in gut innervation, Kbp-/- mice also had smaller olfactory bulbs and died shortly after birth.

We have previously shown that some ENCCs migrate with high caudal directionality while others migrate non-directionally. To identify the intracellular signalling pathways involved in directional ENCC migration, we examined the effects of inhibition of JNK and MEK and activation of PKA on the speed and directionality of ENCCs. Inhibition of JNK (using SP600125, 30 μM) significantly decreased ENCC speed without affecting directionality. Inhibition of MEK (PD184352, 10 μM) had no significant effect on ENCC speed or directionality. Activation of PKA (SP-8-Br-cAMP, 120 μM) increased ENCC directionality without affecting speed.

We conclude that KBP, JNK and PKA are required for development of gut innervation.

Wednesday 7th December
Symposia 16: Neurophysiology and neuropathology at the nanoscale

TRAFFIC AND SURFACE DYNAMICS OF VOLTAGE GATED CALCIUM CHANNELS

Jennifer Heck[1], Romy Schneider[1], Ulrich Thomas[2], Arthur Bikbaev[1], Anna Ciuraszkiewicz[1], Andreas Voigt[3], **Martin Heine[1]**
[1]Molecular Physiology group; [2]Depeartment of Neurochemistry, Leibniz Institute for Neurobiology, Magdeburg, Germany; [3]Otto-von-Guericke-University Magdeburg, Systemverfahrenstechnik, Universitätsplatz 2, D-39106 Magdeburg, Germany

Neuronal activity and plasticity is based on the function of chemical synapses. The release of neurotransmitters is highly variable between synapses and depends on the molecular architecture of the active zone. Here, the spatial relation between ready releasable vesicles and voltage gated calcium channels (VGCC) is very critical. In order to monitor the presynaptic organisation of VGCC we used single particle tracking photo activation localisation microscopy (sptPALM). We identified that about 60% of VGCC in the synapse are mobile. Further analysis of the VGCC dynamics in the presynaptic membrane revealed a transient confinement (100-200 ms) in membrane areas of ~80 nm in diameter. Using different C-terminal splice variants with altered affinity to presynaptic scaffold proteins as RIM and RIM binding protein (RBP) did not change the confinement of VGCC. Increasing the number of VGCC in the presynaptic membrane by coexpression of α2δ1-subunits did alter the size and number of synapses as well as the release probability, but did not change the dynamics of VGCC. In order to probe the functional impact of mobile VGCC in synaptic transmission we used light induced clustering of VGCC. This manipulation drastically changed the local organisation of VGCC and altered synaptic release probability. From these experiments we conclude that VGCC are at least partially mobile in the active zone of the presynaptic membrane, participate in the release of synaptic vesicles and give the possibility to tune on a millisecond time scale the release probability of synapses. We hypothesis that mobile VGCC are used to tune the release probability dependent on the activation history of the synapse.

PRION-LIKE POLYMERIZATION OF MUNC18-1 AND INTERACTIONS WITH SYNUCLEIN

Dr Yann Gambin[1], Dr Emma SIERECKI[1], Miss Ye Jin Chai[2], Prof Frederic Meunier[2]
[1]University Of New South Wales, [2]University of Queensland

In this study we have used independent experimental approaches to demonstrate that Munc18-1 missense mutations leading to Early Infantile Epileptic Encephalopathy create a gain-of-function with a dramatic cascade of cellular effects. Our results point to a prion-like property of Munc18-1 mutants, highlighting their ability to nucleate aggregation of the wild-type Munc18-1 protein. The discovery of Lewy body-like structure positive of Munc18-1 led us to investigate a potential link between Munc18-1 and Synuclein, a hallmark of Lewy bodies in Parkinson's Disease. We identified a novel interaction between native Munc18-1 and Synuclein, and showed that aggregates formed by Munc18-1 mutants also incorporate Synuclein. We further reveal that Parkinson-disease mutations in Synuclein that cause Lewy body formation also induces Munc18-1 aggregation, suggesting an unexplored underlying overlap between the different neurological diseases. To the best of our knowledge, our study is the first to indicate a functional link between Munc18-1 and Synuclein. Finally, we demonstrate that Munc18-1 controls Synuclein's propensity to aggregate. The aggregation of Synuclein has previously shown to be regulated by chaperone proteins such as Hsp70, and our results point to a critical role for endogenous Munc18-1 in controlling the formation of toxic Synuclein oligomers. Considering that Synuclein was recently shown to be a prion, targeting the Munc18-1 Synuclein binding site may have therapeutic values for a number of neurodegenerative diseases including PD, EIEE, and multiple system atrophy (MSA).

SYNAPTIC TRAFFICKING OF GABAA RECEPTORS WITH EPILEPSY-CAUSING MUTATIONS VISUALIZED WITH SUPER-RESOLUTION MICROSCOPY

Dr. Nela Durisic[1], Professor Joe Lynch[1]
[1]Queensland Brain Institute

GABAA receptors are ligand-gated ion channels that mediate both phasic synaptic and tonic perisynaptic inhibition. Several different idiopathic epilepsy syndromes have been associated with mutations in GABAa receptor subunit genes including GABRA1 and GABRG2 but the mechanisms of epileptogenesis caused by these mutations are still unknown.
To determine the impact of GABRA1 and GABRG2 mutations on GABAa receptor function we formed synapses between neurons and HEK 293 cells and measured synaptic currents produced by these receptors. We then mapped the distribution of single GABAaRs with mutant subunits in neurons using localisation-based super-resolution techniques.
All tested GABAaRs containing the mutant α1 and/or γ2L subunits impaired receptor function and changed synaptic current properties. In neurons GABAaRs containing wild-type α1 and γ2L subunits showed confined movement at the synapse, rapid diffusion in extrasynaptic regions and dynamic exchange of the receptors between these two regions. In contrast, GABAaRs with epilepsy-causing mutations were found immobilised at synaptic sites. Partial retention in endoplasmic reticulum reduced surface expression of the receptors. This in turn lowered the number of GABAaRs found at the synapse and depleted the extrasynaptic population of the receptors. In addition, perturbed ER homeostasis by mutant subunits increased cell death.

NANOSCALE ODYSSEY INTO THE PRESYNAPSE

Adekunle T. Bademosi[1], Ravi Kasula[1], Merja Joensuu[1], Andreas Papadopulos[1], Elsa Lauwers[2], Pranesh Padmanabhan[3], Andreas Papadopulos[1], Geoffrey J. Goodhill[3,4], Patrik Verstreken[2], Bruno van Swinderen[3] and **Frédéric A. Meunier[1]**

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With the advent of super-resolution microscopy, the door is now opened to dissect the mechanism of neurotransmitter release using nanoscale imaging techniques. We demonstrate in neurosecretory cells that Munc18-1, a key presynaptic protein, controls the engagement of Syntaxin1A into the SNARE complex via the opening of a critical hinge loop in domain 3A (1). We then turn to the drosophila synapse to demonstrate that syntaxin1A forms nanoclusters which are dynamically regulated during neurotransmitter release by poly-phosphoinositides and NSF-dependent SNARE disassembly (2). Finally, we venture into the hippocampal presynapse to establish the first technique capable of imaging single synaptic vesicles in the crowded environment of the hippocampal synapse. Our results reveal that synaptic vesicles dynamically oscillate between several diffusion states with distinct rates of transition between these states (3).

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Wednesday 7th December
Oral Session 11: Sensory Systems

PREDICTIVE CODING AND THE 'SILENT' SURROUND OF DRAGONFLY FEATURE DETECTING NEURONS

Mr Joseph Fabian[1], Dr Steven Wiederman[1], Prof David O'Carroll[2]
[1]The University Of Adelaide, [2]Lund University

Biological visual systems excel at encoding the movement of small targets. This is highly evident in dragonflies, formidable predatory insects that perform acrobatic pursuits of small prey or conspecifics with success rates >90%. However, detecting small targets is inherently challenging. At any instant a small target stimulates a limited number of photoreceptors, potentially resulting in low signal-to-noise. Given this constraint, how does the dragonfly brain extract reliable target information?
Here we present intracellular responses from Small-Field Small Target Motion Detecting (SF-STMD) neurons within the dragonfly optic lobe. These neurons have small classical receptive fields (5-10°), and show exquisite size tuning with optimal responses for features spanning ~1°. We show that presenting a drifting target within the receptive field elicits moderate responses. However, when the same stimulus was preceded by a 'priming' target moved along a continuous trajectory outside and towards the classical receptive field, responses were facilitated by 41% ($p < 0.01$, n=15). Interestingly, if we reverse the direction of the primer such that it moves away from the classical receptive field, responses to targets within the receptive field are not facilitated.
These results suggest that the classical receptive fields of SF-STMD neurons have 'silent' surrounds. These silent surrounds allow SF-STMD's to gauge the context of a stimulus, enhancing responses to a stimulus predicted by a trajectory in the surround. We propose that across a population of SF-STMD neurons, selective facilitation by the surround boosts the signal-to-noise ratio for targets that move on realistic target trajectories.

PRIMARY VISUAL CORTEX IS HOME TO AN INTRINSIC NEURAL "EYE TRACKER"

Dr. Adam Morris[1], Prof. Bart Krekelberg[2]
[1]Monash University, [2]Rutgers University

Neurons in primary visual cortex (V1) respond to light within a restricted region of the retina – their receptive fields. An object in a visual scene therefore projects to different positions in the V1 map every time the eyes move. The cortical image thus depends more on gaze direction – which is almost constantly changing – than it does the positions of objects in the visual scene! How does stable vision emerge from these fickle, viewpoint-dependent cortical representations? Here we show that V1 has an embedded non-visual "pointer" indicating the momentary direction of gaze, providing the crucial link between each fleeting image and its true position in the world. We recorded extracellular activity of macaque V1 neurons during a task requiring fixations, fast eye movements, and slow tracking movements. Many neurons showed different levels of activity depending on the direction of gaze during fixation. We trained a decoder to translate patterns of activity across a population of such neurons into metric estimates of the current (stationary) gaze direction. Remarkably, this decoder not only accurately reported the direction of gaze during fixation (for which it was trained), but also during fast and slow eye movements and with low latency. The results show that V1 neurons provide a reliable representation of eye position that can be read-out by downstream structures using a fixed pooling rule regardless of oculomotor state. This robust cortical "eye tracker" could provide the crucial link between the ever-changing positions of objects on the retina and their stable positions in the world.

CHARACTERIZATION OF GANGLION CELL POPULATIONS IN MARMOSET RETINA

Miss Rania Masri[1,2], Dr. Kumiko Percival[1], Dr. Amane Koizumi[3], Prof. Paul Martin[1,2], Assoc. Prof. Ulrike Grünert[1,2]
[1]Department of Ophthalmology and Save Sight Institute, The University of Sydney, [2]Australian Research Council Centre of Excellence for Integrative Brain Function, The University of Sydney, [3]National Institutes of Natural Sciences

Three well described types of ganglion cells in primate retina form the bulk of the ganglion cell population; they are midget, parasol, and small bistratified cells. In addition, there is a variety of low density wide-field ganglion cell types, but the exact numbers and proportions of these cells are not known. Here, we characterized retinal ganglion cells in the marmoset using particle-mediated gene-transfection. Quadrants of retinal tissue were transfected using expression plasmid for the postsynaptic density 95-green fluorescent protein. The retinas were cultured for 3 days, fixed, then processed with bipolar and amacrine cell markers in order to determine ganglion cell dendritic stratification. Ganglion cells were classified based on dendritic field size, morphology and stratification in the inner plexiform layer. In total over 500 ganglion cells were classified into at least 17 types, including midget, parasol, broad thorny, small bistratified, large bistratified, recursive bistratified, recursive monostratified, narrow thorny, smooth monostratified, large sparse, giant sparse ganglion cells, and a group that may contain several as-yet uncharacterized types. Assuming each characterized type forms a hexagonal mosaic, midget and parasol cells account for over 80% of all ganglion cells in central retina but only ~50% of cells in peripheral (>2 mm) retina. Thus, in primate, the fovea is dominated by midget and parasol cells, but outside the fovea the ganglion cell diversity is likely as great as that reported for non-primate retinas.

FROM FEATURE PERCEPTION TO SPATIAL ATTENTION IN THE MACAQUE LATERAL INTRAPARIETAL CORTEX (LIP)

Dr. Ekaterina Levichkina[1,3], Mr. Mojtaba Kermani[1], Dr. Yuri Saalmann[1,2], **Professor Trichur Vidyasagar[1]**
[1]Department of Optometry & Vision Sciences, University Of Melbourne, [2]Department of Psychology, University of Wisconsin, [3]Institute for Information Transmission Problems, Russian Academy of Sciences

INTRODUCTION: We previously reported the existence of two cell-groups in LIP, cells with significant (Wilcoxon, p>0.05) attentional enhancement and no orientation selectivity (AE+ cells) and orientation selective cells with no modulation by attention (AE- cells). These cells might represent 2 stages of a Guided Search model of attention (Wolf, 1994), AE- cells constructing a saliency map and AE+ cells providing top-down attentional feedback.
METHODS: We tested this in two macaques trained on a delayed-match-to-sample task by analysing neural network activity during the delay.
RESULTS: AE– cells maintained moderately elevated response for the whole delay period, consistent with a role in feature memory. However, AE+ cells showed markedly increasing activity towards second stimulus, consistent with a role in focal attention. We also studied spike-field coherence of LIP cells in the delay period and found that coherence in the beta to low gamma range is significantly higher in the late delay period (Wilcoxon, $p < 0.05$) for the AE+ group than for AE– group. We then determined frequency bands related to attention and feature discrimination (Bokil et al., 2007) and studied the LFP coherence between LIP and MT areas in these frequency bands. We found that after the first stimulus is presented feature-related coherence rises for 300-400ms and then drops, while attention-related coherence rises in the late delay period (after 500 ms).
CONCLUSION: Our results suggest that the AE- cells extract and retain feature information which is used by a second stage composed of AE+ cells for top-down modulation of spatial attention.

DOMINANT-NEGATIVE R373C PROMININ-1 IS A POST-TRANSLATIONAL DISEASE OF THE PHOTORECEPTOR OUTER SEGMENT

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Retinal photoreceptors (rods and cones) are highly specialised neurons containing an expanded ciliary membrane (outer segment) consisting of membranous disks that are required for vision. Mutations in proteins operating in the photoreceptor outer segment can lead to debilitating vision disorders such as retinitis pigmentosa and macular degeneration. In many cases, autosomal recessive mutations of core outer segment genes, lead to a defective protein that precipitates neurodegeneration. In the case of prominin-1, an integral membrane protein required for photoreceptor survival, an autosomal dominant mutation (R373C) in patients leads to severe photoreceptor specific degeneration.

Prominin-1 is concentrated in the nascent plasma membrane protrusions of rods, and on the edge of the cone outer segment plasma membrane. It was believed that R373C prominin-1 is mislocalised in all cells and in the photoreceptors throughout the entire outer segment and potentially trapped in the endoplasmic reticulum. Upon closer examination of R373C we have found that R373C can be transported correctly to the base of the outer segment *in vivo* and to plasma membrane protrusions of other cells *in vitro*. We have found that R373C aggregates membranes, which we hypothesise is the cause of the photoreceptor specific degeneration. Furthermore, in transfected cells we found that the turnover of R373C containing membranes into the medium as exosomes and ectosomes was significantly impaired, with the majority being retained on the cell surface. In this study we have identified a novel post-translational cause for the mutant R373C phenotype.

MACHINE LEARNING OF DORSAL COLUMN NUCLEI SURFACE POTENTIALS TO PREDICT TACTILE AND PROPRIOCEPTIVE PERIPHERAL STIMULI

Alastair Loutit[1], **Dr Jason Potas[1,2]**
[1]The Australian National University, [2]The University of New South Wales

Despite current achievements, motor prostheses currently lack natural sensory feedback which limits their usefulness. We propose the dorsal column nuclei (DCN) as a potential neuroprosthetic target for the restoration of somatosensation and proprioception. We used machine learning to demonstrate that DCN surface potentials provide reproducible information associated with joint position and tactile discrimination. Urethane anaesthetised 8 week-old male Wistar rats were prepared for 7-electrode array surface recordings. All four limbs were 1) moved into flexion and extension, and 2) stimulated on the palm with a dowel and brush (20 g force) in 10 repeated trials of each stimulus at ~2.4 second intervals. Each limb was presented with10 cycles of the 10 repeated trials over the course of the experiment. DCN surface potential recordings acquired from all 7 electrodes, were divided into 2.4 second trials and subjected to feature extraction. Input/output data sets, obtained following feature extraction, were randomly allocated into training (70%), validation (15%) and testing (15%) data sets to determine the mean accuracy of 10 learning/testing cycles of a supervised back-propagation neural network algorithm. The neural network was able to classify (greater than chance, 12.5%): 1) the correct limb and movement (flexion or extension) with 85 ± 4.8% accuracy, and 2) the correct limb and tactile stimulation (dowel or brush) with 95 ± 2.2% accuracy. These findings demonstrate

that machine learning can accurately classify movements of limbs as well as identify distinct tactile stimuli by generalising an array of DCN surface potential signal features.

GLUN2B-CONTAINING NMDA RECEPTORS IN INTERNEURONS MODULATE AUDITORY-EVOKED HIGH FREQUENCY NEURAL OSCILLATIONS IN ADULTHOOD

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NMDA receptors containing the GluN2B subunit have been considered to play their most important roles in early life, and outside the synapse. However, recent evidence suggests that they may play a greater role during adulthood at the synapse than previously thought. Here, we investigated the effect of pharmacological blockade of GluN2B-containing NMDA receptors (using GluN2B-selective antagonist Ro25-6981) on neural circuit activity in adult mice. To do this, we measured Ro25-6981-induced changes in baseline and auditory-evoked high frequency (gamma) oscillations using electroencephalography (EEG). Furthermore, to test whether any effects of GluN2B blockade were due to effects on pyramidal neurons or interneurons specifically, we compared effects of Ro25-6981 in wildtype mice and mice with conditional knockout of GluN1 from pyramidal neurons ($GRIN1^{CAMKII\text{-}KO}$). Baseline gamma power was increased in homozygous $GRIN1^{CAMKII\text{-}KO}$ mice (n=20) compared to wildtypes (n=20) (main effect genotype; $p<0.01$). GluN2B blockade further increased baseline gamma power, but only in $GRIN1^{CAMKII\text{-}KO}$ mice (Ro25-6981 x genotype interaction; $p<0.00005$). In contrast, there was no effect of $GRIN1^{CAMKII\text{-}KO}$ genotype on auditory evoked gamma power. GluN2B blockade decreased evoked gamma power in both genotypes (main effect Ro25-6981; $p<1x10\text{-}5$). These findings suggest that GluN2B-containing NMDA receptors play an important role in modulating neural circuit activity in adulthood. In particular, GluN2B-containing NMDA receptors in interneurons, but not NMDA receptors on pyramidal neurons, are critical for generating evoked neural responses to auditory stimuli.

NEURONS IN MT ARE LESS LIKELY TO BE DIRECTION SELECTIVE TO RANDOM DOT STIMULI AFTER CHRONIC V1 LESION IN ADULT MARMOSET

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Damage to the primary visual cortex (V1) results in a scotoma in the corresponding parts of the visual field. However, patients retain some unconscious visual faculties, or "blindsight", within scotoma. The motion-sensitive, Middle Temporal Area (MT) is a candidate to mediate blindsight. However, no data exist on long-term effects of V1 lesions in MT. This is important, as visual faculties in humans take ~6 months to stabilize.

We recorded from 69 MT cells in 3 anesthetized, adult marmosets, 7-11 months after unilateral V1 lesions. The boundary of each scotoma was determined by recording from V1 cells around the lesion border. MT cells were classified by whether they responded to a visual stimulus placed inside (15%), along boundary of (59%), or outside (26%) the scotoma. All categories had fewer direction selective cells (ANOVA $p<0.05$) than controls (80%, 40 cells): cells with receptive fields (RFs) inside (20%; $p = 7.37x10\text{-}5$, Binomial distribution), along the boundary (49%; $p=6.5x10\text{-}6$), and outside the scotoma (50%; $p=3.3x10\text{-}3$). Consistent with visuotopic reorganisation of MT after V1 lesion, cells with RFs along the border and even outside the scotoma included units from inside the lesion projection zone, which contributed to the lower number of direction selective cells.

Thus, MT exhibits dramatic changes after long-standing V1 lesions. While the overall prevalence of direction selectivity decreased, a small number of direction selective cells persist, including RFs inside the scotoma. These cells are likely to underlie blindsight, and may be recruited through training to recover visual faculties within the scotoma.

Wednesday 7th December
Oral Session 12: CNS Disorders

INDUCED PLURIPOTENT STEM CELL MODEL OF PCDH19-GIRLS CLUSTERING EPILEPSY REVEALS ROLES FOR THIS PROTOCADHERIN IN REGULATION OF NEURONAL POLARITY AND DIFFERENTIATION

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Induced pluripotent stem cells (iPSC) provide a unique opportunity to study neurological disorders using disease relevant cells usually unattainable from the patients. We have generated iPSCs from PCDH19-Girls Clustering Epilepsy (PCDH19-GCE) patient skin fibroblasts. PCDH19-GCE is a female specific epilepsy associated with a spectrum of neurodevelopmental and behavioural problems. It is caused by a variety of loss of function mutations in an X-chromosome gene, Protocadherin19 (PCDH19), with 100s of cases reported to date. PCDH19-GCE is a disorder of cellular mosaics, that is females who undergo X-inactivation and males with somatic mosaicism (3 cases reported). In addition to generating PCDH19-GCE iPSC we also developed an optimised protocol of cortical development based on a model of dual-SMAD inhibition, which we found reproducible across multiple PSC lines. Using this protocol and PCDH19-GCE iPSCs, we modelled PCDH19-GCE by replicating the cellular mosaicism of the patient brain. We found that PCDH19 is important for the maintenance of neural stem cell polarity during cortical development, with loss-of-function mutation in PCDH19 being able to form neural rosettes, but unable to properly maintain these structures as evidenced by a decrease in lumen size and number of polarised structures/rosette area. A significant increase in the number of neurons at the edge of the rosettes was also observed suggesting increased neuronal differentiation. We also found that PCDH19 regulates axonal extensions with mutant neurons having an increased primary neurite length. Taken together this work identifies novel roles for PCDH19 in neuronal polarity during cortical development and neuronal differentiation and morphology.

NEUROPEPTIDE Y (NPY) IS REQUIRED SPECIFICALLY IN THE CENTRAL AMYGDALA FOR THE DEVELOPMENT OF STRESS-INDUCED OBESITY IN MICE

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Chronic stress has adverse consequences on many organ systems as well as on a variety of physiological processes including eating behaviour, adiposity and fat distribution. A main centre in the brain responsible for mediating these effects is the amygdala. We found that mice under conditions of a high fat diet combined with cold stress (HFD-S) treatment for two weeks, showed an increase in food intake and body fat mass, and the expression of neuropeptide Y (NPY) was strongly upregulated in the central amygdala (CeA), the key centre for stress processing. Consistent with the known orexigenic effect of NPY, global deletion of NPY in the mice, results in reduced food intake and body fat mass under HFD-S when compared to the control group. In order to delineate the neurons-specific requirement of NPY function in regulating food intake and fat metabolism, we re-introduced NPY specifically in the NPY neurons of the CeA in the NPYKO mice using flip-excision (FLEX) technology and exposed these mice to the HFD-S conditions. Importantly, these mice with NPY re-introduction resulted in higher food intake and increased in respiratory exchange ratio, subsequently leading to increased body weight and body fat mass, supporting the crucial role of NPY in the CeA for the development of obesity during chronic stress situations. Consistent with this, selective ablation of NPY in the CeA had the opposite phenotypic outcome. Together, our data suggest that NPY in the central amygdala is essential in additional modulation of food intake and energy homeostasis under stress conditions.

SCHIZOPHRENIA PATIENTS CARRYING NEUREGULIN 1 (NRG1) RISK ALLELES (HAPICE) WITH ELEVATED LEVELS OF NRG1 TYPE III HAVE EXACERBATED INHIBITORY INTERNEURON PATHOLOGY IN THE DORSOLATERAL PREFRONTAL CORTEX

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Two pathological hallmarks for schizophrenia in the prefrontal cortex are reductions in inhibitory interneuron (IIN) expression and increased density of IIN+ cells within the superficial white matter (SWM), however the mechanism behind these pathologies is underexplored. Schizophrenia-associated gene, Neuregulin-1, encodes for numerous NRG1 protein isoforms that can uniquely mediate aspects of migration and differentiation of IINs. Elevated levels of the NRG1 type III (III) isoforms have been observed in the dorsolateral-prefrontal cortex (DLPFC) of schizophrenia patients carrying NRG1-HAPICE alleles; however the role of upregulated NRG1-III in schizophrenia pathophysiology is unknown. As NRG1 signalling can directly impact IINs, we hypothesised that IIN pathology would be exacerbated in schizophrenia patients that have predicated increases in NRG1-III based on genotype. To test this, IIN mRNA expression from grey matter and IIN density in SWM in post-mortem DLPFC (34-

schizophrenia/34-controls) was quantified using qPCR, in-situ-hybridization and immunohsitochemistry. ANCOVAs using factors: diagnosis and NRG1-HAPICE carrier-status was used to analyse data. NRG1-HAPICE carrying patients had significantly larger reduction of somatostatin (SST) mRNA expression in the DLPFC and reduced density of SST mRNA+ cells in layer-II of the DLPFC compared to schizophrenia non-NRG1-HAPICE carriers ($F[1,54]=5.02, p=.029$). Additionally, the density of glutamate-decarboxylase-65/67 mRNA+ cells within the SWM of the DLPFC was elevated in schizophrenia NRG1-HAPICE patients compared with schizophrenia non-NRG1-HAPICE carriers patients. These results indicate that elevated levels of NRG1-III in NRG1-HAPICE carrying patients relates to the degree of IIN pathology in schizophrenia suggesting that genetic factors may increase risk by perturbing IIN development or differentiation in the disease state.

MUNC18-1 CONTROLS A-SYNUCLEIN SELF-REPLICATING AGGREGATION IN EARLY INFANTILE EPILEPTIC ENCEPHALOPATHY

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Munc18-1 is an essential element of the exocytic machinery controlling neurotransmitter release. Munc18-1 knockout mice show no developmental phenotype but die at birth from paralysis. Surprisingly, Munc18-1 heterozygous mutations are responsible for developmental defects, neurodegenerative and epileptic phenotypes including infantile epileptic encephalopathy (EIEE), suggestive of a gain of pathological function. Here, we used single molecule analysis, gene-edited cells and neurons to demonstrate that Munc18-1 EIEE-causing mutants promote the formation of large polymers that co-aggregate wild-type Munc18-1 *in vitro* and in neurosecretory cells. Surprisingly, Munc18-1 EIEE mutants also form Lewy body-like structures that contain α-synuclein (α-Syn). We reveal that not only Munc18-1 binds α-Syn but also its mutants can co-aggregate with α-Syn. Likewise, removal of endogenous Munc18-1 increases the aggregative propensity of $\alpha\text{-Syn}^{WT}$ and that of Parkinson's disease-causing $\alpha\text{-Syn}^{A30P}$ mutant, an effect rescued by $Munc18^{WT}$ expression indicative of chaperone activity. Co-expression of $\alpha\text{-Syn}^{A30P}$ mutant with Munc18-1 reduced the size of $\alpha\text{-Syn}^{A30P}$ aggregates. Munc18-1 mutations may therefore lead to a pathogenic gain of function through both the corruption of native Munc18-1 and a perturbed chaperone activity for α-Syn. Our results uncovers an unexpected function of Munc18-1 in controlling α-Syn propensity to aggregate.

DIFFERENTIAL EFFECTS OF ANTI-PRION TREATMENTS HIGHLIGHT DIFFERENCES IN THE PATHOGENESIS OF SPORADIC AND FAMILIAL FORMS OF PRION DISEASE AND IDENTIFIES NOVEL MODE OF ACTION FOR PENTOSAN POLYSULFATE

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Early detection and disease modifying treatment of neurodegenerative disorders is essential for the preservation of nervous system function and patient quality of life. Familial forms of neurodegeneration are arguably the most attractive candidates for timely prophylactic intervention. However very few studies have considered whether therapies that are effective against misfolded forms of proteins with a wildtype sequence (representing sporadic disease) are also effective against misfolded proteins encoding a disease associated mutation (representing familial disease).

In the current study we found that cells infected with prions propagated from a wildtype prion protein were susceptible to treatment with congo red, chlorate, pentosan polysulfate (PPS) and cholesterol. In contrast, prions propagated from a mutant prion protein were susceptible to congo red and chlorate treatment but unaffected by treatment with PPS and cholesterol.

Further investigation revealed a change in the lipid raft association of the mutant prion protein relative to the wildtype protein and further revealed that treatments that failed to treat prions propagating from a mutant prion protein significantly affected cell associated cholesterol levels and localisation.

We therefore conclude that PPS and cholesterol cure prions propagating from a wildtype prion protein through affects on lipid raft integrity and that failure to clear prions propagating from a mutant prion protein reflects the presence of a non-lipid raft associated pool of protein. This study highlights a difference in susceptibility of sporadic and familial disease to treatment and further offers an alternative mode of action for the anti-prion affects of PPS.

ALS-LINKED MUTANT CYCLIN F IMPEDES ER-GOLGI TRAFFICKING AND INDUCES ER STRESS MEDIATED APOPTOTIC CELL DEATH

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Intracellular protein aggregates are characteristic of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) which is characterized by selective death of upper and lower motor neurons. The exact role of protein aggregates in disease

pathology is still under debate. Previously, we demonstrated that inhibition of ER-Golgi transport induces ER stress and apoptosis in ALS. Several proteins are linked genetically and pathologically to neurodegeneration in ALS, including SOD1, TDP-43 and FUS (Parack et al., 2016). Recently, missense mutations in the CCNF gene encoding cyclin F, were identified at similar frequencies to those reported in TARDBP and FUS in cohorts of familial and sporadic ALS and FTD cases from diverse geographic populations. However, the pathogenic mechanisms induced by mutant CCNF remain to be established. Here, we demonstrate that expression of ALS-associated mutant S621G cyclin F leads to its mislocalisation to the cytoplasm. Furthermore, mutant cyclin F inhibits protein transport between the endoplasmic reticulum and the Golgi apparatus, impairs ERAD degradation and enhances ER stress, leading to apoptotic neuronal death in SH-SY5Y neuronal cells. Packaging of proteins into COPII vesicles is essential for ER-Golgi transport and the ubiquitination of COPII subunit Sec31A determines the size and function of ER-derived vesicles. We also demonstrate that Sec31A is ubiquitinated less in mutant cyclin F expressing cells compared to controls, suggesting that the reduction of the ubiquitination of Sec31A could impact the assembly of COPII vesicles in ALS. Together, these results provide evidence for ER to Golgi trafficking dysfunction as a pathogenic pathway in cyclin F-mediated disease.

BEHAVIOURAL AND ELECTROPHYSIOLOGICAL OUTCOMES IN THE POLY-I:C MOUSE MODEL OF SCHIZOPHRENIA FOLLOWING RALOXIFENE TREATMENT

Ms Anna Schroeder[2], Mr Jay Nakamura[1,2], Mr Mathew Hudson[3], Dr Xin Du[1,2], A/Prof. Nigel Jones[3], **Dr Rachel Hill[1,2]**
[1]Monash University, [2]Florey Institute for Neuroscience and Mental Health, [3]University of Melbourne

Schizophrenia shows sex differences with females presenting with a later age of onset and less severe symptoms - thought to be due to the neuroprotective actions of estrogens. The selective estrogen receptor modulator, raloxifene, has shown promise in the clinic, but lacks mechanistic understanding.
Alterations in brain high-frequency neural oscillatory activity are a prevalent pathophysiological finding in schizophrenia and may be an underlying mechanism of cognitive dysfunction. Our novel data demonstrate that following ovariectomy, both estradiol and raloxifene recover gamma frequency oscillation deficits in the brain to enhance spatial memory. This project investigated behavioural and electrophysiological effects of raloxifene in a mouse model with high relevance to schizophrenia; the poly-I:C-induced maternal immune activation model.
Mice were exposed to the viral mimetic, Poly I:C, or saline at gestational day 17. Slow release raloxifene or placebo pellets were subcutaneously administered at early adulthood. Learning, memory, anxiety and sensorimotor gating were probed using the y-maze, cheeseboard maze, elevated plus maze and pre-pulse inhibition (PPI) chambers. Electrophysiological recordings were simultaneously taken from the dorsal hippocampus.
Male Poly I:C mice showed increased anxiety, disrupted spatial learning, and decreased PPI. Female mice demonstrated perseverative behaviour and decreased PPI. Raloxifene reversed the poly-I:C-induced deficits in male spatial learning and female preservative behaviour. Disrupted gamma power was found during acquisition memory in males and decision making in female Poly I:C treated mice which was reversed with raloxifene treatment.
In summary this study revealed sex-specific effects of both prenatal poly-I:C exposure and adult raloxifene treatment on behaviour and electrophysiology.

INTERLEUKIN-1 RECEPTOR SIGNALLING MEDIATES SEIZURE SUSCEPTIBILITY AFTER TRAUMATIC INJURY TO THE IMMATURE MOUSE BRAIN

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Post-traumatic epilepsy is common after traumatic brain injury (TBI) at a young age, and has been associated with poorer functional outcomes. However, most existing models of post-traumatic epileptogenesis examine injury to the adult brain. Here, we first investigated susceptibility to induced seizures after TBI in mice at postnatal day 21, approximating a toddler-aged child. By 2 weeks, brain-injured mice showed a more pronounced seizure response to the convulsant pentylenetetrazol (PTZ) compared to sham-operated controls. A heightened seizure response persisted to 3 months post-injury, associated with abnormal mossy fibre sprouting in the ipsilateral hippocampus. The inflammatory mediator interleukin-1β has been implicated in both neurodegeneration and epilepsy, and a robust elevation of the ligand and signalling receptor IL-1R were detected after paediatric TBI. Acute post-injury treatment with an IL-1R antagonist (IL-1Ra; 100mg/kg s.c. at 2, 8 and 24h) reduced seizure susceptibility at 2 weeks after paediatric TBI, and was associated with a reduction in reactive gliosis, but no preservation of hippocampal granule cells or interneurons. Next, we extended the treatment window (vehicle or IL-1Ra at 100mg/kg s.c. at 2h, then 100mg/kg/day by s.c. osmotic pump for 7d), and evaluated a chronic time point of 6 months post-injury, after implantation of subdural electrodes for video-EEG monitoring. Administration of PTZ induced generalized seizures in 50% (7/14) of vehicle-treated TBI mice but 0% (0/12) of IL-1Ra-treated TBI mice. Together, these results provide evidence of persistent hyperexcitability in the brain after early life injury, and support interleukin-1 signalling as a mediator of post-traumatic epileptogenesis.

Wednesday 7th December
Oral Session 13: Environment and Epigenetics

PATERNAL ENVIRONMENTAL ENRICHMENT ALTERS AFFECTIVE BEHAVIOURAL AND PHYSIOLOGICAL PHENOTYPES IN F2 OFFSPRING BUT NOT IN F1 OFFSPRING

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Recent studies have demonstrated that paternal stress in rodents can result in modification of offspring behaviour. Environmental enrichment, which enhances cognitive stimulation and physical activity, modifies various behaviours and reduces stress-responses in adult rodents. This project aimed to investigate the transgenerational influence of paternal environmental enrichment on offspring behaviour and physiological stress response. Adult C57BL/6J male mice (F0) were exposed to either environmental enrichment or standard housing for four weeks and pair-mated with naïve females thereafter. The F2 generation was generated using F1 male offspring. Male and female F1 and F2 offspring were tested for anxiety using the elevated plus maze and light-dark box at 8 weeks of age. Depression-related behaviour was assessed using the forced-swim test. HPA axis function was determined by quantification of serum corticosterone and adrenocorticotropic hormone levels at baseline and after forced-swim stress. Paternal environmental enrichment did not alter F1 offspring anxiety and depression-related behaviours. In contrast, despite no observed changes to anxiety, F2 offspring had reduced latency to immobility in the forced-swim test, suggesting greater propensity for depression. F2 females had significantly higher serum corticosterone levels post-stress but not ACTH levels, indicating hyper-responsiveness of the adrenal glands. These results show that paternal environmental enrichment exerts a sex-specific transgenerational impact on the behavioural and physiological response to stress.

NOVEL CHROMATIN STATE TRANSITIONS OCCUR DURING NEURAL DEVELOPMENT

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A key question in developmental biology is how cellular differentiation is controlled during development. Particular interest has focused upon changes in chromatin state, with transitions between Trithorax-group (TrxG) and Polycomb-group (PcG) states vital for the differentiation of ES cells to multipotent stem cells. Recently a number of other chromatin states have been shown to exist in cell culture, including a repressive "Null" chromatin state devoid of common chromatin marks. However, little is known as to the role of chromatin states during the development of complex organs such as the brain.
In order to understand the role chromatin states play in neural development, we used the Targeted DamID system to profile chromatin states within the developing fruit fly brain. We obtained genome-wide binding profiles of five key chromatin proteins in three separate cell types – neural stem cells (NSCs), immature neurons and mature neurons – and we determined chromatin states through a Hidden Markov Model approach.
We demonstrate that the majority of genes that are activated during neuronal differentiation are repressed by the Null chromatin state in NSCs. Furthermore, almost all key NSC genes are switched off via a transition to HP1-mediated repression. Interestingly, PcG-mediated repression does not play a significant role in regulating either of these transitions; instead, PcG chromatin specifically regulates lineage-specific transcription factors that control the spatial and temporal patterning of the brain. Combined, our data suggest that forms of chromatin other than canonical PcG/TrxG transitions take over key roles during neural development.

EXERCISE IMPROVES LEARNING AND INCREASES NEUROGENESIS AFTER AN ENDOTHELIN-1-INDUCED HIPPOCAMPAL STROKE IN ADULT MOUSE

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[1]Queensland Brain Institute

The hippocampus is involved in learning and memory functions but is vulnerable to damage in stroke. Impaired cognition is common following stroke and this is associated with decreased hippocampal volume (Xie et al., 2011, Blum et al., 2012, Schaapsmeerders et al., 2015). Ongoing neuronal production (neurogenesis) in the hippocampus continues throughout life. Reduced neurogenesis is associated with impaired cognitive functions, whereas exercise stimulates neurogenesis and improves learning in aged or injured animals. Reports of the impact of exercise-induced neurogenesis on cognitive functions following stroke have been inconsistent so we examined this in our localised hippocampal stroke model. Mice underwent unilateral intrahippocampal injection of the vasoconstrictor Endothelin-1 followed by free access to a running wheel. Exercise-induced neurogenesis was disrupted by ablating immature neurons in a cohort of animals using a transgenic approach. Spatial learning was tested using the hippocampal-dependent Active Place Avoidance (APA) task and immunohistochemistry performed. We found that endothelin-1-induced stroke resulted in damage to the dentate gyrus, reduced numbers of immature neurons, and

an observable spatial learning deficit. Post-stroke voluntary running was associated with increased levels of neurogenesis and improved APA learning performance when compared to stroked animals that did not run. Furthermore, ablation of the immature neurons arising following exercise abrogated this exercise-induced improvement in learning. Exercise following hippocampal stroke improves cognitive recovery and this is dependent on increased neurogenesis.

MATERNAL OBESITY–ASSOCIATED HYPERACTIVITY IN THE HIPPOCAMPUS AND CORTEX OF THE OFFSPRING: ONLY PARTIAL RESCUE BY DIET REVERSAL AT WEANING

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Approximately 40% of pregnant Australian women present with high body mass index (BMI), the offspring develop high BMI early and are at increased risk of neurodevelopmental delay, lower cognitive performance and poor executive function and memory as adults. Our aim was to determine the effects of maternal obesity in pregnancy on cognitive function and hippocampal electrophysiology in the offspring, insights into the mechanisms involved, and the extent to which diet reversal at weaning might alter outcomes.
Long Evans rats were fed western high-fat diet (HF) or control chow (CC) for 4 weeks before mating and throughout pregnancy and lactation. At weaning half of the offspring were maintained on the maternal diet, the other half were crossed onto the other diet. Offspring were tested in an 8-arm radial maze at 12 weeks of age. Multi-electrode array (MEA) was used to test electrophysiological activity in hippocampal and prefrontal and motor cortical slices at 16 weeks.
Female and male offspring raised on HF diet had poorer reference and working memory outcomes than CC offspring. Working memory followed current diet and was restored following HF?CC switch. Reference memory appeared set in early life rather than current diet. Hippocampal and cortical MEA recordings revealed hyperactivity in HF offspring, particularly striking in males. HF was associated with an increase in astrocyte activation and reduced glutamic acid decarboxylase (GAD), indicating GABA interneuron disturbances.
Maternal diet in pregnancy has a significant influence on cognitive function in the adult offspring, and some of these effects appear programmed for life.

SYSTEMIC REGULATION OF EXERCISE-INDUCED ADULT HIPPOCAMPAL NEUROGENESIS

Dr Tara Walker[1,2], Ruslan Rust[1,2], Lisa Groennert[1,2], Susann Ruhwald[1,2], Vijay Adusumilli[1,2], Dr Rupert Overall[1,2], Odette Leiter[1,2], Dr Alex Sykes[3], Prof Gerd Kempermann[1,2]
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Physical activity has been widely demonstrated to act as a potent stimulator of hippocampal precursor cell proliferation, however the complex underlying regulatory mechanisms remain poorly understood. Physical exercise is associated with increased blood flow and the proximity of the hippocampal stem cell niche to blood vessels allows the crosstalk between neural stem cells and peripheral factors. Using the in vitro neurosphere assay we show that circulating systemic factors that are released into the blood stream in response to physical activity increase hippocampal precursor proliferation. Proteomic screening of plasma isolated from running and standard-housed mice revealed a number of interesting blood-born factors that were either up- or downregulated following exercise.
One protein that was highly upregulated in the plasma of running mice was Selenoprotein P, the major transporter of the trace element selenium from the plasma into the brain. Selenium is necessary for normal brain function and its deficiency is correlated with a number of neurodegenerative diseases. Using the neurosphere assay and adherent monolayer cultures we demonstrated that selenium can significantly increase hippocampal precursor proliferation and neuronal differentiation in vitro. Following selenium infusion into the brain we observed a significant increase in both proliferation and net neurogenesis in the subgranular zone of the hippocampal dentate gyrus. Furthermore, we show that this is likely mediated by selenium's antioxidant capacity, being able to significantly reduce levels of reactive oxygen species in vitro. Altogether, selenium is a promising proneurogenic factor that may be useful in cases of reduced neurogenesis associated with aging and neurodegeneration.

MATERNAL OBESITY EXACERBATES BRAIN INJURY RESPONSES FOLLOWING HYPOXIA-ISCHEMIA IN RAT OFFSPRING

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In humans, maternal obesity is associated with an increase in the incidence of birth related difficulties, but the impact on brain injury severity in offspring is not known. Recent studies have shown enhanced inflammation in brains of obese rodents. Here, we have studied the effects of maternal obesity on various brain injury responses following neonatal hypoxia-ischaemia (HI) in offspring. Female Sprague Dawley rats were allocated to high fat (HFD, n=8) or chow (n=4) diets and mated with lean males. On postnatal day 7 (P7), pups were randomly assigned to HI injury (right carotid artery occlusion followed by 8% oxygen (3h) or control (C) groups. Control pups were kept under ambient conditions for 3h. Brain injury was quantified in brain sections from

P14 pups using cresyl violet staining and glial markers measured using immunohistochemistry. The Chow-HI pups (n=31) showed an 8.9±3.3% loss in ipsilateral brain tissue while the HFD-HI group had significantly greater loss (20.5±3.2% n=44, $p<0.05$, 2 way ANOVA). There was no significant injury detected in the HFD-C (n=16) and Chow-C (n=25) pups. When analysed on a per litter basis, the size of the injury was significantly correlated with maternal weight ($r=0.89$; $P<0.05$). We observed a similar pattern of changes in glial markers. Our data clearly demonstrate that maternal obesity can exacerbate brain injury severity caused by HI in neonatal offspring. Given that previous studies have shown enhanced inflammatory responses in offspring of obese mothers, these factors including gliosis and microglial infiltration may contribute to the enhanced brain injury.

EPIGENETIC REGULATION OF NICOTINE SEEKING IN RATS

Dr Kelly Clemens[1], Dr Neil Youngson[1], Dr Timothy Bredy[2,3], Galina Shevchenko[1], Dr Kevin Morris[1,4], Matthew Castino[1]
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Drugs of addiction lead to a wide range of epigenetic changes throughout the brain. Many of these changes occur within the promoter regions of genes directly implicated in learning and memory processes. We have previously shown (Castino et al. 2015, PLoS One) that the histone deactylase inhibitor sodium butyrate (NaB), accelerates the extinction of nicotine seeking and provides resistance to relapse. Here we explore the potential molecular mechanisms underlying this effect. Rats received intravenous nicotine or saline self-administration followed by 6 days of extinction training, with each extinction session followed immediately by treatment with NaB or vehicle. On the last day of extinction, rats were sacrificed and the medial ventral prefrontal cortex retained for processing. Quantitative PCR identified changes in several genes associated with NaB treatment that were independent of nicotine exposure. An interaction of nicotine history and NaB treatment was detected only in the expression of BDNF exon IV and exon IX. Chromatin immunoprecipitation (ChIP) was then used to identify changes in epigenetic marks at the BDNF exon IV promoter. A history of nicotine exposure was associated with a significant decrease in H3K14 acetylation that was normalised following NaB treatment. Furthermore, nicotine resulted in a significant decrease in histone methylation at the H3K27me3 and H3K9me2 marks, indicative of a permissive chromatin state. Together these results suggest that nicotine self-administration leads to a number of epigenetic changes at the BDNF promoter, and that these changes may contribute to the enhanced extinction of nicotine-seeking by NaB.

EXPLORING THE ROLE OF THE EPIGENETIC REGULATOR ING1 IN EXTINCTION MEMORY

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Our understanding of the effects of experience on brain function has advanced in recent years with the realization that epigenetic processes regulate gene expression underlying learning and memory. Here, using lentiviral-mediated gene transfer, we show that Inhibitor of growth family member 1 (ING1), a reader protein for the DNA modification 5-formylcytosine (5fC), is critically involved in fear extinction memory in mice. To investigate the mechanism underlying regulation of extinction memory by ING1, we have developed a novel fluorescence-activated cell sorting (FACS)-based method to enrich for neurons that have been selectively activated by learning. We applied this method to dissociated cells from the mPFC of mice immediately following extinction training, then performed ING1 ChIP-seq on protein-DNA complexes derived from those activated neurons. In this way, we targeted ING1 binding exclusively in neurons that are involved in the formation of the memory trace, and elucidate genes that are directly involved with extinction memory. Most of the targets identified have been demonstrated to be involved in various brain functions, but not previously been implicated in fear extinction. Importantly, we have discovered that some, not all, ING1 binding sites are associated with the accumulation of 5fC during extinction learning. These finding represent a potentially new mechanism of gene regulation in the adult brain, and suggest an important role for ING1 in the formation of extinction memory.

Wednesday 7th December
Oral Session 14: Dementia and Aging

NEUROPATHOLOGICAL MARKERS OF ALZHEIMER'S DISEASE IN VIETNAM WAR VETERANS WITH TRAUMATIC BRAIN INJURY & POST-TRAUMATIC STRESS DISORDER

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Background: Epidemiological research indicates that amongst veterans, both Traumatic Brain Injury and Post-Traumatic Stress Disorder are associated with a 2-4-fold increase in risk of dementia; however, mechanisms contributing to this relationship are poorly understood. The aim of this study was to investigate if Vietnam war veterans without mild cognitive impairment or dementia, but with TBI and PTSD show evidence of Alzheimer's disease pathological markers, as assessed by amyloid, tau and glucose metabolism using PET.

Method: 82 male participants -41 veterans with chronic PTSD (aged 68.12 ±2.43 years), 18 with a TBI (aged 68.19 ±2.44 years) and 22 controls (aged 69.63 ±5.29 years)- underwent FDG, tau (18F-AV1451) and amyloid PET (18F-Florbetaben). The Standardized Uptake Value Ratio (SUVR) was calculated using the cerebellar cortex as reference region for all tracers.

Results: The TBI cohort demonstrated significantly higher 18F-AV1451 retention than the control group in the temporo-parietal region (1.23 ± 0.10 vs 1.17 ± 0.08, p=0.044) and frontal cortex (1.18 ± 0.10 vs 1.11 ± 0.09, p=0.044). In addition, 18F-FDG retention in the frontal cortex was significantly lower in the PTSD group when compared to the controls (1.03 ±0.06 vs. 1.07 ±0.07 p=0.014). There was no significant difference in A burden between the groups.

Conclusions: These preliminary findings suggest that TBI is associated with later life tau deposition, whilst chronic PTSD is associated with hypometabolism later in life. More studies to confirm these results are warranted.

ACTIVATION OF TOLL-LIKE RECEPTOR 2 INCREASES ALPHA-SYNUCLEIN LEVELS IN NEURONAL CELLS

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Parkinson's disease (PD) is a progressive neurodegenerative disorder with the formation and development of Lewy bodies (LBs) and Lewy neurites (LNs) as its pathological characteristics. The most prominent and well-studied component of LBs and LNs is -synuclein, which is thought to propagate through PD brain in a prion-like manner contributing to neural dysfunction. New evidence suggests that toll-like receptor 2 (TLR2), a member of the innate immune pattern recognition receptor family, may contribute to the spread of -synuclein in PD brain [1]. In this study we used differentiated SHSY5Y cells, and primary IPS-derived neural progenitor cells and treated them with PAM3CSK4, a potent agonist for TLR2. Over a one-week period we observed a significant increase in levels of endogenous -synuclein by both imaging and immunoblotting. The increase in -synuclein was associated with an increase in the selective autophagy marker p62/SQSTM1, suggesting -synuclein may be increased due to impaired autophagy-lysosomal clearance. Moreover, the TLR2-stimulated accumulation of -synuclein could be prevented by promoting autophagy with rapamycin. Finally we found that a number of small molecule inhibitors that target the TLR2 pathway could also prevent the accumulation of -synuclein in neural cells. These results suggested that targeting TLR2 and/or the AKT-mTORC1 signaling pathway might be a potential therapeutic option for preventing α-synuclein accumulation in PD.

1 Kim, C., E. Rockenstein, B. Spencer, H.K. Kim, et al., *Antagonizing Neuronal Toll-like Receptor 2 Prevents Synucleinopathy by Activating Autophagy.* Cell Rep, 2015. **13**(4): p. 771-82.

P75NTR MEDIATES AMYLOIDOGENESIS THROUGH INTERACTIONS WITH BOTH B-SITE AMYLOID PRECURSOR PROTEIN CLEAVING ENZYME 1 AND AMYLOID PRECURSOR PROTEIN

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Neurotrophin receptor, p75 (p75NTR), is a receptor for neurotrophins and proneurotrophins, as well as amyloid beta (Aβ). P75NTR expression is elevated in the hippocampus of patients with Alzheimer's Disease (AD), thus is involved in the pathogenesis of AD. Our recent study shows that the shedding of p75 extracellular domain is dysregulated leading to the

upregulation of the full-length p75NTR. However, how the full-length p75NTR participates in the pathogenesis of AD remains unclear. We aimed to demonstrate the amyloidogenic role of p75NTR and propose a plausible mechanism on how it regulates Aβ generation. Here we show that the p75NTR signaling activated by Aβ and proneurotrophins causes the upregulation of amyloidogenesis via increased interactions with both β-site amyloid precursor protein cleaving enzyme 1 (BACE1) and amyloid precursor protein (APP). We have found that Aβ increased p75NTR/APP and p75NTR/BACE1 interactions and enhanced APP and BACE1 internalization from the cell surface in a p75NTR-dependent manner. Furthermore, Aβ also increases the phosphorylation of BACE1 S498 and APP T668 and increased Aβ generation through p75NTR. In conclusion, our data demonstrates that p75NTR mediates the amyloidogenic processing of APP triggered by neurodegenerative ligands and therefore may represent a therapeutic target in AD.

DEFINED INDUCTION OF HUMAN NEURAL ORGANOIDS FROM INDUCED PLURIPOTENT STEM CELLS USING GELATIN METHACRYLATE: A THREE-DIMENSIONAL MODEL FOR STUDYING DEVELOPMENT, DISEASE AND REGENERATIVE MEDICINE

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Gelatin methacrylate (GelMA) is a versatile semisynthetic matrix that incorporates the intrinsic bioactivity of natural matrices with the fidelity of synthetic biomaterials for more defined and clinically-compliant cell support. Here we describe the first example of using GelMA hydrogel for rapid induction of human neural organoids from human induced pluripotent stem cells (iPSCs). Differentiation of iPSCs was staged following initial culture on GelMA, forming early neural precursors by day 5, early stage neurons and neural rosettes by day 14, and organoids with mature neurons by day 28. Organoids comprised densely packed cell soma that self-organised to form hollow neural tube-like structures, as well as active neurons with prolific neurite outgrowth that formed networks with synaptic contacts. Consistent with recent reports of optimal substrate stiffness for iPSC survival, induction of neuronal cell fate and neurite protrusion, GelMA was mechanically tuned to ~500 Pa for a softer gel, also resembling the physiological stiffness of brain tissue. The optimised method provides a defined and simplified platform for both research and translation of iPSCs, neural derivatives and neural/brain organoids, including in vitro modelling of brain development and disease, tissue engineering and regenerative medicine.

SKILLED MOTOR LEARNING IN MICE – MODULATION BY REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION

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Repetitive transcranial magnetic stimulation (rTMS) can non-invasively modulate cortical plasticity via pulsed extrinsic magnetic fields, and can induce long-lasting changes. Complex pattern rTMS such as intermittent theta burst stimulation (iTBS) can enhance motor learning tasks in humans when applied to the motor cortex. However, the underlying biological mechanisms remain poorly understood.
This study examined the effect of low-intensity rTMS on the motor cortex of mice as they learned a skilled motor task.
We used a custom-built rodent-specific circular coil (8mm dia) to deliver iTBS (120mT field strength, 600 pulses delivered over 190s) over the motor cortex of awake, restrained mice. For skilled motor learning, we used a pellet reaching task in which adult male C57Bl6/J mice were tested for 20mins daily for 10 consecutive days, and scored for accuracy of reaching using video analysis. Three experimental groups were tested:

1. Sham – motor learning only
1) Priming – iTBS given daily immediately before motor learning
2) Consolidating - iTBS given daily immediately after motor learning

Linear mixed modelling was used to analyze the relationship between pellet reaching accuracy, time and treatment with iTBS. Motor learning showed the expected increase of ~15% in accuracy over the 10 days, with all groups becoming slightly more accurate each day. Compared to sham, consolidating rTMS did not significantly alter accuracy, but priming rTMS caused a significant (p=0.02) increase in accuracy of ~9% (+/-3.5% SE). However, treatment showed no significant interaction with time, indicating a transient effect of priming rTMS upon accuracy lasting less than 24hrs.

A ZEBRAFISH MODEL OF FAMILIAL ALZHEIMER'S DISEASE (FAD) SHOWS DRAMATIC, EARLY CHANGES IN BEHAVIOUR AND GENE EXPRESSION AND A PREDISPOSITION TO PATHOLOGICAL CHANGE

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Alzheimer's disease (AD) develops over decades but the early, initiating pathological stresses are undefined. We report the first detailed molecular analysis of young adult, pre-AD histopathology brains from a heterozygous, endogenous fAD PRESENILIN (PSEN) mutation model (i.e. closely modelling the human fAD genetic state rather than attempting to phenocopy late-stage AD pathology). fAD mutation K115Efs truncates the coding sequence of PSEN2 to resemble the hypoxia- and cholesterol-inducible PSEN2 isoform PS2V. We modelled this mutation in zebrafish (K97Gfs in psen1). Heterozygous K97Gfs brains show a reduced unfolded protein response (UPR) consistent with inappropriate PS2V-like expression despite the existence of a brain-specific homeostatic mechanism upregulating transcripts from the non-mutant allele to maintain normal expression. The homeostatic mechanism does not exist in skin or liver. Large, highly statistically significant differences are observed between the brain transcriptomes/proteomes of young adult heterozygous fAD-like mutant and non-mutant fish. Changes in behaviour and gene expression are observable in very young heterozygous mutant zebrafish larvae (facilitating future exploitation in unbiased drug screens). Systems biology analyses indicate that RNA/protein expression changes driven by the mutation might make the brain more susceptible to initiation of neuroinflammatory, metabolic and phosphorylation-state responses that would then be self-reinforcing. Our results support a model of early onset fAD whereby PSEN fAD mutations force changes in cellular state that prematurely occupy a proportion of cells' homeostatic capacity thereby lowering the threshold at which that homeostatic capacity is overwhelmed by cellular stressors such as hypoxia. Self-reinforcing pathological loops then drive the brain into a neurodegenerative state.

INNATE PHAGOCYTOSIS BY PERIPHERAL BLOOD MONOCYTES IS ALTERED IN ALZHEIMER'S DISEASE

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Sporadic Alzheimer's disease (AD) is characterised by the deposition and accumulation of specific protein aggregates. Failure of clearance could underlie this process, and recent genetic association studies point towards involvement of the phagocytosis and autophagy pathways. We developed a real-time tri-color flow cytometry method to quantitate the phagocytic function of human peripheral blood monocyte subsets including non-classic CD14dimCD16+, intermediate CD14+CD16+ and classic CD14+CD16- monocytes. Using this method, we have measured the phagocytic ability of fresh monocytes in a study of preclinical, prodromal and clinical AD, matched with cognitively normal healthy control subjects. Basal levels of phagocytosis in all three subsets of monocytes were similar between healthy controls and AD patients, while a significant increase of basal phagocytosis was found in subjects with high Aβ-amyloid burden as assessed by PET scans. Pre-treating cells with Copaxone (CPX, to stimulate phagocytosis) or ATP (an inhibitor of P2X7-mediated phagocytosis) showed a differential response depending on clinical or Aβ-burden status, indicating a relative functional deficit. Overall the results are consistent with a perturbation of basal and stimulated innate phagocytosis in sporadic AD.

TAU REGULATES POST-SYNAPTIC SIGNALLING AND AMYLOID-BETA TOXICITY IN ALZHEIMER'S DISEASE

Professor Lars Ittner
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Alzheimer's disease is characterized by deposition of the microtubule-associated protein tau in neurofibrillary tangles, and amyloid-β (Aβ) in extracellular plaques. We and others have shown that tau plays a role in neuronal dysfunction prior to its deposition. Furthermore, we have previously shown that tau mediates Aβ toxicity at the post-synapse. In the present study, we investigated the molecular pathways regulated by tau during onset and progression of deficits in Alzheimer's disease mouse models. Using unbiased next generation whole genome sequencing we have identified specific candidate pathways differentially regulated in tau-deficient comparted to wild-type mice during neuronal toxicity. Using biochemical, histological and molecular methods together with a range of specific gene knockout mice, we show in detail how tau regulates Aβ-mediated NMDA receptor-dependent toxicity at the neuronal post-synapse, by orchestrating both toxicity-promoting and -limiting signalling pathways. We then used CRISPR-mediated point mutations to demonstrate the role of specific pathways *in vivo*. Furthermore, we generated new transgenic mice with specific activation of a disease-limiting pathway as well as adeno-associated virus-mediated gene expression to prevent memory deficits and aberrant neuronal network activity in mouse models of Alzheimer's disease. Taken together, we revealed that tau is critically involved in mediating specific signalling pathways involved in Aβ toxicity in Alzheimer's disease, providing new targets for therapeutic intervention.

Lawrie Austin Plenary Lecturer – David Small (University of Tasmania)

EMERGING IDEAS IN ALZHEIMER'S DISEASE RESEARCH: ABETA, APP AND NEURAL NETWORK DYSFUNCTION

Small DH

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

The amyloid hypothesis has dominated Alzheimer's disease (AD) research for decades. Genetic studies demonstrate that Abeta aggregation and accumulation is the primary cause of all forms of AD. However, the selective vulnerability of certain populations of neurons, the reason for the precise spatiotemporal pattern of neurodegeneration and the key mechanisms that drive disease progression remain a mystery. AD can be understood as a molecular nexopathy in which neurodegeneration progresses based on connectivity of neurons within neural networks. Ultimately, network failure explains the clinical symptoms of the disease. The talk will review our current understanding of the molecular mechanisms which underlie neural network dysfunction in AD and it will cover the central role of Abeta and the amyloid precursor protein in this process.

Wednesday 7th December
Symposia 17: The exciting life of neuronal dendrites

ACTIVE DENDRITES IN GRID CELLS

Michael Häusser
University College London

Neuronal dendrites are electrically excitable, endowing them with active properties. Understanding how active dendrites are exploited for behaviourally relevant computations is a fundamental challenge in neuroscience4. Grid cells in medial entorhinal cortex represent an attractive model system for addressing this question, as the computation they perform is clear: they convert synaptic inputs into periodic, spatiallymodulated firing. The resulting hexagonally organized activity map may provide the brain with an accurate metric of space. Whether active dendrites are engaged in the transformation of synaptic input into the dual temporal and rate code characteristic of grid cell output is unknown. I will describe work from in vitro 2-photon glutamate uncaging experiments, and in vivo patch-clamp recordings from grid cells, that show that the dendrites of these neurons are highly excitable and exhibit a supralinear input-output function. By incorporating these nonlinear dynamics into grid cell models we show that they can sharpen the precision of the temporal code and enhance the robustness of the rate code, thereby supporting a stable and accurate representation of space under varying environmental conditions. Active dendrites may therefore constitute a key cellular mechanism for ensuring reliable spatial navigation.

GNRH NEURONS ELABORATE A LONG-RANGE PROJECTION WITH SHARED AXONAL AND DENDRITIC FUNCTIONS

Dr Karl Iremonger[1], Dr Michel Herde[1], Professor Allan Herbison[1]
[1]Centre For Neuroendocrinology, University Of Otago

Gonadotropin-releasing hormone (GnRH) neurons project to the median eminence where they release GnRH peptide from their nerve terminals into the circulation. GnRH neurons have very long, thin and unbranched dendrites. We have set out to understand how these unique dendrites integrate synaptic inputs, regulate spike generation and ultimately control GnRH peptide secretion. We have used electrophysiology and functional imaging in acute brain slices in combination with reconstructions of GnRH neuron morphology. These studies have revealed that the previously termed dendrite is the site of action potential initiation and that the dendrite projects uninterrupted to the median eminence. In addition to initiating and actively propagating sodium action potentials, the dendrite also receives and integrates synaptic inputs along its entire path to the median eminence. Imaging of genetically encoded calcium indicators in GnRH nerve terminal boutons has revealed that nerve terminal excitability can be regulated several different ways. Bursts of action potentials drive large but short-lived elevations in nerve terminal Ca2+ (seconds in duration). Remarkably, local neuropeptide signals can also induce robust Ca2+ elevations. Neuropeptide evoked Ca2+ responses are action potential independent and can persist for upwards of one hour. Overall, these data reveal that GnRH neurons possess a long-range projection that functions simultaneously as a dendrite and axon (termed the dendron). This allows GnRH neurons to integrate inputs at all parts of their projection, including at the nerve terminals. These unique adaptations allow for greater dynamic control of GnRH secretion.

DENDRITIC INTEGRATION OF SENSORY INFORMATION: A BALANCING ACT

Lucy Palmer

In the living animal, sensory systems are generally not stimulated in isolation but are instead activated collectively. During this multi-sensory stimulation, pyramidal neurons in the primary somatosensory cortex receive both feedforward input from the thalamus and feedback input from other cortical areas. Since the synaptic location of the different input streams are morphologically and functionally isolated, how multi-sensory input is integrated and computed at the level a single neuron is currently unknown. Here I will present recent results investigating dendritic activity in the hindpaw somatosensory cortex during hindpaw stimulation alone and during activation of additional sensory-evoked feedback input generated by forepaw stimulation. Using both single-cell electrophysiology and dendritic two-photon calcium imaging in vivo, we show that sensory input from the stimulation of the forepaw has different effects on the dendritic sub-domains of layer 2/3 pyramidal neurons. Namely, forepaw stimulation causes a decrease in the Ca2+ activity of apical tuft dendrites whereas there is an increase in synaptic input in proximal basal dendrites. Combined, these multi-sensory input streams led to a balancing of sensory information and had a negligible effect on the action potential output of the neuron. This balance between excitation and inhibition during multi-sensory information could be modulated by altering the strength of feedback input by photo-activation/inactivation of the forepaw somatosensory cortex. These results not only illustrate the counterbalancing interaction of multi-sensory input in cortical neurons but it also highlights the complexity of local dendritic activity during sensory perception.

DECONSTRUCTING RETINAL CIRCUIT COMPUTATIONS

Stephen Williams
University of Queensland

Wednesday 7th December
Symposia 18: Exploring the role of zinc in cognition

HUNTINGTIN OVER-EXPRESSION CAUSES ZINC DEFICIENCY

Dr Scott Ayton[1], Dr. Thibault Renoir[1], Dr Ambili Appukuttan[1], Professor Anthony Hannan[1], Professor Ashley Bush[1]
[1]*Florey Institute Of Neuroscience And Mental Health*

Objectives: Huntington's disease (HD) is an intractable genetic neurological disorder. We recently published that the zinc ionophore, PBT2 prevented cognitive and motor decline, and extended lifespan, in multiple models of HD, and improved cognitive performance in a phase II clinical trial. But the neuroprotective mechanism of PBT2 in HD is not understood.
Results: We performed a body-wide full elemental survey of the R6/1 mouse model of Huntington's disease using inductively coupled plasma mass spectrometry (ICP-MS). Compared to WT controls at the same age, R6/1 mice aged 12, 16 and 20 weeks (n=11-32 each) exhibited marked ($0.0008<P<0.048$) zinc deficiency in cerebral cortex, striatum, and hippocampus. There were modest or no changes to zinc in the midbrain, cerebellum, and peripheral tissues. Immortalised striatal neurons over-expressing mutant huntington (STHdHQ 111/111) also had lower zinc levels than cells expressing WT huntington (STHdHQ 7/7), and were resistant to zinc intoxication.
Conclusions: These data implicate Huntingtin protein in zinc regulation and point to a role for zinc dyshomeostasis in HD. Drugs that restore zinc homeostasis, such as PBT2, could provide the disease-modifying treatments for HD.

EXTRACELLUR ZN2+ PLAYS A KEY ROLE FOR β-AMYLOID-MEDIATED COGNITIVE DECLINE

Ph.D. Atsushi Takeda[1]
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Soluble amyloid-β (Aβ) oligomers are readily formed in the presence of Zn^{2+}, Cu^{2+}, and Fe^{3+} and thought to be causative of Alzheimer's disease (AD). The present study examined whether these metal ions are involved in $A\beta_{1-42}$-induced impairment of long-term potentiation (LTP) in the dentate gyrus where is vulnerable in early AD. In anesthetized rats, LTP was induced at perforant path-dente granule cell synapses under local perfusion of the dentate gyrus with artificial cerebrospinal fluid. Extracellular Zn^{2+} concentration in the brain is estimated to be approximately 10 nM. LTP was not impaired by perfusion with 5-1000 nM Aβ, but impaired by perfusion with 5 nM Aβ and 10 nM $ZnCl_2$. The co-perfusion with $CuCl_2$ or $FeCl_3$ had no effect on LTP. The impairment of LTP was rescued by co-perfusion with CaEDTA, an extracellular Zn^{2+} chelator, suggesting that extracellular Zn^{2+} is essential for Aβ-mediated impairment of LTP. Intracellular Zn^{2+} level measured with intracellular ZnAF-2 was increased by injection of Aβ and the increase was blocked by co-injection of CaEDTA. In vitro Aβ staining with Aβ monoclonal antibody (4G8) was enhanced in the presence of $ZnCl_2$, but not in the presence of $CuCl_2$ and $FeCl_3$, and blocked in the presence of CaEDTA. The present study suggests that interaction of Zn^{2+} and $A\beta_{1-42}$ in the extracellular fluid increases the uptake of Zn^{2+} and $A\beta_{1-42}$ into dentate granule cells and impairs perforant path LTP, followed by cognitive decline. Extracellular Zn^{2+} may play a key role for transiently Aβ-induced cognitive decline.

ZINC AS A CRITICAL PLAYER IN NORMAL AND PATHOLOGICAL COGNITIVE DECLINE

Associate Professor Paul Adlard[1]
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There is an emerging view that one of the key cellular processes that becomes dysregulated with age and participates both directly and indirectly in cognitive function, is metal homeostasis and the neurochemistry of metalloproteins. This is particularly true for zinc, in which 10-15% of brain zinc exists in a chelatable form primarily within synaptic vesicles at glutamatergic synapses, highlighting its potential importance in synaptic plasticity/cognition. During neuronal activation, zinc (up to 300μM) is released into the synaptic cleft alongside glutamate where it interacts with synaptic receptors (including NMDA and AMPA receptors), ion channels, transporters and other post-synaptic targets (such as the growth factor receptors, eg. TrkB) to modulate synaptic transmission and plasticity. Our studies have proven that alterations to this available zinc pool, via ablation of the synaptic zinc transporter (ZnT3), results in an age-dependent cognitive phenotype that is underscored by significant deficits in key proteins involved in synaptic plasticity (eg NMDA and AMPA receptors). Furthermore, the partial pharmacological restoration of zinc levels within key brain structures (using both ZnT3 KO and normal aged WT mice) is sufficient to normalise cognition in the whole animal, restore long-term potentiation (an electrophysiological surrogate of memory) and elevate key proteins involved in learning and memory. Taken together with other supporting data in the literature, this demonstrates a critical role for zinc in learning and memory.

ESTIMATING THE FREE ZINC CONCENTRATION IN THE GLYCINERGIC SYNAPTIC CLEFT

Prof Joe Lynch

Test: Zn2+ is concentrated into presynaptic vesicles at many central synapses and is released into the synaptic cleft by nerve terminal stimulation. There is strong evidence that synaptically released Zn2+ modulates glutamatergic neurotransmission,

although there is debate concerning its peak concentration in the cleft. Glycine receptors (GlyRs), which mediate inhibitory neurotransmission in the spinal cord, are potentiated by low nanomolar Zn2+ and inhibited by micromolar Zn2+. A mutation that ablates Zn2+ potentiation of GlyRs results in a hyperekplexia phenotype suggesting that Zn2+ physiologically modulates glycinergic neurotransmission. There is, however, little evidence that Zn2+ is stored presynaptically at glycinergic terminals and it is possible that GlyRs are modulated by tonically bound Zn2+. We sought to estimate the peak Zn2+ concentration in the glycinergic synaptic cleft as a means of evaluating whether it is likely to be synaptically released. We employed 'artificial' synapses because they permit the insertion of engineered ??? GlyRs with defined Zn2+ sensitivities into synapses. By comparing the effect of Zn2+ chelation on glycinergic IPSCs with the effects of defined Zn2+ plus glycine concentrations applied rapidly to recombinant GlyRs in outside-out patches under simulated synaptic activation conditions, we inferred that synaptic Zn2+ rises to at least 1 ?M following a single presynaptic stimulation. Moreover, using the fast high-affinity chelator, ZX1, we found no evidence for tonic Zn2+ bound constitutively to high affinity GlyR sites. We conclude that diffusible Zn2+ reaches 1 ?M or higher and is therefore likely to be phasically released at glycinergic synapses.

Wednesday 7th December
Symposia 19: Blood, inflammation and neurodegeneration

COMPLEMENT-MEDIATED INFLAMMATION DRIVES NEURODEGENERATION IN MOTOR NEURON AND PARKINSON'S DISEASE AND IS ASSOCIATED WITH ALTERATIONS IN PERIPHERAL IMMUNITY

A/Prof Trent Woodruff[1]
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There is now convincing evidence that components of the innate immune system are upregulated in the degenerating brain, and contribute to neuroinflammation which drives neuronal death. This lecture will provide an overview of our research demonstrating a key role for the innate immune complement system in driving Parkinson's and motor neuron disease, and offer novel therapeutic targets and strategies to mitigate disease progression. It will also present our emerging data demonstrating peripheral blood immunological alterations in mouse models and human samples from these diseases, and highlight potential processes leading to activation of these blood inflammatory cascades, and their link to CNS neuroinflammation.

TARGETING TYPE-1 INTERFERON SIGNALING TO LIMIT NEUROINFLAMMATION

Juliet Taylor[1]
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Current therapies for the treatment of Alzheimer's disease (AD) and Parkinson's disease (PD) are inadequate, therefore new strategies to slow the cellular degeneration are required. Activation of the innate immune response occurs in both diseases with studies suggesting this "neuroinflammation" contributes to the neuronal cell damage. The type-1 interferons (IFNs) are master regulators of the neuroinflammatory process, however their contribution to the progression of these neuropathologies is still largely unknown. We recently demonstrated a key role for the type-1 IFNs in mediating the neuroinflammatory response in both AD (APP/PS1)1 and PD (MPTP)2 mouse models. We have also confirmed that blocking type I IFN signaling with a monoclonal antibody reduces neuroinflammation and neuronal cell loss induced by MPTP, with subsequent improvement in behavioural outcome2.
Our current studies are focused on further characterising the cellular source and target of the type-1 IFNs in AD and PD. We have confirmed the type-1 IFNs, through interferon-regulatory factor-7 (IRF-7), are critical modulators of the microglial reactivity displayed in these mouse models. The type-1 IFNs have been well characterised in the periphery, and utilising a bone marrow chimera approach we have now demonstrated they also modulate the peripheral immune response induced by MPTP. Gaining a greater understanding of the neuroinflammatory response and its contribution to the neuronal cell death seen in AD and PD will aid in the identification of future therapeutic targets for the treatment of these diseases.

AGING AND MICROGLIA: UNDERSTANDING RESIDENT MACROPHAGE COMMUNICATION IN THE BRAIN

Saul Villeda[1]
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Microglia are appreciated to have roles in neural development, cognition, and neurodegenerative diseases; however, mechanisms underlying microglia aging are unknown. While microglia surveillance of their local environment is important for brain homeostasis, it has recently been suggested that the local environment drives region-specific differences in microglia during aging. To gain insight into the regulation of microglia aging by local extrinsic cues, we investigated the role of membrane bound extracellular vesicles called exosomes in regulating microglia function. Exosomes mediate bulk transfer of bioactive protein, RNA and lipid cargo, and in the brain are implicated in neurotransmitter release, neuropeptide catabolism, and pathological spread of protein aggregates. Notwithstanding, the role of exosomes in microglia communication under normal or aging conditions is unknown. We hypothesized that age-related changes in brain exosome profile alter the local environment to promote microglia aging. Using Western blot, nanoparticle tracking, and mass spectrometry analysis we detected differences in extracellular exosome abundance and content between the aged and young brain. In aged microglia we detected higher levels of exosome-associated proteins, CD63 and LAMP2, and exosome release gene, Rab27a, compared to young microglia. Functionally, in vitro and in vivo stimulation of purified microglia-derived exosomes resulted in altered activation, actin dynamics and phagocytic function in recipient microglia. Collectively, our data provide evidence that brain exosomes serve an immunosuppressive role in the aged brain raising the possibility of targeting exosomes to resolve microglia inflammation in aging.

TRANSCRIPTIONAL DETERMINANTS OF MICROGLIA FUNCTION - ROLE OF IRF8

Professor Iain Campbell[1]
[1]*University of Sydney*

Microglia, the resident myeloid cells of the brain have diverse roles ranging from neuronal maintenance to immunosurveillance and host defence. The major intrinsic molecular determinants of microglia function in the healthy and diseased brain are largely

unknown. We identified the haematopoietic transcription factor IRF8 as a constitutive and IFN-β-stimulated nuclear factor in microglia and have used IRF8 KO mice to further delineate the functions of this factor in these cells. In IRF8 KO mice, microglia were increased in number and exhibited profound changes in morphology as well as significantly altered molecular properties including decreased Iba1 and P2RY12 while tomato lectin binding was increased. Examination of the response of microglia to sterile nerve injury (SNI) or CNS infection with West Nile virus (WNV) revealed there was gross impairment in IRF8 KO mice. In response to SNI, microglia activation, neuronal wrapping and proliferation were significantly reduced in IRF8 KO mice compared with wild type (WT). Compared with WT, following WNV infection in IRF8 KO mice there was markedly reduced infiltration of the CNS with leukocytes, particularly Ly6Chi inflammatory monocytes, while microglia activation was almost absent. In conclusion, IRF8 is a major intrinsic molecular constituent of microglia that: (1) is not essential for the colonization of the brain by these cells, (2) regulates microglia homeostasis in the healthy brain and, (3) is a pivotal regulator of the functional response of microglia to injury and infection. Support: NHMRC Project grant APP1007757.

Wednesday 7th December
Symposia 20: Autism spectrum disorders: from human genetics to animal models

IDENTIFICATION OF DE NOVO VARIATION GENOME-WIDE IN AUTISM FAMILIES FROM WHOLE GENOME SEQUENCING

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Previous work on the genetic etiology of autism spectrum disorder (ASD) has clearly demonstrated a role for de novo (dn) and inherited exonic variants, but the contribution of single nucleotide variants (SNVs) and small insertions and deletions (indels) in noncoding regions, and many categories of structural variation (SV), is largely unknown. By analyzing deep whole-genome sequencing (WGS) data from 519 ASD families from the Simons Simplex Collection, we have identified and investigated SNVs, indels, and SVs, genome-wide, for association with ASD risk. We have integrated eight variant discovery algorithms, and initial validation experiments estimate true positive rates of ~90% for SNVs and indels and 86% for large SVs. We identify an average rate of 68 dnSNVs and 16 dnIndels per child, both of which correlate positively with paternal age. After correction for paternal age and multiple testing, these small de novo variants did not show enrichment in probands versus their unaffected siblings in any tested features (e.g. enhancers) of the noncoding genome. A statistically significant 2:1 excess of small de novo deletion SVs was observed in probands versus unaffected siblings and de novo dnSVs in ASD probands were more likely than dnSVs in siblings to disrupt constrained genes and known ASD risk loci. Notably, the vast majority of these gene-disrupting dnSVs were only discoverable from WGS data, as opposed to alternative approaches, a finding that has significant implications for future ASD genetic research and diagnostics. Lessons gleaned from these analyses will be of value to WGS studies more generally.

ENHANCER RNA EXPRESSION IN THE HUMAN BRAIN

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The unexpected complexity of the human transcriptome is being increasingly recognized. This is in part due to uncovering the transcriptional output of non-coding genomic regions. Among non-coding regions, enhancers carry out well-defined regulatory functions. These distal regulatory regions are highly conserved and are critical for tissue-specific and developmentally-regulated gene expression. Despite major progress in identifying enhancer regions on a genome wide scale, the majority of available data is limited to model organisms and human transformed cell lines. Here we identified a robust set of enhancer RNAs (eRNAs) expressed in the human brain, and characterized their expression variation across brain regions. We also found that regions expressing eRNA in the human brain were enriched for genetic variants associated with autism spectrum disorders, but not adult onset disorders such as major depression, bipolar disorder or schizophrenia.

TESTING THE PLAUSIBILITY OF MODIFIABLE RISK FACTORS FOR ASD IN MOUSE MODELS

A/Prof. Thomas Burne[1], Ms Mia Langguth[1], Ms Suzy Alexander[1], Ms Michelle Sanchez Vega[1]
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There are many environmental and genetic risk factors that impact on brain development and behaviour of relevance to neuropsychiatric disorders. Autism Spectrum Disorder (ASD) is a group of neurodevelopmental disorders commonly characterised by verbal and nonverbal communication deficits, impaired social interaction and repetitive, stereotypic behaviours. We have embarked on a program of research focusing on translational neuroscience, to model candidate risk factors in animal models relevant to ASD. Our aim is to examine candidate risk factors, such as the effects of developmental vitamin D (DVD) deficiency and exposure to moderate doses of ethanol in different inbred mouse strains on vocalisations, social and repetitive behaviours. We have used three different inbred strains; C57BL/6J, BALB/c and BTBR T+tf/J (BTBR) mice and measure isolation-induced ultrasonic vocalisations to measure communication, a social interaction task to observe social novelty and preference, the open field test to examine repetitive movements and hyperlocomotion, and the active place avoidance task to assess hippocampal-dependent learning and memory. We have characterised the phenotype at P7-21 which includes increased calling rate in BTBR and prenatal ethanol-exposed mice, and altered distribution of call types among DVD-

deficient and ethanol-exposed mice. We show that BTBR have behavioural deficits in communication, social interaction, repetitive, stereotypic movements and cognitive impairments in spatial memory in comparison to C57BL/6J and BALB/c mice. Using different mouse models, we can explore the behavioural phenotype and better understand gene and environmental risk factors and how they interact to contribute to the aetiology of ASD.

NOVEL METHOD TO STUDY THE FUNCTION OF HUMAN AUTISM (ASD) RISK ALLELES
John Rubenstein

A rapidly increasing number of genes are implicated in autism spectrum disorder (ASD). While null alleles (e.g. termination codon truncations) that are tightly linked to ASD are extremely helpful in identifying causative genes, one can't predict the in vivo effects of other types of alleles (e.g. nonsynonymous mutations). Thus, the field greatly needs to develop moderate-throughput assays to identify in vivo functional effects of nonsynonymous mutations. I will discuss a cell transplantation method to investigate in vivo autonomous functions of two ASD genes (Pten and Caspr2) in developing cortical interneurons. We chose this assay because of its versatility, ease, and the increasing evidence that cortical interneuron dysfunction contributes to neuropsychiatric disorders including ASD, schizophrenia and epilepsy. We and our collaborators have demonstrated that transplantation into the neonatal cortex of immature interneurons obtained from their embryonic source (medial ganglionic eminence, MGE), leads to efficient migration followed by synaptic and circuit integration of fully differentiated interneurons. Here I will discuss this approach to study the functions of Caspr2 and Pten mutations on interneuron development, and the functions of ASD alleles of these genes.

Wednesday 7th December

Symposia 21: Integrated approaches to treating pain and other diseases of the central nervous system: From targets to circuits and beyond

ALLOSTERIC MODULATION OF GPCRS IN CNS DRUG DISCOVERY: CHALLENGES AND OPPORTUNITIES

Dr Christopher Langmead[1]
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G protein-coupled receptors (GPCRs) represent attractive drug targets for CNS disorders due to their wide array of central roles, including regulation of synaptic transmission. A number of highly attractive GPCR targets have been identified in various CNS pathologies, including the adenosine A1 receptor in neuropathic pain, the muscarinic acetylcholine M1 receptor in Alzheimer's disease and schizophrenia and the muscarinic acetylcholine M5 receptor in addiction and dependence. However, in all of these cases, the lack of sufficiently subtype-selective ligands has limited either pharmacological validation or drug discovery efforts. Targeting allosteric binding sites that are topographically distinct from orthosteric binding sites utilised by endogenous ligand(s) offers the opportunity to selectively target and modulate GPCR function. Allosteric modulators can either positively or negatively modulate GPCRs, to varying degrees as well as exhibit intrinsic agonist (or inverse agonist) behaviour. This presentation will outline recent developments in the validation of positive allosteric modulation of adenosine A1 and muscarinic M1 receptors and negative modulation of muscarinic M5 receptors in vitro and in vivo. Furthermore, for muscarinic M1 receptor positive allosteric modulators (PAMs) the presentation will review recent efforts to establish those molecular properties that are related to efficacy versus adverse effects to enable more predictive drug discovery.

IDENTIFYING AND MODULATING NOVEL INHIBITORY CIRCUITS IN THE SPINAL CORD IN NEUROPATHIC PAIN

Wendy Imlach
University of Sydney

The development of neuropathic pain involves persistent changes in signalling within pain pathways. Reduced inhibitory signalling in the spinal cord following nerve-injury has been used to explain sensory signs of neuropathic pain but specific circuits that lose inhibitory input have not been identified. This study shows a specific population of spinal cord interneurons, radial neurons, lose glycinergic inhibitory input in a rat partial sciatic nerve ligation (PNL) model of neuropathic pain. Radial neurons are excitatory neurons located in lamina II of the dorsal horn, and are readily identified by their morphology. The amplitude of electrically-evoked glycinergic inhibitory post-synaptic currents (eIPSCs) was greatly reduced in radial neurons following nerve-injury associated with increased paired-pulse ratio. There was also a reduction in frequency of spontaneous IPSCs (sIPSCs) and miniature IPSCs (mIPSC) in radial neurons without significantly affecting mIPSC amplitude. A subtype selective receptor antagonist and western blots established reversion to expression of the immature glycine receptor subunit GlyRa2 in radial neurons after PNL, consistent with slowed decay times of IPSCs. This study has important implications as it identifies a glycinergic synaptic connection in a specific population of dorsal horn neurons where loss of inhibitory signalling may contribute to signs of neuropathic pain. This raises the challenge of identifying selective targets within this sub-circuitry to optimize therapeutics for minimal side effects.

TARGETING NEURONAL CHLORIDE HOMEOSTASIS FOR THERAPEUTICS; IMPLICATIONS AND CHALLENGES

Dr. Yves De Koninck[1]
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Beyond receptor signalling, a key determinant of excitability are the transmembrane ion gradients maintained by cells. In particular, disrupted Cl- transport is emerging as an important substrate of neuropathic pain, but also of several other disorders such as epilepsy, stress, anxiety, motor spasticity and perhaps even schizophrenia and autism. This lecture will address the challenges associated with targeting ion transport mechanisms, measuring them in a context-dependent manner as well as assessing the multiple, often unintuitive functional impacts of Cl- dysregulation on neural processing. I will discuss how taking into account the dynamics and regulation of intracellular Cl- is important for understanding how synaptic inhibition fails, how to best detect that failure, why chloride regulation is so important for information coding, and the overall consequences one can derive for therapeutics.

THE "TOLL" OF KNOWING YOU ARE SICK: MICROGLIAL INNATE IMMUNE SIGNALLING AS A KEY CONTRIBUTOR TO SEX DIFFERENCES IN PAIN AND ANALGESIA.

Mark Hutchinson
University of Adelaide

The aetiology of persistent pain in humans is comprised of a complex, twisted and multi factorial journey that culminates in a "cancer of the soul". Recent advances in the basic science underpinning our mechanistic understanding of persistent pain have embraced "the other brain" as an integrator of multiple life stimuli. This complex integration of life experiences, which are translated into neurokine signals cause the neuroimmune cells of the central nervous system to adapt and change the

environment in which the neuronal system operates. If these adaptations present in the somatosensory neuroanatomical locations then this can present as hypernocicpetion and eventual persistent pain. Our appreciation for this neuroimmune signalling and its contributions to the health and disease of the brain has its origins in the study of the illness response. It is now apparent that these specialised brain-immune processes are engaged in a range of other disparate responses, including the pharmacodynamic failure of analgesics. This presentation will explore recent studies originating from the Australian Research Council Centre of Excellence for Nanoscale BioPhotonics exploring how sex differences in innate immune signalling contribute to substantial differences in allodynia and analgesia in a “humanised" preclinical model of pain.