The Australian and New Zealand Society for Neuropathology

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34th Annual Scientific Meeting
NEUROPATHOLOGY IN RESEARCH

Medical Sciences Lecture Theatre 2
Menzies Research Institute

v

Hobart,
Sunday December 4th, 2016

This Satellite meeting will:

- Interconnect and engage those working in Neuroscience in the area of Neuropathology
- Provide an update on advances in Neurodegenerative diseases
- Allow neuropathology trainees, young researchers and students to present their work in a relaxed and amiable forum
- Advance knowledge in new nervous system imaging techniques
- Showcase the neuropathology of difficult clinical neurodegenerative cases
- Registration cost - $50

Program

9 am – 10.45 am– Session 1

9.00 - 9.20

Glenda Halliday - Are neurodegenerative diseases really infectious?
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Are neurodegenerative diseases really infectious?

Glenda Halliday
Brain and Mind Centre, Sydney Medical School, The University of Sydney, Australia

Prions are proteins that can take on an infectious form and spread through the brain of anyone infected, and also can infect others if ingested. After infection, there is a long incubation period and the infectious form of the protein builds up in the brain, the neurons swell and burst, and the brain becomes inflamed. It is now apparent that the spread of pathological forms of normal brain proteins in association with inflammation occurs in many neurodegenerative diseases, and recent publications have suggested that pathological proteins can be transmitted through contact with already infected brain material. This has now been suggested for Alzheimer's disease, but the data is strongest for multiple system atrophy (MSA), which has some similarities to prion disease. There include considerable inflammation, considerable neuronal death, and transmission along white matter tracts. Compared to Parkinson's disease, MSA is usually rapidly progressive, similar to prion disease. Unlike Parkinson's disease, extracts from the brains of patients with MSA appear to be able to transmit MSA pathology to particular genetic mice, and then the aggregates from these mice can also be transmitted in similar mice [1]. However, unlike prions, not all mice can be infected [1], indicating that a predisposition is important for the phenomenon. What is it that could predispose people to MSA? Genetic polymorphisms in the prion protein predispose people to prion diseases, although recent data suggests that common genetic variations predisposing to MSA are unlikely [2]. It will be important to determine what may predispose people to the more rapidly progressive alpha-synucleinopathy of MSA, or other forms of neurodegeneration.

[1] Prusiner *et al.* Proc Natl Acad Sci U S A. 2015;112(38):E5308-17.
[2] Federoff *et al.* Parkinsonism Relat Disord. 2016;22:35-41

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9.20 – 9.40

Tracey Dickson - A new target mechanism for Motor Neuron Disease.
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Inhibitory Dysfunction: A new target mechanism for MND

Rosemary M. Clark[1], Catherine A. Blizzard[1], Kaylene M. Young[1], Catriona McLean[2] and **Tracey C. Dickson[1]**

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In amyotrophic lateral sclerosis (ALS), cortical hyperexcitability is a prominent event, often preceding motor neuron degeneration. While many factors may be responsible for this pathophysiology, a possible candidate, the interneuron, has largely been overlooked.
Here, in a systematic immunohistochemical study of interneuron subsets, we demonstrate unique regional- and lamina-specific alterations in the cortex of transgenic mice and human patients with end-stage ALS. In *SOD1*^{G93A} mice we examined the motor and somatosensory cortex from presymptomatic ages through to end-stage disease. We report that two distinct interneuron populations are altered in the motor cortex and both exhibit presymptomatic, but contrasting pathology. NPY^{+} interneuron number was decreased by ~17% in *SOD1*^{G93A} mice relative to controls prior to motor symptom onset (8 weeks). However, by late disease

stages (20 weeks) *SOD1*^{G93A} mice had ~30% more NPY^{+} interneurons than controls. By contrast the number of calretinin^{+} interneurons in the cortex of *SOD1*^{G93A} mice progressively declined and was ~31% less than controls from symptom onset (16 weeks). Branch complexity was similarly reduced, but this change preceded the decline in cell number. Interneurons were unaltered in the somatosensory cortex, and no changes were identified in other interneuron populations in either region, suggesting that NPY and calretinin interneurons may drive a motor-specific inhibitory phenotype from early stages of disease. Analysis of human ALS post-mortem brain tissue revealed that the 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, early alterations to these specific inhibitory populations in the motor cortex is likely to have a wide-reaching effect on pathophysiology within the region, driving motor neuron vulnerability in ALS.

9.40 – 10.00

Brad Turner - SMN gene therapy approach for SMA and ALS MND
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Survival motor neuron gene therapy approach for SMA and ALS

Rebecca K. Sheean,[1] Nirma D. Perera,[1] Kevin S. Smith[2], Lauren Jones[2], Kevin Talbot[3], Mary-Louise Rogers[2], Bradley J. Turner[1]

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Insufficient levels of the widely expressed survival motor neuron (SMN) protein, which is essential for gene splicing, causes selective degeneration of spinal motor neurons, muscle weakness, wasting and infant death in spinal muscular atrophy (SMA). Given that low levels of SMN confer vulnerability of spinal motor neurons, we first addressed whether SMN expression is also abnormal in amyotrophic lateral sclerosis (ALS) which shows both pathological and pathogenic overlap to SMA. Full-length, but not spliced, SMN protein levels were significantly reduced by ~50% in post-mortem lumbar spinal cords of sporadic ALS patients (n=10), compared to controls (n=5). SMN protein depletion in spinal motor neurons correlated with cytoplasmic TDP-43 pathology in ALS cases. Abnormal SMN expression in spinal motor neurons was confirmed in both mutant superoxide dismutase 1 (SOD1) and TAR DNA binding protein 43 (TDP-43) mouse models of ALS. To test the impact of augmenting SMN levels on the ALS phenotype, human SMN was neuronally overexpressed in mutant SOD1 and TDP-43 mice. SMN overexpression significantly delayed symptom onset, extended lifespan and improved spinal motor neuron survival, notably in mutant TDP-43 mice. These results suggest that restoration of SMN levels may be beneficial for ALS, in addition to SMA. In an effort to build a non-viral gene therapy for ALS and SMA, we have employed a novel approach using immunogenes as vectors for targeted motor neuron delivery of SMN from the periphery. Our results demonstrating highly efficient, sustained and broad SMN immunogene expression in spinal cords of mice will be presented, highlighting the therapeutic promise of immunogenes to correct inadequate SMN expression levels and function for both SMA and ALS.

10.00-10.20

Steven Petratos - Targeting the Nogo Receptor-dependent mechanisms of axonal degeneration in multiple sclerosis
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Nogo receptor 1 (NgR1) deletion in axons halts axonopathy and demyelination during experimental autoimmune encephalomyelitis (EAE)

Lee J.Y.[1], Thomas S.[1], Kim M.J.[1], Aui P.M.[1], Magee K.A.[1], Harvey A.R.[2], Strittmatter S. M.[3], McClean C., **Petratos S**[1].

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Background
We have previously shown that deletion of the *ngr1* allele limits experimental autoimmune encephalomyelitis (EAE) severity by preserving central nervous system (CNS) axons. What is unknown is whether this preservation is governed by myelin being intact thereby protecting axons, or in fact axonal degeneration is limited, thereby preventing demyelination.

Objectives
We investigated how neuronal deletion of *ngr1*, may prevent axonal degeneration during EAE. In a parallel study, we investigated whether neuronal over-expression of *ngr1* in *ngr1*$^{-/-}$ mice potentiates axonal degeneration during EAE. This approach may differentiate the role of NgR1 in promoting axonal damage during EAE.

Design Methods
Conditional deletion of ngr1 in axons was produced by intraocular injection of AAV2 encoding Cre (AAV2-iCre-eGFP) in *ngr1*$^{flx/flx}$ mice. Conversely, conditional re-introduction of NgR1 in axons was produced by intraocular injection of AAV2 encoding full-length mouse NgR1 (AAV2-NgR1-eGFP) in *ngr1*$^{-/-}$ mice. Animals were induced with EAE and culled at the peak stage of disease. The degree of axonal degeneration and demyelination were assessed by immunohistochemistry. Molecular mechanisms of axonal degeneration were verified with immunoblot.

Results
Axonal degeneration is limited in AAV2-iCre-eGFP injected *ngr1*$^{flx/flx}$ whereas, significant axonal damage is found in AAV2-NgR1-eGFP injected *ngr1*$^{-/-}$ optic nerves during EAE. As a corollary, the preservation of myelin integrity was a prominent feature in AAV2-iCre-eGFP injected *ngr1*$^{flx/flx}$, whereas significant demyelination was found in AAV2-NgR1-eGFP injected *ngr1*$^{-/-}$ optic nerves. Furthermore, the interaction between the axonal motor protein, kinesin-1 (KIF5) and collapsin response mediator protein 2 (CRMP-2) was reduced in AAV2-NgR1-eGFP injected *ngr1*$^{-/-}$ optic nerves.

Conclusions
Our data suggest that NgR1 governs axonal degeneration in the context of inflammatory-mediated demyelination through phosphorylation of CRMP-2, abrogating axonal vesicular transport. Moreover, the axon-specific deletion of *ngr1* preserves axons blunting the induction of demyelination during EAE, thereby suggesting that NgR1-dependent neurodegeneration maybe a primary mechanism during neuroinflammation.

Keywords: Nogo; Neurodegeneration; Demyelination; Kinesin

10.20 – 10.40

Michael Lardelli – **Zebrafish models of AD: Why, how and what**
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Zebrafish models of Alzheimer's disease: Why, how, and what.

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An internet search using the strict phrase "mouse model of Alzheimer's disease" finds over 325,000 hits. A similar search in Pubmed finds over 6,000 papers spanning decades of research. So Hargis and Blalock's recent transcriptome data analysis showing little relationship between human Alzheimer's disease (AD) brains and brains from five commonly used transgenic AD mouse "models" has enormous implications for our understanding of the disease and for ongoing research using these mice (*Behav Brain Res*. 2016 May 4. pii: S0166-4328(16)30273-X). A "back to basics", objective approach is needed where we model as closely as possible the genetic state of familial Alzheimer's disease (fAD) rather than attempting to recreate what we believe are necessary histopathological signs of the disease state. Genome engineering technologies such as TALENs and CRISPR-Cas9 allow introduction of fAD-like mutations into a wide range of organisms including the increasingly popular laboratory model, the zebrafish. The biology of zebrafish presents a number of
advantages in disease modelling that are superior to any other commonly used backboned animal. These include outstanding reduction of genetic and environmental "noise" in 'omics analyses, their facility in screening of chemical libraries to identify therapeutic drug candidates, and their relatively low maintenance costs. Disadvantages include their poikilothermic physiology

and regenerative ability. I will describe the recent success of the Alzheimer's Disease Genetics Laboratory in generating fish with fAD-like mutations in endogenous genes. Our analyses of these genetic models of AD point to changes in glucose use and energy production as initial cellular stresses leading to the disease. Our results support a model of early onset fAD whereby 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 stressors such as hypoxia. Selfreinforcing pathological loops would then drive the brain into a neurodegenerative state that may not require accumulation of Amyloidβ.

10.45 – 11 am – Morning tea

11.00 -11.20

Jillian Kril – **Alcohol and the brain.**
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Alcohol and the brain

Discipline of Pathology, Sydney Medical School, University of Sydney, Sydney 2006

The harmful effects of excessive alcohol use have been recognised for many years and our early work showed regional specificity in the susceptibility of the brain to alcohol toxicity. In addition, we also demonstrated the important role of thiamin deficiency in the aetiology of alcohol-related brain damage. Since 2000 the NSW Brain Tissue Resource Centre (BTRC) has collected, characterised, stored and distributed tissue from patients with alcohol use disorders. Over 500 projects have received tissue from the BTRC resulting in more than 450 publications. The projects cover a broad range of research questions and have necessitated the continual refinement of the characterisation of donors. Recently, the Diagnostic and Statistical Manual (DSM) criteria for alcoholism were updated leading to a revision of diagnosis for all alcohol use disorders. On-going improvements in tissue quality and the breadth of clinical data available to researchers has markedly increased the scope of techniques applied to the study of alcohol-related brain damage.

11 am – 1.00pm – Session 2

11.20 – 11.40

Robert Vink – CTE after repeated concussive injury: mechanisms and targeted intervention.
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CTE after repeated concussive injury: mechanisms and targeted intervention

***Robert Vink**, Sansom Institute for Health Research, University of South Australia, Adelaide SA.*

Repeated concussion in contact sport has been associated with the development of the neurodegenerative condition known as chronic traumatic encephalopathy (CTE). CTE is characterized by accumulation of hyperphosphorylated tau protein, particularly around blood vessels within the depths of the sulci, and these tau tangles are thought to be responsible for the cognitive and behavioural manifestations that appear as the condition progresses. However, the mechanisms linking repeated concussive injury with tau hyperphosphorylation are unknown. In this presentation, we demonstrate the association between substance P (SP) release following repeated concussive injury and hyperphosphorylation of tau. In a rodent model of acceleration impact brain injury, three concussive (100 g) brain injuries resulted in SP release and accumulation of hyperphosphorylated tau to a level equivalent to that following a single moderate to severe brain injury. The perivascular SP release and associated tau hyperphosphorylation was prevented by pretreatment with a TRPV1 (mechanoreceptor) antagonist, but not by posttreatment. In contrast, a SP NK1 receptor antagonist administered after the injury prevented tau hyperphosphorylation by modulating the activity of several kinases linked to tau phosphorylation. Our results demonstrate that mechanical stimulation of perivascular sensory nerve fibres causes tau hyperphosphorylation via SP release and kinase activation. Given that maximal mechanical stress following a concussive injury occurs in the depths of the sulci, this NK1 mediated mechanism accounts for the tendency for tau accumulation in CTE to initially occur around blood vessels at the base of the sulci. Moreover, administration of an NK1 receptor antagonist after suspected concussive or sub-concussive injuries might represent the first pharmacological approach to attenuate the development of CTE.

11.40 – 12.00

Scott Ayton - **Biological metals as contributing factors in neurodegenerative diseases.**
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Iron in the pathogenesis of Alzheimer's disease

Scott Ayton, Martha Clare Morris, Ashley Bush

Background: Cortical iron deposition was first identified in post mortem cases of Alzheimer's disease (AD) in the 50's, and while this observation has been confirmed by multiple studies, it is not yet known whether elevated iron contributes to disease pathogenesis. Iron has the potential to aggravate the disease process by causing oxidative stress and accelerating aggregation of amyloid beta and tau, but the association between cortical iron levels and disease pathology, and clinical symptoms, has not been directly assessed. Here, we investigate the relationship between cortical iron and AD risk factors (amyloid plaque, neurofibrillary tangles, APOE ε4) and clinical presentation in the Memory and Aging Project (MAP).

Participants: MAP is an on-going clinical-pathologic, prospective epidemiologic cohort study of retirement community residents that began in Chicago in 2001. All participants receive annual evaluations including extensive interview data on a comprehensive set of AD risk factors, health and hospitalization history, cognitive testing, genomic data, clinical measurements and neurological evaluation. Brain donations from deceased participants are analysed for neuropathology using standardized disease criteria.

Results: In a cohort of 197 post mortem cases, iron level in the inferior temporal lobe was positively associated with tangle burden (not amyloid); an effect that was stronger in APOE ε4 carriers (p=0.0005) than non-carriers (p=0.03). The level of iron in the inferior temporal lobe was inversely associated with cognitive performance in the years prior to death (ave 6 years) as measured by annual cognitive composites of global cognition (p=0.0001), episodic memory (p=0.0085), sematic memory (p=0.0012) and working memory (p=0.0024; models were controlled for age, sex, APOE ε4, neuropathology burden).

Conclusions: Cortical iron load is strongly associated with AD pathology and clinical presentation.

12.00 – 12.20

Victor Villemagne – **Aβ and Tau in-vivo imaging**
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Aβ-amyloid and tau imaging

Victor L. Villemagne, MD

The introduction of in vivo imaging of Aβ–amyloid ((Aβ) pathology more than a decade ago, using derivatives of histopathological dyes, transformed the assessment of Alzheimer's disease (AD) allowing the evaluation of Aβ deposition over time by providing highly accurate, reliable, and reproducible quantitative statements of regional or global Aβ burden in the brain, and is currently being used for both patient recruitment and outcome measure in many anti- Aβ therapeutic trials. Aβ imaging validation against neuropathology paved the way for being approved for clinical use by the FDA and EMA. Aβ imaging studies has deepened our insight into Aβ deposition, showing that Aβ accumulation is a slow and protracted process extending for more than two decades before the onset of the clinical phenotype. Although cross sectional evaluation of Aβ burden does not strongly correlate with cognitive impairment in AD, Aβ burden does correlate with memory impairment and a higher risk for cognitive decline in the ageing population and MCI subjects. These associations suggest that Aβ deposition is not a benign process, part of normal aging. The recent addition of selective tau imaging is allowing ascertaining if these effects are directly associated with Aβ deposition or if they are mediated, *in toto* or *in parte*, by tau as it spreads out of the mesial temporal lobe into neocortical association areas. The combination of Aβ and tau imaging studies will likely help elucidate the relationship and/or interplay between the two pathological hallmarks of the disease. Longitudinal observations to assess their potential independent and/or synergistic, sequential and/or parallel effects on cognition, disease progression, and other disease-specific biomarkers of neurodegeneration will be required to further clarify the respective role of Aβ and tau deposition play in the course of Alzheimer's disease.

12.20 -12.40

Jamie Flynn – **Clearing the spinal cord and 3D imaging**
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Anatomical, structural, and molecular interrogation of biological structures has classically been performed using thin, essentially 2D sections of tissue to limit light scatter. Recent advances in whole-tissue clearing, such as CLARITY, CUBIC and 3DISCO, have made 3D histology possible in large tissue blocks. These procedures transform biological matter into a optically transparent, macromolecule-permeable material while maintaining the structure and position of proteins and nucleic acids. Furthermore, they are completely compatible with standard immunostaining techniques and small molecule dyes. Running in parallel with this development, lightsheet microscopy has progressed to the point where large, cleared 3D tissue samples can be rapidly visualised (minutes/hours) with single cell resolution. This is in contrast to traditional imaging methods such as confocal microscopy which can take days or even weeks to image large 3D tissue samples, and is associated with significant photobleaching. This talk summarises the latest developments in chemical tissue clearing methods, staining protocols, and lightsheet microscopy for application on animal and human tissues.

Lunch and poster viewing - 12.40 - 1.30pm

1.30 pm - 3.30 pm – Session 3

1.30 – 1.45
Shelley Forrest - FTLD-tau: Recent developments in pathology and pathogenesis.
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FTLD-tau: recent developments in pathology and pathogenesis

Shelley Forrest[1], Glenda Halliday[2], John Hodges[2], John B. Kwok[2], Maria Spillantini[3] and Jillian Kril[1].

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Genetic and familial forms of neurodegenerative disorders have provided important insights into the pathogenesis of many sporadic neurodegenerative disorders. A positive family history is present in up to 40% of frontotemporal dementia (FTD) patients and heritability varies between clinical syndromes. *MAPT* gene mutations account for ~20% of familial FTD cases and are associated with FTLD-tau of which four main pathological subtypes are recognised: 1) Pick's disease (PiD), 2) corticobasal degeneration (CBD), 3) progressive supranuclear palsy (PSP), and 4) globular glial tauopathy (GGT). Similarities in neuropathology between genetic and sporadic FTLD-tau suggest cases with a *MAPT* gene mutation should be considered as familial forms of FTLD-tau and on a disease continuum with sporadic FTLD-tau. According to Goldman pedigree classification criteria, 15% of FTLD-tau cases in the Sydney-Cambridge cohort have a suggested autosomal dominant pattern of inheritance, 23% have some family history and 62% are apparently sporadic. *MAPT* mutations were found in 9% of cases. FTLD-tau cases with a *MAPT* mutation were younger at symptom onset by 10 years (P=0.001), but did not differ in disease duration from cases without a *MAPT* mutation. GGT has the strongest degree of heritability, 40% having a suggested autosomal dominant pattern of inheritance followed by CBD (21%), PiD (11%) and PSP (6%). All four familial PSP cases were negative for a mutation in the *MAPT* gene. These data suggest that heritability influences age of symptom onset in FTLD-tau and varies between pathological subtypes. Further identification of a genetic link in cases with strong heritability await discovery.

1.45 – 2.00
Rachel Tan - TDP-43 pathology in behavioural variant frontotemporal dementia and amyotrophic lateral sclerosis.
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TDP-43 Proteinopathies: Pathological Identification of brain regions differentiating clinical phenotypes

Rachel H Tan[1,2], Jillian J Kril[3,4], Manaal Fatima[1,2,3], John BJ Kwok[1,2], John R Hodges[1,2,5], Matthew C Kiernan[6], Glenda M Halliday[1,2]

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Background. The pathological sequestration of TAR DNA-binding protein 43 (TDP-43) into cytoplasmic pathological inclusions characterises the distinct clinical syndromes of amyotrophic lateral sclerosis and behavioural variant frontotemporal dementia, while also co-occurring in a proportion of patients with Alzheimer's disease, suggesting that the regional concentration of TDP-43 pathology has most relevance to specific clinical phenotypes. This has been reflected in the three different pathological staging schemes for TDP-43 pathology in these different clinical syndromes, with none of these staging schemes including a preclinical phase similar to that that has proven beneficial in other neurodegenerative diseases. To apply each of these three staging schemes for TDP-43 pathology, the clinical phenotype must be known undermining the potential predictive value of the pathological examination.

Objective. The present study set out to test whether a more unified approach could accurately predict clinical phenotypes based solely on the regional presence and severity of TDP-43 pathology.

Methods. The selection of brain regions-of-interest was based on key regions routinely sampled for neuropathological assessment under current consensus criteria that have also been used in the three TDP-43 staging schemes. The severity of TDP-43 pathology in these regions-of-interest was assessed in four clinicopathological phenotypes: amyotrophic lateral sclerosis (n=27, 47-78 years, 15 males), behavioural variant frontotemporal dementia (n=15, 49-82 years, 7 males), Alzheimer's disease (n=26, 51-90 years, 11 males) and cognitively-normal elderly individuals (n=17, 80-103 years, 9 males).

Results. Our results demonstrate that the presence of TDP-43 in the hypoglossal nucleus discriminates patients with amyotrophic lateral sclerosis with an accuracy of 98%. The severity of TDP-43 deposited in the anterior cingulate cortex identifies patients with behavioural variant frontotemporal dementia with an accuracy of 99%.

Discussion and conclusion. This identification of regional pathology associated with distinct clinical phenotypes suggests key regions on which probabilistic pathological criteria, similar to those currently available for Alzheimer's disease and dementia with Lewy bodies, can be developed for TDP-43 proteinopathies. We propose and validate a simplified probabilistic statement that involves grading the presence of TDP-43 in the hypoglossal nucleus and the severity of TDP-43 in the anterior cingulate for the pathological identification of TDP-43 proteinopathy cases with clinical amyotrophic lateral sclerosis and behavioural variant frontotemporal dementia.

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2.00 – 3.00
Presentations – 1 hour

3.00– 3.30
Clinical Pathologic case studies – *When the neuropathology diagnosis is unexpected*

Catriona McLean, Alfred Health, Anatomical Pathology

Clinicopathological Conference - Disseminated necrotising leucoencephalopthy (DNL)

1975, Rubinstein et al., coined a new term, disseminated necrotizing leukoencephalopathy (DNL).
This was typically a progressive and fatal form of the neurological disease induced by intrathecal MTX injection therapy and irradiation. Cerebral white matter, cerebellum and brainstem, especially the pons were involved.
In 1995 Morris et al., described a similar process of multifocal calcifying leucoencephalopathy as a cause of progressive dementia in the absence of a history or MTX or irradiation. Further more recent studies have shown further linkage with intrathecal MTX and without radiaton. In a series of 185 primary CNS lymphoma patients,
the 5 year incidence of DNL was 24%. Clinically, this is characterised by a rapidly progressive subcortical dementia. A similar pathology has also been reported in glioma patients treated with BCNU. Grossly DNL shows multiple
chalky foci within the white matter. DNL consists of multiple small foci of demyelination and coagulative necrosis
that are randomly distributed in the cerebral white matter, cerebellar white matter, and brainstem white matter.
Axonal swellings (spheroids) are usually detected in the lesions. Disruption of the blood-brain barrier is a proposed mechanism in radiation induced change.

4.00 – 4.30

Winners of the Bill Evans Memorial "Young Investigator" Awards announced

4.30 – 5.00

ANZSNP Special General Meeting

5.00 - Close

Abstracts

Anti-hypertensive medication use is associated with less Alzheimer's and small vessel disease neuropathology

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Previous studies have suggested that antihypertensive medication use reduces the prevalence of Alzheimer's disease pathology in the brain, although this has often not been assessed in association with the severity of all forms of cerebrovascular disease. This study aimed to assess the effect of hypertension and anti-hypertensive medication use on both Alzheimer's disease and cerebrovascular disease neuropathology. Following ethics and tissue approvals, 149 brain autopsy cases from the Sydney Brain Bank were included using criteria of 1) 80 years of age or older at the time of death, 2) a neuropathologically confirmed diagnosis of Alzheimer's disease (n=96, 56 with cerebrovascular disease), cerebrovascular disease (n=92, 56 with Alzheimer's disease) or some age-associated pathologies (n=53, 36 with cerebrovascular disease), and 3) sufficient clinical information from medical records and longitudinal research reviews on hypertension status and medication use. Severity of neuropathologies was assessed using standardized staging, and non-parametric and stepwise regression statistics were used. The largest association of pathology was with reported antihypertensive medication use, with those not taking these medications being 12 times more likely to have a severe stage of small vessel disease and 3.7 - 3.8 times more likely to have a severe stage of neurofibrillary tangle and neuritic plaque formation. There was a correlation between increased severities of small vessel and neuritic pathologies. In contrast, a history of hypertension had no significant association with these pathologies. Overall, our data suggest that anti-hypertensive medication use is potentially protective, with users having significantly lower severities of both Alzheimer's and small vessel disease neuropathologies. These findings extend previous studies showing reduced Alzheimer neuropathology with the use of angiotensin receptor blockers for treating hypertension, but also suggest that studies concluding that hypertension increases Alzheimer pathology need to be reassessed to include anti-hypertensive medication effects.

Novel Mouse Model of an ALS-associated PFN1 Mutation

Keywords: Profilin 1, ALS

Merryn Brettle[1,2], Holly Stefen[1], Josephine Chan[1], Aleksandra Djordjevic[1], Fabien Delerue[2,3], Yazi Ke[4], Lars Ittner[2,3,5], Thomas Fath[1]

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Amyotrophic lateral sclerosis is the most common form of motor neuron disease. Sporadic and familial forms of disease present with similar clinical symptoms and histopathology. Understanding the underlying pathogenesis of the disease is essential for the development of treatments. Mutations in profilin 1 have been identified as a rare cause of familial ALS, but how these mutations cause ALS is unknown. We have developed a novel mouse model to elucidate the role that PFN1^{C71G} plays in ALS. Expression of V5-tagged PFN1^{C71G} was targeted to α-motor neurons in the spinal cord. Initial data shows V5- PFN1^{C71G} expression in the anterior horn of the neural tube starting from embryonic stages in transgenic mice. Motor testing shows that transgenic mice have progressive motor deficits on RotaRod commencing at 2 months of age. This novel mouse model of PFN1^{C71G} will provide a potential tool to understand the role that PFN1 plays in the pathogenesis of ALS and
could be used for testing future ALS therapeutics.

Alzheimer's disease cerebrospinal fluid biomarkers are not influenced by gravity drip or aspiration extraction methodology

Alan Rembach[1**], Lisbeth A. Evered[2], Qiao-Xin Li[1], Tabitha Nash[1], Lesley Vidaurre[1], Christopher J. Fowler[1], Kelly K. Pertile[1], Rebecca L. Rumble[1], Brett O Trounson[1], Sarah Maher[2], Francis Mooney[2], Kevin Taddei[3], Stephanie Rainey-Smith[3], Simon M. Laws[3], S. Lance Macaulay[5], William Wilson[6], David G. Darby[1], Ralph Martins[3], David Ames[7], Steven Collins[4], Brendan Silbert[2], Colin L. Masters[1], James D. Doecke[5,6] and the AIBL Research Group[8]

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Cerebrospinal fluid (CSF) biomarkers, although of established utility in the diagnostic evaluation of Alzheimer's disease (AD), are known to be sensitive to variation based on pre-analytical sample processing. We assessed whether gravity droplet collection versus syringe aspiration was another factor influencing CSF biomarker analyte concentrations and reproducibility. Standardized lumbar puncture and CSF collection using gravity fed collection followed by syringe aspirated extraction was performed in a sample of individuals participating in a large long-term observational research trial. Analyte assay concentrations were compared. For the 44 total paired samples of gravity collection and aspiration, reproducibility was high for biomarker CSF analyte assay concentrations (concordance correlation): A [illegible]42 (Aβ42) 0.83, T-tau 0.99, and phosphorylated tau (P-tau) 0.82; and Bonferroni corrected paired sample t-tests showed no significant differences in group means (SD): Aβ42 366.5 (86.8) vs 354.3 (82.6), p = 0.10; T-tau 83.9 (46.6) vs 84.7 (47.4) p = 0.49; P-tau 43.5 (22.8) vs 40.0 (17.7), p = 0.05). The mean duration of collection was 10.9 minutes for gravity collection and <1 minute for aspiration. Our results demonstrate that aspiration of CSF is comparable to gravity droplet collection for AD biomarker analyses but could considerably accelerate throughput and improve the procedural tolerability for assessment of CSF biomarkers.

The effect of alcohol exposure following repeated concussion

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A history of repeated concussion has been linked to the later development of the neurodegenerative disease, chronic traumatic encephalopathy (CTE), which is characterised by the accumulation of hyperphosphorylated tau. How repeated concussion promotes neurodegeneration remains poorly understood and external factors may play a role in disease development. Of particular interest is the role of alcohol consumption, with up to a quarter of people consuming alcohol following a concussion, which enhances neuroinflammation and worsens outcome. However the effect of alcohol following repeated concussion is unknown. To investigate male Sprague-Dawley rats were subject to three concussions 5 days apart using the diffuse impact-acceleration model to generate ~100G. Sham animals underwent surgery only. 5 days following last injury alcohol (3.5mg/kg or 5mg/kg) or dextrose via oral gavage was administered, with rats perfuse-fixed 24hrs later for examination of levels of inflammation and phosphorylated tau (ptau). A significant increase in ptau within the cortex was only seen in repeated concussion animals administered 5mg/kg of alcohol ($p<0.05$ compared to vehicle controls), with no effect of the lower dose of alcohol. Intriguingly enhanced inflammation, as assessed by the number of GFAP positive cells was only seen in the repeated concussion animals treated with 3.5mg/kg of alcohol, with the higher dose (5mg/kg) appearing to reduce the number of GFAP positive cells. No effects of alcohol were seen in sham animals. Alcohol appears to have dose-dependent and contradictory effects on inflammation and tau phosphorylation following repeated concussion, with further research needed to determine whether this has functional consequences.

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.

Co-existing Lewy body disease and clinical parkinsonism in frontotemporal lobar degeneration

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Frontotemporal lobar degeneration (FTLD) is associated with two underlying pathologies; phosphorylated tau (FTLD-tau) or TAR-DNA binding protein-43 (FTLD-TDP). Within each group there are distinct pathological subtypes based on the morphology, distribution and biochemical composition of cellular inclusions. The most common subtypes of FTLD-tau include Pick's disease (PiD); corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP). Four FTLD-TDP subtypes are recognised: Types A-D. A limited number of studies report FTLD cases with additional alpha-synuclein pathology in the form of multiple system atrophy (MSA) or Lewy body disease (LBD). This study investigated the prevalence of MSA pathology and clinically relevant LBD in subtypes of FTLD cases from the Sydney Brain Bank (n=126). Using a variety of histochemical stains to characterise pathology, cases were also subtyped and scored for Alzheimer's disease (AD) neuropathologic change according to current recommendations. No cases were identified with co-existing MSA. Nine cases were identified with co-existing LBD ≥ Braak stage IV. These were associated with PiD (n=2), CBD (n=2), PSP (n=2) and TDP-Type A (n=3), all of which had similar distributions of FTLD pathologies as pure FTLD cases. AD neuropathological change was absent in seven cases and low in two cases with coexisting LBD. Co-existing MSA is rare in FTLD, and coexisting LBD in FTLD comprises a small proportion of cases with implications for clinical and neuropathological diagnoses. Accurate prediction of underlying pathology, or combination of pathologies, is needed for developing therapeutic interventions and identifying biomarkers for FTLD.

Alpha-Synuclein toxicity is abolished by liproxstatin-1 through a ferroptosis-independent mechanism

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Background: There is good evidence that **a**lpha-synuclein (αsyn) and iron both contribute to neurotoxicity in Parkinson's disease (PD). However, it is unknown how they cause cell death, and whether the toxicity of αsyn and iron is synergistic or independent.

Objective: To investigate whether αsyn causes neurodegeneration by ferroptosis (a recently identified iron-dependent cell death pathway).

Methods: Toxicity of pre-formed fibril preparations of αsyn was assessed in multiple immortalised and primary culture cell lines (C57Bl/6/129sv mouse primary cortical neurons, STHdh$^{Q7/7}$ cells and immortalised astrocytes). To determine whether αsyn caused cell death via ferroptosis, αsyn was co-administered with ferroptosis inducers (iron and buthionine sulphoximine), and ferroptosis inhibitors (desferrioxamine, reduced-glutathione, ferrostatin-1 and liproxstatin-1). The impact of these ferroptosis modulators on the toxicity of αsyn was compared to their impact on the toxicity of erastin (potent ferroptosis inducer). Ferroptotic markers (lipid peroxidation, iron and glutathione levels) were measured in the intoxicated cells.

Results: Liproxstatin-1 (potent ferroptosis inhibitor) dose-dependently abolished αsyn and erastin toxicity. However, unlike erastin, αsyn toxicity was not modulated by any of the other inducers or inhibitors of ferroptosis. This implied that αsyn causes cell death by a ferroptosis-independent pathway, despite the complete rescue by liproxstatin-1. We hypothesise that liproxstatin-1 binds αsyn to confer neuroprotection.

Conclusion: That αsyn and iron act independently to cause death in cell models of PD. Liproxstatin-1 could be a compound that protects against both types of lesion.

Synapse dysfunction of layer V pyramidal neurons precedes neurodegeneration in a mouse model of TDP-43 proteinopathies

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TDP-43 is the primary component of characteristic neuronal inclusions in frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS); the TDP-43 proteinopathies. Previously, research has focused on the nuclear role of the protein and the toxicity of cytoplasmic aggregates-however, research now indicates TDP-43 mis-processing pathologically impacts the synapse. We report a crucial role for TDP-43 in cortical dendritic spine formation at the synapse. Immunohistochemistry, confocal microscopy and Neurolucida spine analysis software were utilised at post-natal (P) days 30, 60 and 90 in Thy1-YFPH and Thy1-YFPH::TDP-43A315T mice to investigate dendritic spine density and morphology in the motor and somatosensory cortices. Dendritic spine density in the motor and somatosensory cortices of Thy1-YFPH mice increased from P30, to peak at P60 prior to significant pruning at P90. Conversely, in Thy1-YFPH::TDP-43A315T mice density was reduced at P60 in the motor cortex, prior to over symptoms and cell death. At P90 (symptom onset), dendritic spine density was apparent in both the motor and somatosensory cortices, and accompanied by motor cortex-specific cell loss. Analysis of dendritic spine morphological subsets revealed impaired development of mature mushroom-type spines within the Thy1-YFPH::TDP-43A315T mouse motor cortex. These impairments to

dendritic spine density and maturation were associated with lowered efficacy of synaptic transmission at P60, indicating the mis-processing of TDP-43 at the synaptic compartment has pathological effects on viable neural transmission and cortical plasticity. To further probe this, cranial window surgical techniques have been optimised in the motor and somatosensory cortices. In conjunction with 2-photon imaging, Thy1-YFPH mice will be employed in order to investigate innate cortical regional differences in the dynamics of dendritic spines. The physiological alterations quantified in these mice will be compared to spine turnover and morphology in Thy1-YFPH::TDP-43A315T mice, to pin-point the earliest pathological effect of protein mutations on spine dynamics in real time.

Is reduced endo/lysosomal function a risk factor for early-onset neurodegeneration?

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Mucopolysaccharidosis type IIIA (Sanfilippo syndrome) is an autosomal recessive childhood-onset neurodegenerative lysosomal storage disorder resulting from a mutation in the gene encoding the lysosomal enzyme sulphamidase (SGSH). Recently, dysfunction of the endo/lysosomal system was suggested to be a factor contributing to the pathogenesis of common later-onset neurodegenerative disorders such as Parkinson's disease (PD), with heterozygous mutations in several lysosomal enzyme genes found in PD patients. To explore this postulate further, we evaluated motor function and brain pathology in mice carrying a D31N mutation in the SGSH gene, which reduces enzyme activity to ~50% wildtype-levels. Female heterozygotes and wildtype mice aged 12, 15, 18 and 21-months of age were examined. There was no difference in the performance of heterozygous and wildtype mice in the open field, elevated plus maze, gait or grip-strength tests. However, impaired performance in the negative geotaxis test was observed, with heterozygous mice exhibiting increased latency to right themselves and increased incidence of falls from the grid. Upon euthanasia, mass spectrometry-based methods determined that there was no evidence of substrate accumulation in SGSH D31N heterozygote brain tissue. Additional brain samples were immunostained with antibodies towards α-synuclein, pTau, Aβ1-42, amyloid precursor protein, cathepsin D, glial fibrillary acidic protein, ubiquitin, lysosomal integral membrane protein-2 and p62. Histochemistry for isolectin B4 was performed, along with PAS, H&E and modified Golgi-Cox stains, and an evaluation of the accumulation of autofluorescent material was carried out. Quantitative or semi-quantitative analyses indicated that whilst expression of many of the disease markers changed significantly with age, there was no difference in staining pattern/intensity seen in heterozygotes and wildtype mice at any age. Similarly, counts of cerebellar purkinje cell numbers returned equivalent outcomes in mice with the two genotypes. Cerebral cortex thickness measurements and pyramidal neuron dendrite analyses however, indicated that there were subtle changes in cortical architecture in mice carrying the D31N mutation. In summary, brain tissue from female SGSH D31N heterozygotes aged 12-21 months does not exhibit the gross features of common neurodegenerative disorders, but appears to undergo subtle structural change.

Overcoming monocarboxylate transporter 8 (MCT8)-deficiency to promote human oligodendrocyte differentiation and myelination

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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.

Expression of MND-linked mutant *CCNF* in zebrafish leads to increased cell death in the spinal cord and an abnormal Motor Phenotype

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Motor Neuron Disease (MND) is a rapidly progressive, fatal neurodegenerative disease characterised by the loss of upper and lower motor neurons. Approximately 10% of MND cases have a known family history of the disease and mutations in multiple genes have been identified. MND-linked mutations in *CCNF* were recently reported, however the pathogenic mechanisms associated with these mutations are yet to be established. Proteomic analysis of a neuronal (Neuro-2a) cell line expressing mutant *CCNF* identified the disruption of several cellular pathways, including caspase-3 mediated cell death and axonal outgrowth. Supporting the *in vitro* findings, transient overexpression of mutant *CCNF* in zebrafish embryos led to increased caspase-3 activity and cell death in the spinal cord and a motor neuron axonopathy characterised by shortened primary motor axons and an increased incidence of aberrant axonal branching. Consistent with these observations, a significantly impaired motor response was identified in these zebrafish. This is the first assessment of an MND-linked *CCNF* mutation *in vivo* and indicates that zebrafish will be a useful tool to model the pathogenesis of *CCNF*-linked motor neuron degeneration. (176 words).

A transgenic zebrafish model of spinocerebellar ataxia-3 develops neuropathology and motor dysfunction

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Spinocerebellar ataxin-3 (SCA-3), also known as Machado Joseph disease, is a neurodegenerative disease characterized by loss of motor co-ordination and balance, paralysis and eventually death. Pathological examination of the patient tissue reveals neuronal protein aggregates. SCA-3 is caused by inheritance of an abnormal form of the ATXN3 gene, which contains a long trinucleotide (CAG) repeat region, encoding a polyglutamine (polyQ) region within the ataxin-3 protein. We have successfully established the first transgenic zebrafish model of SCA-3. These zebrafish express human ataxin-3 containing either 23 glutamines (23Q, wild-type) or 84Q (SCA-3). Because SCA-3 is a progressive disease with adult onset we monitored our transgenic zebrafish throughout aging and identified a marked motor phenotype from 4 months old. From 4 months to 12 months old ataxin-3 84Q zebrafish tended to swim shorter distances than ataxin-3 23Q zebrafish during monthly swim tests. At 12 months old (mid-adulthood for zebrafish) the SCA-3 zebrafish (n=28) swam significantly shorter distances than ataxin-3 23Q (n=28) and non-transgenic controls (n=13) ($p<0.004$). Euthanasia and immunohistochemical processing of the brains and spinal cords of the zebrafish revealed a neuritic beading aggregation phenotype within the medulla of ataxin-3 84Q zebrafish, but not ataxin-3 23Q or non-transgenic controls (n=6 each group, $p<0.0001$). Immunostaining for ataxin-3 produced a similar result ($p<0.001$). An ataxin-3-positive neuritc beading staining phenotype has previously been reported in SCA-3 patients samples. Our results demonstrate that our transgenic zebrafish model of SCA-3 is relevant to human SCA-3 and could be used in the search for a cure for the disease.

Whole transcriptome analysis shows involvement of immune response and inflammation in TDP-43-related frontotemporal lobar denegeration (FTLD-TDP)

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Frontotemporal lobar degeneration (FTLD) is characterized by the progressive changes in behavior and personality, executive dysfunction, and decline of language skills. Although histopathologically diverse, the majority of FTLD cases have abnormal cytoplasmic accumulation of the nuclear RNA-binding protein TDP-43 (FTLD-TDP). TDP-43 plays crucial roles in several steps of RNA metabolism, but the extent to which TDP-43 pathology impacts the brain transcriptome during disease remains unclear. In this study, we used strand-specific RNA-Seq technology to investigate changes in the transcriptome profile of FTLD-TDP human brain tissue from the NSW Brain Banks (following institutional ethics and tissue approvals). Our results revealed that genes differentially expressed (DE) in FTLD-TDP are mostly upregulated (69%) and protein-coding genes (78%). We found that cell death and survival, cell signaling and interaction, protein synthesis, and molecular transport are the most enriched cellular functions altered in FTLD-TDP, and that immune response and inflammation-related pathways are particularly affected in this disease. We analyzed publically available single-cell RNA-Seq datasets derived from healthy brain cell types, and found a greater level of dysregulation among microglia- and neuron-specific genes (10 and 9 genes dysregulated, respectively). Furthermore, we

saw that microglia- and astrocyte-specific genes are mostly upregulated in FTLD-TDP, while genes specifically expressed in neurons and oligodendrocytes show a trend for downregulation. Together, our data suggest that immune response and inflammation might play a major role in FTLD-TDP, and that the impact is greatest on the transcriptomes of neurons and microglia.

The correlation between ß-amyloid PET-imaging and cerebrospinal fluid biomarkers is affected by the assay design

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Attempted neuropathological confirmation of Alzheimer's disease (AD) can reveal the presence of co-morbidities or potential discrepancies with the clinical diagnosis. Cerebrospinal fluid (CSF) biomarkers provide a low cost alternative to PET imaging for *in vivo* quantification of different hallmarks (e.g., Aβ amyloidopathy, neurofibrillary tangles, axon loss) in the brains of affected subjects. By assessing CSF biomarker scores derived from three different versions of immunoassays, the current study explores the premise that the degree of correlation between ^{11}C-Pittsburgh Compound B (PiB) PET-Imaging and the CSF biomarkers might be affected by the assay design. CSF was extracted via gravity feed as per standard procedures and analysed using CE-registered single analyte ELISAs (EUROIMMUN (Aß1-40, Aß1-42, total tau); INNOTEST (Aß1-42, total tau, P-Tau181P)) or the multiplex INNO-BIA AlzBio3 xMAP assay (Aß1-42, tau, P-Tau181P). Statistical analyses of biomarkers focused on group-wise comparisons for PET status (PET –ve N=37, PET +ve N=42) within platform, and predictive performance for combinations of markers. The newly designed assays from EUROIMMUN showed a stronger separation capability for Aß1-42 group comparisons as compared to the INNOTEST and AlzBio3 platforms. PET status comparisons for the INNOTEST and AlzBio3 assay were comparable. Of the individual markers, Aβ42 performed the strongest, with sensitivity and specificity ranging between 71 and 94%. The performance of the Aβ42/t-tau ratio was stronger than Aß1-42 alone, with sensitivity and specificity highest for the EUROIMMUN platform (sensitivity 100%, 84.4% specificity, compared with the INNOTEST (sensitivity 87.5%, specificity 90.6%), and the AlzBio3 (sensitivity 93.8%, specificity 74.2%)). Selection of optimal multivariable models to predict PET status using the EUROIMMUN assays confirmed the use of the Aß1-42/t-tau ratio with age, gender and apoe4 status as the strongest combination, with cross validated sensitivity and specificity of 97%.

According to statistical assessment of the three platforms, we hypothesise that the design of the assay has an impact on the correlation between Aß-PET imaging and CSF biomarker analysis.

Enhanced Heparin Binding Affinity Of APP96-110 Results In Increased Neuroprotection Following Traumatic Brain Injury

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Following traumatic brain injury (TBI), neurological damage is serious and ongoing. Recently, the amyloid precursor protein (APP) derivative APP96-110 has shown encouraging neuroprotective activity following TBI, believed to be due to its heparin-binding properties. It is hypothesised that mutation of key amino acid residues responsible for these properties could enhance its binding ability and subsequent therapeutic efficacy. To determine this, key residues were mutated on wildtype APP96-110, and its heparin-binding affinity assessed via chromatography assay. Its efficacy was then assessed *in vivo* following moderate-severe diffuse impact-acceleration injury in rats. A single dose of either wildtype APP96-110 (0.05m/kg or 0.5mgkg) or mutated APP96-110 (0.05mg/kg, 0.25mg/kg, 0.1mgkg or 0.5mg/kg) was administered intravenously at 5 hours post-TBI. Rats were assessed daily for motor deficits using the rotarod, before brains were perfused fixed following 3 days. Following TBI, rats treated with wildtype APP96-110 demonstrated significant improvements in motor outcome over 3 days when compared to vehicle control rats. Treatment with 0.25mg/kg of mutated APP96-110 significantly improved motor performance post-TBI when compared to vehicle control rats, reaching a similar level as the 0.5mg/kg wildtype APP96-110 treatment. A similar trend was seen throughout, where lower doses of mutated APP96-110 showed improved motor outcome on a par with higher doses of wildtype APP96-110. Unexpectedly, the highest dose of mutated APP96-110 (0.5mg/kg) was unable to improve motor outcome, indicating a maximal therapeutic threshold. These results indicate that through enhancing the heparin-binding affinity of APP96-110, lower doses may be used to improve its neuroprotective efficacy *in vivo* post-TBI.

Involvement of striatal indirect pathway beyond motor control

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The striatum is the brain region critical in regulating movement and has two major outputs, known as the direct and indirect pathways. The direct pathway directly inhibits dopamine neurons, which facilitates movement. Whereas the indirect pathway projects to the globus pallidus (GP) which then relays to the subthalamic nucleus (STN), glutamatergic inputs from the STN excites dopamine neurons and inhibits motor output. These pathways work in concert to exert well-balanced control over movement. Disturbances in these neural pathways are hallmarks of Parkinson's Disease. In addition to motor control, basal ganglia connectivity also influences motivation, cognition and reward seeking behaviour. PD patients also suffer from anxiety, motivation and cognition deficits. Deep brain stimulation of Subthalamic nucleus (STN) and Globus pallidus (GP) are current therapeutic surgical procedures for patients with Parkinson's disease. Yet the effects of STN and GP manipulation on non-motor behaviours are clear. We found simultaneous chemogenetics inhibition of ventral GP and STN has no effect on locomotor, however significantly reduces motivation for reward (alcohol) seeking. Consistent with other studies our results show that inhibition of these brain regions does not effect on motor control. These results demonstrate that these brain regions are important for motivational behaviour.

Hyperactivity and Dysinhibition-like behaviour in a P301S Tau-Transgenis mouse model of frontotemporal dementia

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Frontotemporal dementia (FTD) is clinically present with behavioral changes, including a loss of interest, social disinhibition and hyperactive behavior. Approximately 40% of FTD patients report a familial history of dementia, with the most frequent mutations found in the tau-encoding MAPT gene. Under physiological conditions, tau promotes and stabilizes microtubule assembly and thereby regulates their dynamics, intracellular trafficking and cell signalling. However, in disease, tau becomes abnormally phosphorylated, dissociates from microtubules and eventually aggregates into so called neurofibrillary tangles, a common neuropathological feature found in FTD patients with tau pathology.

Following the discovery of mutations in the MAPT- gene, transgenic mice expressing human mutant tau have been generated and used to study disease pathogenesis of the human condition. We have recently reported a detailed neuropathological and functional characterization of a novel tau transgenic mouse strain, TAU58/2, carrying the mutant human P301S mutation. The TAU58/2 mice develop early- onset motor deficits accompanied by axonal pathology prior to tau deposition, features reminiscent of human FTD with tau pathology.
In present study, we show that TAU58/2 mice develop early onset of disinhibition-like behaviour in the elevated plus maze and hyperactivity in the open field arena. Furthermore, those behavioural changes were accompanied by an early onset of tau pathology in the amygdala, characteristic features of human FTD with tau pathology.

Learning deficits in the TAU58/2 mouse model correspond to deficits in long-term potentiation formation

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Neuronal functional deficits and neurofibrillary tangle formation occur in Alzheimer's Disease (AD) and frontotemporal lobar degeneration (FTLD) in the hippocampus. The hippocampus is a brain region important for learning and memory. During learning, hippocampal neurons undergo synaptic plasticity with long-term potentiation (LTP). Behavioral and electrophysiological experiments were undertaken to establish whether the novel TAU58/2 transgenic mouse model, which mimics the histopathological features of AD and FTLD, presents with impairments in learning and memory formation due to functional deficits.

Tau58/2 male mice (2, 4 and 6 month-old) underwent learning/memory testing using Morris Watermaze (MWM). Electrophysiological analysis with theta-burst stimulation on brain slices from 1 and 4 month-old TAU58/2 mice were used to determine LTP in the hippocampus (Schaffer collateral pathway to CA1 region) compared to wildtype littermates.

The 4 month-old and 6 month-old Tau58/2 mice show significantly impaired learning/memory formation compared with wildtype littermates in the MWM ($P<0.05$). These mice take longer to find the platform at both ages and 6 month-old mice spend less time moving towards the platform ($P<0.05$). Furthermore, we found deficits in LTP formation observed in brain slices from the 4 month-old mice ($P<0.01$). Taken together, this data is indicative of progressive worsening of tau pathology and neuronal functional deficits seen in the TAU58/2 mouse model.

Learning/memory formation deficits are observed at the same age when decreased LTP formation was detected in the hippocampus of TAU58/2 mice.

Characterising the temporal profile of cerebral oedema and intracranial pressure following stroke in an ovine model

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Background and Aims: Stroke is a leading cause of mortality and the most common cause of neurological disability worldwide largely attributed to abnormal accumulation of fluid in the cerebral parenchyma, or cerebral oedema. Cerebral oedema is the principal cause of death within one week of stroke and carries a mortality rate of up to 80%. The deleterious effect of space-occupying swelling precedes elevations in intracranial pressure (ICP) and secondary neurological deterioration, resulting in irreversible brain tissue damage and death. Despite the devastating consequences of cerebral oedema and elevated ICP, current treatments are limited and fail to address the underlying pathophysiology of the swelling, thus highlighting the need for development of targeted treatments. When screening novel agents it is essential to use clinically relevant models and establish the temporal profile of cerebral oedema and elevated ICP evolution following stroke. To address this, our laboratory has established a novel large animal model of stroke in the sheep, to take advantage of the large human-like brain, which has a gyrencephalic structure, extensive white matter domains and strong tentorium, all of which more closely replicate the human brain than largely adopted rodent models. As such, the aim of the current study was to determine the temporal profile of cerebral oedema and elevated ICP following transient stroke in our clinically relevant ovine model.

Methodology: 46 anaesthetised Merino sheep (21M, 25F; 64.70kg±7.42kg; 18-36mths) underwent 2hrs middle cerebral artery occlusion (MCAo) with reperfusion or sham surgery. Animals were randomized into either sham or 1, 2, 3, 4, 5, 6, 7 day survival time-points. At 4hrs prior to the terminal time-point animals were re-anaesthetised and ICP, blood gas and blood pressure monitored for a period of 4hrs before undergoing magnetic resonance imaging (MRI) to determine volume of infarct (T2) and cerebral oedema (FLAIR). Following MRI, animals were perfused under anaesthesia and brains removed and processed for hisopathological analysis.

Results: ICP recordings and MRI scans were normal in sham animals. Following stroke, ICP rose gradually over time and by 5d was significantly elevated compared to sham animals ($p<0.0001$). Profound cerebral oedema was observed as early as 2d post-stroke and continued to evolve out to 5d, in keeping with the increasing ICP. At 7d post-stroke, both the ICP and extent of cerebral oedema showed a trend towards return to baseline.

Conclusions: Our study has shown that ICP and cerebral oedema peak at 5d following transient stroke in our clinically relevant Ovine model. Such findings suggest that novel therapeutic agents targeting cerebral oedema and elevated ICP will likely be effective in reducing complications when administered prior to 5d post-stroke. As such, our future studies will focus on examining the efficacy of an NK1 tachykinin receptor antagonist at 5d post-stroke. This treatment aims to prevent pathological development of cerebral oedema and elevated ICP and thus improve the likelihood of successful clinical translation to reduce death and disability following stroke.

Diagnostic evaluation of Alzheimer's disease CSF biomarkers in the AIBL cohort and development of a CSF oligomeric -synuclein assay for Parkinson's disease

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The cerebrospinal fluid (CSF) Aβ42, total-tau (T-tau) and phosphorylated-tau (P-tau) profile has been established as a valuable biomarker for Alzheimer's disease (AD). The current study aimed to determine CSF biomarker normative reference ranges using positron emission tomography (PET) Aβ imaging screened subjects from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of ageing, and evaluate the diagnostic utility in our laboratory. The study included 157 AIBL participants who underwent CSF collection and PET imaging, utilizing ^{11}C-Pittsburgh Compound B, ^{18}F-flutemetamol, or ^{18}F-florbetapir, to determine the Aß pathology. Using an INNOTEST assay, normative references were established (Aβ42 >544 ng/L, T-tau <407 ng/L and P-tau <78 ng/L) employing a rank based method to define a "positive/abnormal" CSF in the sub-cohort of amyloid-PET negative healthy participants (N=97), and compared with the presence of PET demonstrated AD pathology. CSF Aβ42 was the strongest individual biomarker, detecting cognitively impaired PET positive mild cognitive impairment (MCI)/AD with 85% sensitivity and 91%

specificity. Cross-validated accuracy, using all three biomarkers or the ratio of P-tau or T-tau to Aβ42 to predict MCI/AD with Aβ pathology, reached ≥92% sensitivity and specificity. CSF Aβ42 levels and analyte combination ratios demonstrated very high

correlation with PET Aβ imaging regardless of ligand. Our study offers additional support for CSF biomarkers in the early and accurate detection of AD pathology, including enrichment of patient cohorts for treatment trials even at the pre-symptomatic stage.

In addition, recognizing an unmet need in relation to clinically accessible diagnostic biomarkers for Parkinson's disease (PD) and building on promising preliminary reports, we are developing CSF assays centred on oligomeric -Synuclein, a key player in PD pathogenesis, with the aim of assisting diagnosis at first presentation or even at the preclinical stage.

Regional and temporal analyses reveal biochemical alterations associated with TDP-43 pathology in a mouse model of motor neuron diease

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Motor neuron disease (MND)/amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease caused by the loss of brain and spinal cord motor neurons. Almost all MND patients are characterised at autopsy by the presence of pathology containing the RNA/DNA-binding protein TDP-43. However, the causes and consequences of TDP-43 pathology development remain unclear. Newly developed transgenic TDP-43 mouse models expressing cytoplasmically-targeted TDP-43 in the brain and spinal cord (rNLS mice) develop, for the first time, both ALS-like TDP-43 pathology and disease phenotype. Using advanced quantitative sequential windowed data-independent acquisition of total high-resolution mass spectra (SWATH-MS) mass spectrometry, we profiled proteomic changes in brain and spinal cord of transgenic rNLS TDP-43 mice compared with litter-matched controls at pre-symptomatic, early-, mid- and late-symptomatic disease stages. Quantitative data were obtained from multiple peptides for >1500 proteins derived from soluble and insoluble protein fractions extracted following sonication and sequential ultra-high speed centrifugation. Of these, >60 proteins were detected with >1.5x statistically significant difference between transgenic rNLS TDP-43 mice and controls, covering novel proteins and those previously associated with MND, including the chaperone protein disulphide isomerase (PDI). Protein changes were validated using immunoblotting and immunofluorescence in neuronal cell culture, mouse brain and spinal cord and human ALS autopsy tissues, revealing a set of protein changes associated with disease onset and progression. These findings provide valuable insight into the early drivers of disease pathogenesis, and highlight multiple new biochemical pathways for investigation of potential disease modifiers with the goal of developing new ALS therapeutics.

Caspase inhibition is protective in an in vivo model of spinocerebellar ataxia type 3

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, is an autosomal dominant neurodegenerative disease, proven to be fatal with no current treatment nor cure. SCA3 is caused by a CAG trinucleotide repeat region within the gene ATXN3, encoding for an expanded polyglutmaine (polyQ) stretch within the ataxin-3 protein. Amongst the healthy population, CAG regions range between 1-40 repeats and SCA3 patients contain CAG repeat regions greater than 40 CAG. We have previously established a transgenic zebrafish model of SCA3. Protein lysates extracted from these transgenic zebrafish contain full-length human ataxin-3, as well as smaller fragments of ataxin-3. Within this study we have tested the effect of prolonged treatment of our transgenic SCA3 zebrafish with a caspase inhibitor compound (zVADfmk). Short-term (2 day) treatment with zVADfmk had no effect on the amount of ataxin-3 cleavage fragments within our transgenic zebrafish samples. However, treating our SCA3 zebrafish with zVADfmk until they were 6dpf resulted in a decrease in the amount of full-length ataxin-3 and a decrease in levels of the autophagy substrate p62 (indicative of positive autophagic flux). Treatment with zVADfmk also produced a significant increase in the distance swum by the SCA3 zebrafish during escape response to darkness tests. These results suggest that, like calpain inhibition within our previous studies, long term caspase inhibition decreases levels of human ataxin-3 and improves motor function, likely due to induction of autophagy. These results suggest that autophagy induction may be a potential target to therapeutic treatment for SCA3 patients.

Attendees

Affleck, Andrew
Ayton, Scott
Beard, Helen
Blizzard, Catherine
Brettle, Merryn
Cappai, Roberto
Chua, Sook Wern
Clarke, Rosie
Collins, Steven
Corrigan, Frances
Crockford, Daniel
Cummins, Tia
D'Arcy, Colleen
Claude Dennis
Dickson, Tracey
Fabian, Vicki
Fiddler, Caroline
Flynn, Jamie
Forrest, Shelley
Guiney, Stephanie
Halliday, Glenda
Handley, Emily
Hemsley, Kim
Hogan, Alison
Kim, Min (Erica)
Kril, Jilian
Laird, Anglea
Lau, Queenie
Lardelli, Michael
Li, Qiao-Xin
Lim, Julia
Leonard, Anna
Lourenco, Guinevere
McLean, Catriona
Naroozi, Ladan
Petratos, Steven
Phipps, Andrew
Plummer, Stephanie
Prasad, Aseeta
Przybyla, Magdalena
Robertson, Thomas
Rodriguez, Michael
Saxon, Sarah
Suh, Lisa
Sutherland, Greg
Sorby-Adams, Annabel
Tan, Rachel
Thomas, Speros
Turner, Brad
Turner, Renee
Van der Hove, Julia
Varghese, Shiji
Villemagne, Victor
Vink, Rob
Volkerling, Alexander
Walker, Adam
Watchon, Maxine
Woodhouse, Adele
Zhao, Ye