Poster Abstracts

Bangfu Zhu

In vivo characterization of candidate genes associated with Alzheimer's disease using Drosophila

We used Drosophila to functionally screen through genes identified by recent AD-GWAS and EWAS for disease-relevant phenotypes. Novel genes identified by GWAS and EWAS were prioritised based on statistical significance. Human genes were inputted to DIOPT, to identify the closest fly ortholog and targetable transgenic RNAi lines to knockdown the candidate gene in flies. We performed STRING analysis for protein-protein interactions and SCOPE analysis for age-dependent changes in gene expression in different neuronal and glia populations. Based on the expression pattern of the fly ortholog, promoter lines were selected to target expression of the respective RNAi. The effect of knockdown of the fly ortholog on neurodegeneration was quantified in the eye comparing their effect to expression of human Tau 0N4R and a secreted form of human Amyloid-beta 42. While the effect on longevity, circadian rhythms, sleep and progressive changes in locomotor behaviour were likewise assessed using the appropriate neuron or glia promoters.

Joshua Harvey

Linking Parkinson's disease and depression, epigenetic insights into midbrain neuronal changes

Parkinson's disease is a highly heterogeneous disorder, encompassing a complex spectrum of clinical presentation including motor, sleep, cognitive and neuropsychiatric symptoms. We aimed to investigate genome-wide DNA methylation networks in post-mortem Parkinson's disease brain samples and test for region-specific association with common neuropsychiatric and cognitive symptoms. Of traits tested, we identify a co-methylation module in the substantia nigra with significant correlation to depressive symptoms and with ontological enrichment for terms relevant to neuronal and synaptic processes. Notably, expression of the genes annotated to the methylation loci present within this module are found to be significantly enriched in neuronal subtypes within the substantia nigra. These findings highlight the potential involvement of neuronal-specific changes within the substantia nigra with regard to depressive symptoms in Parkinson's disease. We further refine the findings, looking at correlation of genetic signals for clinical depression and Parkinson's disease, as well as comparing the cell types of the midbrain implicated in each.

Oliver Milner

Investigating the relationship and modelling the impact of neuroinflammation on cerebral vascular function in dementia using novel 3D models of cerebral vasculature.

Cerebral vascular abnormalities, including reduced or dysregulated cerebral blood flow, pericyte loss, endothelial activation, and leakiness of the blood-brain barrier (BBB), are major contributors to cognitive decline and disease pathology in the early stages of Alzheimer's disease (AD). Asby et al. (2021) recently reported that cerebral hypoperfusion and BBB breakdown were exacerbated in early AD, independently of Aβ, in people with systemic infection. The severity of vascular dysfunction was strongly related to raised levels of brain cytokines, particularly IL-15 and IL-17 which are key regulators of microglial and T-cell recruitment to blood vessels. We predict that the interaction between immune and vascular cells, driven by neuroinflammation, which is induced by systemic infection, is a major cause of cerebral vascular dysfunction and contributes to cognitive decline and disease pathology in AD. This PhD will aim to (i) characterise the distribution and explore the relationship between perivascular microglia activation, neuroinflammation, vascular dysfunction and dementia aetiopathogenesis in human post-mortem brain tissue and (ii) design an in-vitro 3D BBB model to directly investigate the impact of cytokines on glial recruitment and cerebral vascular degeneration in Alzheimer's Disease models.

Caroline Sarah Gregory

In-vivo characterisation of novel risk genes for Alzheimer's Disease using Drosophila

Alzheimer's Disease is one of the most prevalent and complex neurodegenerative diseases. Novel risk genes for Alzheimer's disease identified by GWAS and EWAS were chosen, and inputted to DIOPT to identify the closest fly orthologue and targetable transgenic RNAI line. The Gal4-UAS transcription system was utilized where different driver lines were selected to target the expression of the RNAi lines in different locations. The effect on locomotor behavior (climbing), lifespan, circadian rhythms, sleep and memory was assessed. qPCR analysis was always carried out to determine if the gene was effectively knocked down.

Emma Jones

Global unbiased proteomic identification of integral membrane proteins that require Retromer for their endosomal sorting in neuronal systems

The efficient transport of membrane proteins such as signalling receptors and ion channels through the endosomal network is essential for maintaining function of all cells and organelles. The role of this system in neuronal cells appears especially critical with proper function shown to be neuroprotective. Functional deficiency of the evolutionarily conserved Retromer complex, a key regulator of sorting through the endosomal network, has been implicated in a number of neurodegenerative diseases including Alzheimer's and Parkinson's diseases. However to understand the role of Retromer in neuroprotection, it is essential that we obtain a detailed understanding of the global array of cargo proteins that rely on this complex for efficient transport.

To address this question and gain a global and unbiased view of the role of Retromer in neuronal function we have used a proteomics-based approach to identify integral membrane proteins whose recycling to the plasma membrane is mediated by Retromer and two associated cargo adaptors, sorting nexin 3 (SNX3) and sorting nexin 27 (SNX27), in primary rat cortical neurons and immortalised human H4 neuroglioma cells. From these approaches we have identified a number of cargo that require the SNX3- and SNX27-Retromer complexes for endosomal recycling, including proteins involved in regulation of synapse organisation (e.g. synaptic adhesion molecule LRFN2, neuronal guidance molecule ephrin B2), chemical synaptic signalling (e.g. GABA and glutamate receptor subunits, calcium regulator STIM1) and solute transport (e.g. human copper transporter SLC31A1). Many of the identified Retromer cargo proteins also have defined links to neuroprotection and dysregulation in neurodegenerative disease.

Our study highlights multiple novel Retromer cargoes across two distinct sub-compalexes, providing further insight into the mechanistic basis of Retromer deficiency in neurodegeneration.

Richard Seager

Investigating the AMPK-MFF pathway as a target for neuroprotection

Cellular hallmarks of neurodegenerative diseases include loss of synapses, neuronal death, inflammation, energy homeostasis defects, oxidative stress, mitochondrial dysfunction, and aberrant proteostasis. AMP-activated kinase (AMPK) maintains energy homeostasis by responding to fluctuating ATP levels, with diverse targets ranging from changing the transcriptional program, promoting glucose uptake, inhibiting protein synthesis, inducing mitochondrial fission, mitophagy and autophagy. Evidence indicates that AMPK is hyperactivated in Alzheimer's disease (AD), and amyloid-β (Aβ) can activate AMPK, leading to mitochondrial fragmentation, mitophagy, and spine loss. In a variety of in vitro and in vivo neurodegenerative models, reducing mitochondrial fragmentation has been shown to be neuroprotective. A key mitochondrial target of AMPK is mitochondrial fission factor (MFF), which is phosphorylated at Ser155 and Ser172 to promote fission. Here we present MFF as a target of SUMOylation (small ubiquitin-like modifier) at Lys151, which is enhanced upon AMPK phosphorylation under stress. Using primary hippocampal neurons, we show that MFF SUMOylation is required to maintain mitochondrial size. We find that sustained AMPK activation, using the AMP analog AICAR, induced loss of pre- and post-synaptic markers in primary cortical neurons. Future work will examine the AMPK-MFF pathway as a target to modulate AMPK-dependent synaptic loss, and whether preventing MFF SUMOylation under stress conditions will impair mitochondrial fission and maintain synaptic integrity.

Emre Tancan

The effects of pharmacological activation of the renin-angiotensin system (RAS) on neuronal function

Alzheimer's disease (AD) is a disorder associated with cognitive deterioration and functional decline, likely caused by the buildup of amyloid beta pathogenic aggregates and neurofibrillary tangles in the brain. Despite many decades of research, a robust treatment has not yet been found. Recent studies have identified the renin-angiotensin system (RAS) as having a potential role in the pathogenesis of AD. RAS is associated with regulation of water and ion balance, and interestingly has been shown to have roles in modulating brain functionality and cognition. Angiotensin-II (Ang-II) is an important mediator within RAS, and its activation of the AT1R receptor is a key regulator in this part of the endocrine system in the brain. Previous studies have found that high levels of Ang-II attenuate cognitive function by promoting oxidative stress and neuroinflammation, and that high expression of ACE2 (Angiotensin II Converting Enzyme 2) activates AT1R by Ang-II, where AT1R influences vasoconstriction in the brain, which worsens cognition function, neurons, and promotes inflammation. However, the underlying mechanism of neuromodulation in RAS has not yet been clarified. To address this, we explored the effects of Ang-II on neuronal function in rat cortical and hippocampal neuronal cultures and acute hippocampal slices. Using electrophysiological assays in vitro, we assayed neuronal network activity and synaptic transmission in response to application of Ang-II to neurons. We found no major effects of Ang-II, indicating that acute exposure to the peptide is unlikely to cause profound dysregulation of neuronal function. Further investigations are underway to pinpoint how Ang-II has a neuroprotective effect via activating AT1R in the brain.

Imogen Targett

Is Dysfunctional Neuronal Differentiation the Link Between Diet and Neurodegeneration?

Type 2 diabetes mellitus (T2DM) has been associated with a nearly 2-fold increased risk of Alzheimer's disease (AD) development. Recent studies have demonstrated dysregulated hippocampal neurogenesis in AD patients during postmortem examinations. Additionally, impaired neurogenesis has been observed in both AD and T2DM mouse models, suggesting this could explain the connection between T2DM and AD. This study aims to investigate how exposure to fatty acids associated with T2DM affects neuronal differentiation, serving as a model of neurogenesis. Human SH-SY5Y neuroblastoma cells were subjected to a 10-day differentiation protocol involving retinoic acid and brain-derived neurotrophic factor. Cells were exposed to oleic or palmitic acid which are the most abundant monosaturated and saturated fatty acids in plasma and shown to be elevated in T2DM and AD. Differentiation and potential signalling markers were evaluated through Western Blotting. Furthermore, the influence of dietary factors on neurite outgrowth was explored using confocal and fluorescent microscopy. Our differentiation protocol produced a reliable differentiated phenotype, expressing neuronal and synaptic markers, with extensive neurite outgrowth. Our results demonstrated that oleate, but not palmitate, significantly modified neurite outgrowth and synaptophysin distribution on day 10 of differentiation. Palmitate exposure led to an increase in activation of 5'-adenosine monophosphate-activated protein kinase (AMPK) by phosphorylation, indicating that palmitate exposure affects metabolic signalling. On day 10, the expression of transcription factors, myocyte-specific enhancer factor 2A (MEF2A) and peroxisome proliferator-activated receptor-γ (PPARγ), were altered in response to palmitate which both contribute to neuronal differentiation. In conclusion our data suggest that exposure to both saturated and unsaturated fatty acids affect the differentiation of SH-SY5Y cells, providing a potential link between T2DM, high fat diets and AD. Future investigations will focus on analysing the secretory products of human-induced pluripotent stem cell (hiPSC)-derived astrocytes in response to dietary factors, and their potential role in altering neuronal differentiation, which could potentially trigger the initiation of AD pathology.