UK Glia 2026
University of Bristol, UK | 08 – 09 June 2026

Experiments on animals and animal tissues

It is a requirement of The Society that all vertebrates (and Octopus vulgaris) used in experiments are humanely treated and, where relevant, humanely killed.

To this end authors must tick the appropriate box to confirm that:

For work conducted in the UK, all procedures accorded with current UK legislation.

For work conducted elsewhere, all procedures accorded with current national legislation/guidelines or, in their absence, with current local guidelines.

Experiments on humans or human tissue

Authors must tick the appropriate box to confirm that:

All procedures accorded with the ethical standards of the relevant national, institutional or other body responsible for human research and experimentation, and with the principles of the World Medical Association's Declaration of Helsinki.

Guidelines on the Submission and Presentation of Abstracts

Please note, to constitute an acceptable abstract, The Society requires the following ethical criteria to be met. To be acceptable for publication, experiments on living vertebrates and Octopus vulgaris must conform with the ethical requirements of The Society regarding relevant authorisation, as indicated in Step 2 of submission.

Abstracts of Communications or Demonstrations must state the type of animal used (common name or genus, including man. Where applicable, abstracts must specify the anaesthetics used, and their doses and route of administration, for all experimental procedures (including preparative surgery, e.g. ovariectomy, decerebration, etc.).

For experiments involving neuromuscular blockade, the abstract must give the type and dose, plus the methods used to monitor the adequacy of anaesthesia during blockade (or refer to a paper with these details). For the preparation of isolated tissues, including primary cultures and brain slices, the method of killing (e.g. terminal anaesthesia) is required only if scientifically relevant. In experiments where genes are expressed in Xenopus oocytes, full details of the oocyte collection are not necessary. All procedures on human subjects or human tissue must accord with the ethical requirements of The Society regarding relevant authorisation, as indicated in Step 2 of submission; authors must tick the appropriate box to indicate compliance.

SA01

Regenerative neural plasticity: neuroprotection and remyelination

Ragnhildur Thora Karadottir[1]

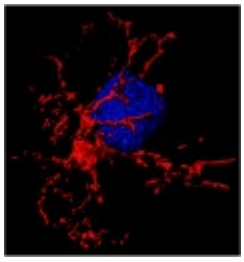
[1]University of Cambridge United Kingdom

Half of the human brain is white matter. Its function relies on oligodendrocytes producing myelin sheaths that are essential for neuronal communication, cognitive function, and motor performance. Throughout life, oligodendrocyte precursor cells (OPCs) differentiate into myelin-forming oligodendrocytes and represent the primary proliferative cells in the adult brain. OPCs can sense neuronal activity through synaptic inputs, voltage-gated ion channels, and neurotransmitter receptors, and they differentiate into myelinating oligodendrocytes in response to changes in neuronal activity—a mechanism increasingly recognised for learning and adaptation.

Whilst myelin's importance is well-established in demyelinating diseases like multiple sclerosis, emerging evidence suggests its critical role in conditions traditionally viewed as neuronal disorders, including dementia. However, with normal ageing, myelin maintenance and regeneration decline, with the primary cause of regenerative failure being the inability of OPCs to differentiate into new myelinating oligodendrocytes. With age focal white matter lesions, characterised by oligodendrocyte loss and myelin damage, accumulate and the number correlate with cognitive decline. Focal white matter lesions are prevalent across neurodegenerative conditions, yet their mechanistic relationship to grey matter pathology remains poorly understood.

Using an anatomically well-defined circuit model, we demonstrate that focal white matter lesions trigger a cascade of events beginning with transient neuronal activity changes and microgliosis. This is followed by synapse loss and increased microglial engulfment in grey matter regions, which can be reversed upon successful myelin regeneration.

Critically, we show that grey matter microgliosis, often considered pathological, is in fact integral to the myelin regenerative process. Experimental prevention of these transient grey matter changes blocks white matter myelin regeneration, whilst myelin regeneration failure results in chronic grey matter neuroinflammation. These findings reveal a bidirectional relationship between white and grey matter pathology, suggesting that myelin regeneration failure may drive the sustained microglial activation characteristic of chronic neuroinflammation in neurodegenerative diseases. This novel mechanism provides a potential unifying framework for understanding multiple neurodegenerative conditions and highlights myelin regeneration as a promising therapeutic target for preventing chronic neuroinflammation.



SA02

Myelin plasticity in nervous system development and disease

David Lyons[1]

[1]University of Edinburgh, United Kingdom

Myelin, made by oligodendrocytes in the central nervous system, is essential to nervous system formation and function, well known for its role in facilitating rapid nerve impulse conduction. Damage to or disruption to myelin is increasingly evident in numerous diseases that span the life course, from neurodevelopmental disorders, neuropsychiatric conditions, through to age-related neurodegenerative diseases. Despite the importance of myelin, we have much to learn about how it is formed on certain axons at certain times of life, how it is dynamically regulated within specific circuits and how this affects their function, and how it responds to different damaging insults across diverse injuries and disease. In this talk I will present our work using zebrafish as a discovery model, which has revealed unexpected plasticity in how myelin sheaths are wrapped around axons, how myelin responds to neuronal activity, and how mature myelin respond to damage. I will show how discoveries in zebrafish led to identification of conserved mechanisms of myelin plasticity in the mammalian and human nervous system that may have important implications for the treatment of disease.

SA03

Ischaemia and amyloid beta evoked damage to the node of Ranvier and myelin

Tania Quintela-Lopez[1], Lorena Arancibia-Carcamo[1], David Attwell[1]

[1]UCL, UK

In human Alzheimer's disease (AD), and the APP^{NL-G-F} mouse model of AD, cerebral blood flow (CBF) is reduced by amyloid beta evoking pericyte-mediated capillary constriction (Nortley et al., 2019). Vascular dementia can also involve a decrease of cerebral blood flow (CBF) (Anderle et al., 2025). The CBF decrease in AD leads to tissue hypoxia which can be reversed by administering the L-type voltage-gated calcium channel blocker nimodipine to increase blood flow (Korte et al., 2024). In human small vessel disease, giving the repurposed drugs cilostazol and isosorbide mononitrate (which are also expected to relax blood vessels) leads to an improved cognitive state (Wardlaw et al., 2023). Maintaining brain energy supply is thus likely to be a prerequisite for avoiding cogniitive decline.

One possible mediator between reduced energy supply and cognitive impairment is damage to myelinated axons (Anderle et al., 2025). White matter hyperintensities on MRI images, which may reflect myelinated axon damage, correlate with cognitive decline and lower blood flow in small vessel disease, but their origin is poorly understood. We find that either ischaemia or amyloid beta leads to a lengthening of the node of Ranvier in mouse and human myelinated axons, which is then followed by an enzyme-evoked disruption of the paranodal structure leading to myelin loss. We are testing whether blocking the culprit enzyme protects the myelin, and hence cognition.

Targeting the damage to myelinated axons as well as the fall in CBF may offer benefits for treating both vascular dementia and AD.

Animal experiments were conducted under a Home Office licence to David Attwell. Human tissue discarded from neurosurgery was used with ethical approval provided to David Attwell from the NHS REC system.

SA04

TRPA1-Dependent Regulation of Oligodendrocyte Potassium Channels Affects Myelin Function and Seizure Susceptibility

Nicola Hamilton[1], Xiangyi Du[1], Xiaolan Wu[1], Grace Flower[1], Vincenzo Giacco[1], Matthew Russell[2], Jackson McLean[1], Mansil Yoon[1], Christlyn Espiritu[1], Navami Sangeeth[1], Marion Cornillot[1], Bilal Cig[1], Craig Sexton[1], Pingting Cao[1], Laren Alphan[1], Svenja Vorthmann[1], Janice Kamajaya[1], Brooke Jones[1], Abinaya Kiritharan[1], Zongda He[1], Maria Artemonova[1], Flavie Derville[1], Ruqaiah Sharif[1], Priscilla Meccheri[1], Alejandra Carbajal[1], Leanne Allison[1], Roland Fleck[1], David Attwell[3], Catherine Hall[4], Robert Wykes[3], Matthew Swire[1], Angela Hodges[1]

[1]King's College London, United Kingdom, [2]King' College London, United Kingdom, [3]University College London, United Kingdom, [4]University of Sussex, United Kingdom

Oligodendrocytes (OLs) help maintain central nervous system homeostasis by siphoning extracellular potassium (K^+) from the periaxonal space after neuronal activity, a process mediated primarily by inwardly rectifying K^+ channels (Kir). Disruption of this mechanism slows axonal conduction and promotes neuronal hyperexcitability and seizures, common in demyelinating conditions. Prior work suggests the transient receptor potential ankyrin 1 channel (TRPA1), expressed by mature OLs, modulates K^+ channel function (Hamilton et al., 2016), but how TRPA1 interacts with distinct K^+ channel populations remains unclear. We combined whole-cell patch-clamp recordings from grey matter (GM) and white matter (WM) OLs with compound action potential (CAP) recordings and behavioural assays in TRPA1-knockout mice to define how TRPA1 regulates K^+ conductance (gK), myelin function, and seizure vulnerability.

Patch-clamp recordings revealed region-specific regulation of K^+ channels. Blocking Kir channels with barium (100 µM) reduced gK in GM OLs but had minimal effects in WM OLs (GM: ΔgK (%) = −30.7±4.2, n=11; WM: ΔgK (%) = −3.2±3.0, n = 29, P<0.0001). Activation of TRPA1 with multiple agonists inhibited gK; in WM OLs, the TRPA1-mediated ΔgK evoked by carvacrol was −42.43 ± 2.66 % (n=55). This inhibition was attenuated by pre-incubation with both Kir and K2P channel antagonists (ΔgK = −24.2±4.5%, n=7, P=0.03), but not by blocking Kir alone (ΔgK = −66.7±6.44%, n=17, P<0.0001) or K2P alone (ΔgK = −38.1±10.8 %, n=6, P=0.82), indicating that TRPA1 regulates both channel types and engages bidirectional compensation in WM.

To evaluate K^+ siphoning, we recorded CAPs from optic nerves of wild-type (WT) and OL-specific TRPA1 conditional-knockout (Sox10-iCreERT2:TRPA1^{flfl}; cKO) mice. High-frequency stimulation (HFS, 100 Hz) induces periaxonal K^+ accumulation, and the recovery from conduction block approximates the rate of K^+ siphoning (Larson et al., 2018). In WT nerves, TRPA1 activation with polygodial (100 µM) slowed recovery (Control: 51.6±6.0 s, n=10; polygodial: 81.2±13.9 s, n=6; P=0.041). Surprisingly, after prolonged HFS (300s), cKO nerves incubated with polygodial recovered even more slowly than WT (WT: 270.9±30.3 s, n=7; cKO: 392.9±19.8 s, n=9; P=0.0043), indicating that TRPA1 loss is not protective and may impair developmental tuning of K^+ channel function or expression.

Preliminary immunohistochemistry supports this: TRPA1 global knockout mice show reduced GIRK1 expression at P14 (P = 0.09, nwt=5, nko=5), increased Kir4.1 at P30 (P=0.008, nwt=5, nko=5), and a transient reduction in MBP at P21 (P=0.01, nwt=5, nko=5), accompanied by reduced gK at P12–18, which recovers by P25-30.

Behaviourally, adult cKO mice exhibit heightened pentylenetetrazole seizure susceptibility. Latency to Racine stage 4 (loss of posture) decreased from 794.3±185.8 s (n=4) in WT to 315.4±41.1 s (n=9) in cKO (P=0.004). Latency to stage 5 (tonic-clonic) decreased from 891.0±178.2 s (n=4) to 385.3±81.1 s (n=6, P=0.02). Preliminary electrocorticography showed increased spike events (>0.6/hr) in motor cortex, but none in somatosensory cortex, of cKO mice (WT: n=1/11; cKO: n=6/14; P=0.08), suggesting circuit-specific vulnerability.

Together, these data identify TRPA1 as a key regulator of oligodendroglial K^+ channel function, reveal region-specific compensatory mechanisms across Kir and K2P channels, and show that perturbing TRPA1 compromises myelin function, slows axonal recovery after activity, and increases seizure propensity.

SA05

Myelin plasticity in adulthood

Cassandra Sampaio-Baptista[1]

[1]University of Glasgow, United Kingdom

Myelin, once considered a static structure, is now increasingly recognized as dynamic in adulthood. Converging evidence from studies in humans and rodents indicates that learning and neural activity modulate white matter structure, oligodendrogenesis and myelination. Recent studies have demonstrated that inhibiting oligodendrogenesis in adult mice selectively impairs certain motor learning tasks and memory consolidation. Using the same conditional knock-out mouse model, we replicated the finding that inhibiting oligodendrogenesis impairs the same motor learning task while leaving motor control unaffected. Ablating oligodendrogenesis over a 6–7 week period also led to increases in EEG power density(1–30 Hz) across wakefulness and sleep. These results suggest that adult oligodendrocyte formation is important not only for skill acquisition but may also contribute to the maintenance of oscillatory brain activity. Task-independent changes following oligodendrogenesis ablation should be considered when interpreting learning and memory deficits in this model.

SA06

Membrane hyperpolarization by Kir2.1 potassium channels drive protective microglial functions in Alzheimer's disease

Ali Rifat[1], Kai Schlepckow[2], Michael Willem[2], Giulia Albertini[3], Jörg RP Geiger[1], Bart De Strooper[4], Christian Haass[2], Christian Madry[1]

[1]Charité - Universitätsmedizin Berlin, Germany, [2]German Center for Neurodegenerative Diseases (DZNE) Munich, Germany, [3]Flanders Institute for Biotechnology (VIB), Belgium, [4]Flanders Institute for Biotechnology (VIB), UK Dementia Research Institute, Belgium, UK

Membrane hyperpolarisation by Kir2 potassium channels drive protective microglial functions in Alzheimer's disease

Introduction

Alzheimer's disease (AD), the most prevalent form of dementia, is strongly shaped by microglial function. Genetic risk variants for late-onset AD are preferentially expressed in microglia, highlighting their central role in disease pathogenesis. In response to amyloid-β (Aβ) deposition, microglia undergo pronounced phenotypic changes and adopt specialized disease-associated states within the plaque microenvironment. These states are critically regulated by local cues, relying on TREM2-dependent signaling pathways that promote transcriptional programs supporting microglial metabolism, phagocytosis, proliferation and survival. While transcriptional signatures of reactive microglia in AD have been extensively characterized, the mechanisms by which these programs are translated into functional responses remain incompletely understood. In particular, the contribution of ion channels as key regulators of cellular electrical properties and intracellular signaling has received comparatively little attention.

Experimental approaches
To investigate the role of ion channels in microglial responses during amyloid pathology, we studied murine models of amyloidosis and human microglial xenografts. Microglia in close proximity to Aβ plaques were analysed using whole-cell patch-clamp electrophysiology to assess changes in membrane potential and ion channel activity. A combination of pharmacological manipulation, single-cell RNA sequencing and genetic deletion approaches was employed to identify a specific potassium channel subtype underlying the observed electrophysiological changes. Integration of these approaches allowed us to investigate the relationship between ion channel activity and disease-associated microglial states, as well as signaling pathways implicated in AD. In addition, histological, biochemical and transcriptomic analyses were used to assess the role of microglial Kir2 on amyloid pathology and inflammatory responses.

Results
Electrophysiological recordings revealed that plaque-associated microglia exhibit a strongly hyperpolarised membrane potential and prominent inwardly rectifying potassium currents compared to microglia located distant from plaques. This electrophysiological phenotype emerged upon microglial

contact with Aβ deposits and was maintained across different stages of pathology. Transcriptomic and genetic analyses identified a specific inwardly rectifying potassium channel subtype of the Kir2 family as a major contributor to these changes in plaque-associated microglia. Functional studies further suggested that this channel is embedded within canonical disease-associated microglial states and is linked to signaling pathways known to regulate reactive microglia in AD. Modulation or loss of Kir2 resulted in marked alterations in amyloid burden, plaque architecture and inflammatory signatures, consistent with changes in microglial function.

Conclusion

Together, our findings identify membrane hyperpolarisation mediated by inwardly rectifying Kir2 potassium channels as a critical biophysical feature of reactive microglia during amyloid pathology, linking transcriptional microglial programs to functional responses in Alzheimer's disease.

SA07

Utilising Live Human Brain Slice Cultures to Study Glia in Health and Disease

Calum Bonthron[1]

[1]The University of Edinburgh, Scotland

The involvement of glia in the development and progression of a wide spectrum of neurodegenerative diseases has become a key focus in translational neuroscience research. To improve the chance of translation from the lab to the clinic, we need models that capture the complexity, heterogeneity and architecture of the adult human brain. Living human brain slice cultures are ideal for the study of glia as they maintain 3D micro-circuit organisation and mixed cellular populations. Our lab specialises in the use of live human cortical tissue to study neurodegenerative disorders, and the involvement of glia. Human brain slice cultures (HBSCs) are generated from surplus neocortical access tissue, taken from consenting patients undergoing tumour debulking surgery. Tissue samples are maintained in ice-cold aCSF, mounted in agar and sliced to 300um, before being plated onto semi-permeable PVDF membranes according to an interface protocol adapted from more common murine models. Optimisation of this culturing protocol has enabled the study of glia in a variety of ways. We have shown that we can observe living human astrocytes and microglia for multiple weeks in culture. Astrocytes can be readily transduced with AAVs for live imaging and RNA knockdown, providing an ideal system to study these cells in a variety of contexts and probe mechanistic pathways. Our lab has utilised such methods to quantify the degree of synaptic engulfment by astrocytes and microglia in response to various experimental insults. We have also shown that they produce a strong neuroinflammatory response when exposed to LPS, indicating the despite their resection and proximity to tumours, the tissue's neuroinflammatory output is not maximised. Together, our model bridges a gap between murine and human post-mortem study, with the aim of increasing the likelihood of success when translating glial mechanisms of disease.

SA08

MRI methods to study glial function: opportunities and pitfalls

Isabel N Christie[1]

[1]University of Sheffield, UK

Translational research attracts funding when the path to human application is clear. Within the UK there is pressure to reduce, refine and ultimately replace animals in research. Non-invasive magnetic resonance imaging (MRI) while expensive, is attractive for human studies because the person is not subjected to any radiation. This talk will explore how MRI can be used in animals and humans to study the role of glial cells in health and disease. The scientific questions we can ask are always limited by the tools available. It will describe some of the practical challenges and pitfalls in preclinical MRI application. For example how to find the expertise and MRI collaborators in the UK? How much will it cost in both time and money? How will you get the data analysed and how will you divide authorship between such a large interdisciplinary team? This talk will describe pitfalls around spatial resolution, choice of anaesthetic and species, imaging artefacts that can and cannot be avoided. This talk will take a deep dive into arterial spin labelling pCASL (pseudo-continuous) and FAIR (flow-sensitive alternating inversion recovery) and the reproducibility of these measurements in different experimental conditions. It will explore how to use MRI to study glymphatic flow and clearance and how new MRI methods are being developed to study the blood-cerebrospinal fluid barrier function and gas exchange between the blood stream and the grey matter. Ultimately this talk will embolden you to embark on MRI research in the future. It will provide you with a greater understanding of potential UK collaborators, the opportunities and pitfalls you may encounter along the way, and it will help you identify a route to move your research from animal to human studies.

SA09

A life course approach to unravelling the role of neuroinflammation in dementia

Diego Gomez-Nicola[1]

[1]University of Southampton, United Kingdom

Research from the Gomez-Nicola lab has provided key findings related to the understanding and targeting of neuroinflammation in Dementia. In recent years, the lab has also explored microglial turnover and development, uncovering significant changes in the dynamics of the microglial population from early development to ageing, and disease. Our findings suggest that disease-associated microglial phenotypes are instructed from early development, and hint at both genetic and environmental determinants underpinning this. Now, the lab has set a direction pivoting to the study of human microglia in health and disease. Our findings, and those in the field, justify a strong need to understand human microglia, based on the demonstrated disparity with rodent microglia in the context of diseases like Alzheimer's. We have a range of techniques, and samples, enabling complex and deep phenotyping and mechanistic studies in human microglia, including Imaging Mass Cytometry (IMC), iPSC-derived microglia and their integration in brain organoids. We are working on other models to enable the study of human microglia in context, which are amenable to higher throughput screening of mechanisms and drugs. Combining our expertise in microglia from development to neurodegeneration, we want to focus on understanding the linkage of cellular trajectories during a healthy lifespan with the onset of cell phenotypes associated to disease initiation and progression. Life-long exposures to environmental stimuli can condition microglia to undergo disease-associated changes, and now we (the field) are in a unique position to uncover these links.

SA10

Molecular alterations of the vasculature-astrocyte interactions in dementia

Blanca Díaz Castro[1]

[1]UK DRI, University of Edinburgh, UK

Astrocytes are remarkably branched brain cells that simultaneously contact neurons and the vasculature, sensing and integrating signals from both. Astrocytes enwrap and interact with the whole brain vasculature to undergo essential brain functions, and both astrocyte and vasculature are altered early in neurodegenerative diseases. The Díaz Castro lab investigates the function of the astrocyte endfoot - the astrocyte subcellular compartment that interacts with the vasculature - and how it communicates with the cells that form the brain blood vessels in health and conditions that lead to cognitive decline.

In this talk, Dr Díaz Castro will share data to show how, using new proteomics methods developed in her lab, they are beginning to understand the molecular communication between the vasculature and the astrocyte endfeet in both in vivo rodent models of disease and postmortem human vasculature. In particular, she will present her lab's most recent data on how the communication of astrocyte endfeet and the brain vasculature is altered during systemic inflammation, ageing and amyloidopathy, conditions that lead to cognitive decline and in which the brain vascular components are altered long before neuronal death.

SA11

Understanding the role of meningeal immune-matrix remodelling in head injury

Andrew Greenhalgh[1]

[1]University of Manchester, United Kingdom

Head injury that results in concussion affects millions of people world-wide each year, and repeated head injury (RHI) is a known risk factor for neurodegenerative disease. How the brain becomes susceptible to acute symptoms or long-term pathology is unclear, and neuroimaging biomarkers are lacking. We propose that the immune response within the meningeal borders of the is key determinant of outcome. We have developed new models of RHI that cause meningeal dysregulation, blood-cerebrospinal fluid (CSF)-barrier breakdown and altered brain function. In this clinically relevant model of a single mild head injury, we found meningeal immuno-matrix dysregulation without brain changes or blood-CSF-barrier breakdown. However, RHI opens the blood-CSF-barrier and causes long-term anxiety-like behaviour and cognitive changes. Blocking regulatory molecule TGF-β drives immune cell accumulation in the cortex, increases microglia activation and prevents adaptive behaviours. In professional athletes with RHI, dynamic contrast enhancement (DCE) MRI of CSF space shows arachnoid associated blood-CSF-barrier breakdown. Together, these results suggest that the physical arachnoid barrier, followed by molecular TGF-β protection, provide a layered defence to head injury, that is overcome by RHI. These data highlight a key role for meningeal immune response in head injury and identify new areas of structural integrity loss, undetectable by standard clinical imaging. Additionally, it opens avenues for neuroimaging biomarker development for those affected by RHI and at risk of neurodegenerative disease.

SA12

Revisiting the glia limitans in the age of single cell and spatial transcriptomics

Philip Hasel[1]

[1]UK Dementia Research Institute Edinburgh, UK

Astrocytes perform critical homeostatic support functions in the central nervous system. It is now clear that astrocytes, similar to neurons, can molecularly, morphologically and functionally specialize to brain areas, circuits and other anatomical domains. We have recently discovered that astrocytes forming brain borders in the brain and spinal cord, called glia limitans astrocytes, are highly specialized. These astrocytes have a highly unusual morphology, a unique gene expression profile and are conserved from fish to mouse to human. Our lab now studies the development, function and dysfunction of these cells using -omics, imaging and transgenic approaches to discover their role in protecting the brain from peripheral insults, injury and neuroinflammation.

SA13

Reciprocal control of neuronal and glial phenotypes in health and disease

Giles Hardingham[1]

[1]University of Edinburgh, UK

The brain is a complex structure comprised of a number of specialised cell types. Our lab is interested in how different cell types in the brain influence each other's properties. A particular interest is in the interactions between neurons and glia, especially astrocytes and microglia. These cell types are subject to regulation by neurons as well as in turn influencing neuronal properties themselves. The talk will provide an overview of examples of reciprocal signaling between neurons and glia during brain development, and the extent to which they influence each other's functional properties. In later-life neurodegenerative conditions like Alzheimer's disease, glia can play a role both as upstream modulator and downstream effector of protein mis-folding pathology, such as that involving ß-amyloid and tau. Examples of recent work that help illuminate this will be shown, as well as the potential for manipulation of glial cells to alter neurodegenerative disease trajectory.

SA14

Astrocyte metabolic dysfunction in Alzheimer's disease

Simon Bell[1], Hollie Wareing[1], Francesco Capriglia[1], Rachel Hughes[1], Ryan Lewis[1], Chris Ratnathurai[1], Alexander Hamshaw[1], Alicja Olejnik[1], Allan Shaw[1], Suman De[1], Daniel Blackburn[1], Heather Mortiboys[1]

[1]University of Sheffield, United Kingdom

Background: Alzheimer's disease (AD) research has long been dominated by the amyloid cascade hypothesis, yet the persistent failure of amyloid-targeting therapies suggests that other early-stage pathological drivers remain under-addressed. A growing body of evidence indicates that **astrocyte-specific metabolic dysfunction** is a primary, rather than secondary, event in AD progression. As the central metabolic hubs of the brain, astrocytes are responsible for the **astrocyte-neuron lactate shuttle (ANLS)**, providing the energetic substrate (lactate) necessary for neuronal synaptic plasticity and memory formation.

Methods: To investigate these glial contributions, we have utilized **patient-derived induced neural progenitor cells (iNPCs)**. By differentiating these cells into mature astrocytes, we can model the disease using the unique genetic and aging signatures of both sporadic (sAD) and familial (fAD) patients. These models allow for the precise measurement of mitochondrial respiration, glycolytic flux, and the transport of metabolites between glial and neuronal populations.

Key Findings: Current research into the glial-metabolic interface has yielded several critical insights:

- **Bioenergetic failure:** Astrocytes derived from AD patients exhibit a "hypometabolic" state characterized by significantly reduced **basal respiration** and **spare respiratory capacity**. This suggests an inability to scale energy production during periods of high neuronal activity.
- **Glycolytic Impairment and Hexokinase 1 (HK1):** A specific deficiency in **Hexokinase 1**, the enzyme responsible for the first step of glucose metabolism, has been identified in sAD astrocytes. The displacement of HK1 from the mitochondria disrupts the coupling of glycolysis and oxidative phosphorylation, leading to a "starved" cellular environment.
- **Lactate Deprivation:** Because AD astrocytes fail to maintain high rates of glycolysis, they export significantly less lactate to the extracellular space. This deprivation has potential to leave neurons energetically vulnerable, directly contributing to the loss of long-term potentiation (LTP) and the eventual death of synapses.
- **Mitochondrial Morphology:** AD-affected astrocytes show increased mitochondrial fragmentation, which correlates with higher levels of oxidative stress and has potential to lead to reduced calcium buffering, further destabilizing the neural microenvironment.

Clinical Significance: We have also shown a correlation between the metabolic health of these patient-derived astrocytes and the donor's actual cognitive performance. Specifically, higher levels of astrocytic

glycolytic reserve in vitro are predictive of better episodic memory scores in vivo. This underscores the potential of astrocytic health as a viable biomarker for disease progression.

Conclusion: Astrocyte metabolic "exhaustion" may be a critical checkpoint in neurodegeneration. Restoring the metabolic link between astrocytes and neurons—specifically by targeting HK1 or augmenting lactate availability—represents a promising therapeutic frontier. By stabilizing the brain's "support system," it may be possible to preserve cognitive function even in the presence of proteinopathy.

SA15

Astrocyte metabolism as a target of antidepressants

Valentina Mosienko[1]

[1]University of Bristol, United Kingdom

Stress-related disorders, including anxiety and depression, affect approximately 8% of the global population, yet around a third of patients do not respond to antidepressant treatment despite a marked increase in their use over the past two decades. The limited success of current therapies highlights major gaps in our understanding of the biological mechanisms underlying stress-related psychopathology.

While research has traditionally focused on neurons, astrocytes are now recognised as key regulators of brain metabolism and emotional behaviour. Chronic stress alters brain metabolism in regions involved in emotional regulation, including the hippocampus and amygdala. Although selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine increase extracellular serotonin levels, their therapeutic effects cannot be explained solely by serotonergic changes, suggesting the involvement of additional signalling pathways. Notably, fluoxetine also enhances the release of astrocytic lactate, a metabolite and signalling molecule essential for emotional regulation; however, the mechanisms driving these metabolic changes remain poorly understood.

In this talk, I will present our ongoing work examining metabolic and morphological adaptations in astrocytes following acute and chronic stress, and show how antidepressants modulate astrocyte function, with particular emphasis on astrocytic metabolism and the release of psychoactive molecules induced by monoaminergic antidepressants, including SSRIs, and rapid-acting antidepressants such as ketamine, and psychedelics.

SA16

Functions and Failures of Astrocytes in Neural Circuits

Baljit Khakh[1]

[1]UCLA, USA

The study of the brain has witnessed important advances in the last few decades. Despite this laudable progress, severe blind spots in our understanding of basic brain function and disease remain. Many cell types that make up the brain are non-neuronal, and many aspects of these cells have been understudied or overlooked. The brain's non-neuronal cells include glia, which represent about half of all brain cells. I will describe recent studies from my laboratory that provide new insights on predominant glial cells called astrocytes. By focusing on the striatum as a model circuitry, I will address long-standing questions concerning the functions of astrocytes in the brain and their failures in disease. The data show that signaling between astrocytes and neurons plays pivotal roles in both normal brain physiology and in disease conditions.

C01

Deconstructing the roles of oligodendrocytes and neurons in mediating TDP43-related neurodegeneration

Marcus Keatinge[1], Sandra Constantinou Juhasz[1], Toby Shaw-McGrath[1], Giles Hardingham[1], David Lyons[1], Siddharthan Chandran[1]

[1]UK DRI, University of Edinburgh, United Kingdom

Introduction

TDP-43 proteinopathies, a group of neurodegenerative diseases encompassing the amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD) spectrum, are characterised by marked oligodendrocyte dysfunction; however, the mechanistic contribution of these cells to neurodegeneration remains unclear. Under physiological conditions, TDP-43 is predominantly nuclear and regulates RNA processing, transport, and stability. In the majority of ALS/FTD cases, TDP-43 mislocalises to the cytoplasm, resulting in both loss of nuclear function and toxic gain of function. Accumulating evidence implicates oligodendrocyte pathology in ALS/FTD. Highly myelinated axons are particularly vulnerable to degeneration, and cytoplasmic TDP-43 aggregation is observed in both neurons and oligodendrocytes, with a substantial subset of patients exhibiting predominant oligodendroglial pathology. Moreover, patient-derived oligodendrocytes are neurotoxic in vitro, and oligodendrocyte-specific disruption of ALS-associated genes induces axonal damage in vivo. Despite this evidence, most studies have focused on neuronal cytoplasmic TDP-43 expression, leaving the contribution of oligodendrocytes relatively underexplored.

Aims/Methods

To investigate the impact of cytoplasmic TDP-43 expression on the CNS from neurons and oligodendrocytes, we generated a series of novel humanised zebrafish models for in vivo analysis. In these models, the endogenous zebrafish TDP-43 gene is replaced with human TDP-43 carrying the Δnls mutation, which drives cytoplasmic mislocalisation. Humanised lines express cytoplasmic TDP-43 at physiologically relevant levels either ubiquitously (Δnls^{ubi}), neuron-restricted (Δnls^{neuro}), or restricted to the oligodendrocyte lineage (Δnls^{oligo}). All regulated zebrafish procedures were sub threshold severity and in accordance with the project licence protocols. 2- anova was conducted for each experiment for significance with at least 5 or more individuals pooled from 3 independent clutches.

Results

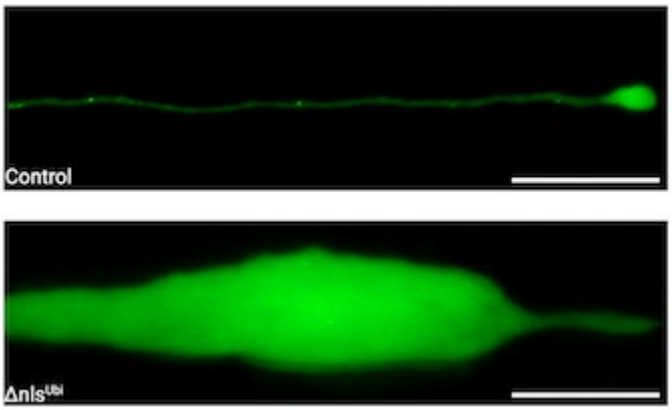
Ubiquitous expression of cytoplasmic TDP-43 resulted in early dysfunction of both neurons and oligodendrocytes, followed by lethality at approximately 10 days post-fertilisation (dpf). Analysis of myelinated upper motor neurons revealed pronounced distal swellings at 5dpf, which preceded motor neuron degeneration several days later. At 5dpf, oligodendrocyte numbers were reduced by ~30%, while myelin levels were decreased by ~60%.

Restricting cytoplasmic TDP-43 expression to neurons similarly caused severe distal axonal swelling and degeneration of myelinated upper motor neurons, although onset was delayed by ~2 days. Notably, oligodendrocyte numbers remained unchanged in the Δnls^{neuro}; however, global myelin levels were reduced by ~35%, indicating impaired oligodendrocyte function. Preventing myelination via Olig2 knockdown directly in the Δnls^{neuro} line exacerbated axonal swellings, suggesting that residual myelin is neuroprotective rather than neurotoxic.

In a similar fashion, oligodendrocyte-restricted cytoplasmic TDP-43 expression did not alter oligodendrocyte numbers but significantly reduced myelin levels by 5dpf. Of note, analysis of highly myelinated upper motor neurons revealed distal axonal structural abnormalities, indicating that cytoplasmic TDP-43 expression exclusively in oligodendrocytes disrupts axonal integrity in a non-cell-autonomous manner.

Conclusions

These findings demonstrate that cytoplasmic TDP-43 expression in either neurons or oligodendrocytes is sufficient to induce axonal dysfunction and reduce myelin levels. In Δnls^{neuro}, myelin reduction precedes axonal degeneration, and inhibition of myelination exacerbates axonal pathology. Furthermore restricting cytoplasmic TDP-43 to the oligodendrocyte lineage also causes a reduction in myelin levels and a disruption to axonal integrity. Taken together, this suggests a pathological feedback loop between neurons and oligodendrocytes, underscoring the reciprocal contributions of neurons and oligodendrocytes to ALS/FTD pathogenesis.



C02

CD4+ T cell-secreted factors suppress phagocytosis and induce antigen presentation machinery in induced pluripotent stem cell-derived microglia-like cells

Andreea-Ingrid Baloc[1], Amelia Brown[1], Catarina de Brito Raimundo[1], Anthony Vernon[1], Shahram Kordasti[2]

[1]Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, [2]System Cancer Immunology, Comprehensive Cancer Centre, King's College London, London, United Kingdom

Background: Microglia, the resident immune cells of the brain, undergo dynamic functional maturation that requires precise integration of both intrinsic genetic programming and extrinsic environmental signals. This maturation process enables microglia to execute distinct developmental functions across different stages of brain development. A critical aspect of microglial maturation involves their transition from a predominantly phagocytic state to an antigen-presenting phenotype (1). Evidence from rodent models has shown that CD4+ T cells play an instrumental role in orchestrating this developmental transition (2). However, comparable human model systems to investigate this immunological crosstalk have been lacking. To address this gap, we utilised human microglia differentiated from induced pluripotent stem cells (hiPSCs) to interrogate whether cytokines derived from activated CD4+ T cells drive the phenotypic shift of microglia from phagocytic to antigen-presenting states.

Methods: Microglia were differentiated from N=4 independent hiPSC lines (2 male, 2 female, all neurotypical) using the Haenseler et al. (2017) protocol (3), and updated Washer et al. (2022) monoculture media (4). Cells were exposed for 7 days to 10% conditioned media (CM) collected from peripheral blood-derived CD4+ T cells activated *in vitro* using CD3/CD28 beads in combination with recombinant interleukin (IL)-2 (30 U/ml). Two treatment applications were administered between days 7-14 of microglial maturation. Control conditions included conditioned media from resting (non-activated) T cells and vehicle control (XVIVO-15 medium supplemented with IL-2). Genome-wide transcriptional profiles were assessed using bulk RNA sequencing, with differential gene expression analysis performed using DESeq2 and pathway enrichment evaluated through Gene Ontology (GO) analysis. To functionally validate transcriptional changes, phagocytic capacity was quantified by measuring fluorescent zymosan bead uptake via flow cytometry and compared to a 24h treatment with the same conditions.

Results: Bulk RNA sequencing analysis revealed 786 differentially expressed genes (adjusted p value < 0.05 and log2 fold change > 1) between activated CM-treated and vehicle control conditions. GO pathway enrichment analysis showed significant upregulation of interferon (IFN)-γ signalling pathways (p.adjust < 0.001), major histocompatibility complex (MHC) assembly processes, and antigen processing and presentation machinery (p.adjust < 0.001). Conversely, pathways associated with phagocytosis were significantly downregulated (p.adjust < 0.001), suggestive of the acquisition of antigen-presenting functional capabilities. Functional validation of these data was performed using zymosan bead phagocytosis. This revealed a significant effect of treatment on normalized mean fluorescence intensity (MFI) values, serving as a proxy measure for phagocytosed bead quantity (2-way ANOVA: $F(2,6) = 15.07$, $p = 0.0046$, partial $eta^2 = 0.75$). Post-hoc comparisons demonstrated that activated CM treatment significantly reduced normalized MFI relative to resting CD4+ T cell media, at both 7 days and 24h treatment timepoints.

Conclusions: Our findings suggest that soluble factors derived from activated CD4+ T cells drive a transcriptional state shift in human microglia from phagocytic to antigen-presenting phenotypes, which

is supported by the functional validation of reduced phagocytosis. These results establish a novel human iPSC-based model system for investigating T cell-microglia interactions and reveal how peripheral immune signals can shape microglial identity during brain development.

C03

Immune stimulation exacerbates cell autonomous microglial dysfunction in a stem cell model of TBK1-associated ALS-FTD

Uroosa Chughtai[1], Raja Nirujogi[2], Daniel Cabezas De La Fuente[3], Gaynor A. Smith[1], Meng Li[3], Dario Alessi[2], Owen M. Peters[1]

[1]Cardiff University/UK DRI, United Kingdom, [2]MRC PPU, University of Dundee, United Kingdom, [3]Cardiff University, United Kingdom

Introduction: Heterozygous loss-of-function mutations in TANK-binding kinase 1 (TBK1) are associated with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (1,2), two devastating and interlinked neurodegenerative disorders that form a continuous ALS-FTD disease spectrum. Whilst TBK1 haploinsufficiency has been shown to contribute to cell autonomous neuronal dysfunction, the impact of TBK1 loss-of-function in microglia is less well understood. We aimed to investigate the function of TBK1 in human microglia and understand how loss of TBK1 activity may intrinsically impair microglial function to contribute to ALS-FTD pathophysiology.

Methods: Using a combination of in-house gene editing, the JAX/iNDI collection (3,4) and well-established differentiation protocols (5), we generated isogenic wild-type ($TBK1^{WT/WT}$) and homozygous TBK1 knockout ($TBK1^{-/-}$) human pluripotent stem cell-derived microglia in two independent genetic backgrounds (HUES9 human embryonic stem cells and KOLF2.1J induced pluripotent stem cells). Using TMT LC-MS/MS, we performed unbiased proteomic and phosphoproteomic characterisation of $TBK1^{WT/WT}$ and $TBK1^{-/-}$ microglia, both at baseline and following immune stimulation (LPS/IFNγ, 100ng/ml, 24 hours) (HUES9: N=4, 1 clone x 4 replicates). Subsequently, we performed targeted functional assays to investigate the cellular consequences of microglial TBK1 loss (HUES9: N=8, 2 clones x 4 replicates; KOLF2.1J: N=4-12, 1-3 clones x 4 replicates). Statistical significance was determined using unpaired t-tests or two-way ANOVA, with post-hoc testing and correction for multiple comparisons as appropriate.

Results: We found that the proteome and phosphoproteome of $TBK1^{-/-}$ microglia was significantly dysregulated, both at baseline and to a greater extent following immune stimulation, with numerous differentially expressed proteins and phosphopeptides being identified (padj<0.01, log2(FC)<-0.6 or >0.6). Functional enrichment analysis highlighted vesicle trafficking as a key dysregulated pathway. Using live cell timelapse microscopy, we visualised the internalisation and processing of pHrodo-tagged cargo, including myelin, dead SH-SY5Ys and dextran. We found that $TBK1^{-/-}$ microglia displayed deficient internalisation and/or processing of several cargo specifically in immune stimulated conditions. Additionally, using sandwich ELISAs on cell culture supernatants, we observed an increase in IL-1α and IL-1β secretion in $TBK1^{-/-}$ microglia specifically upon immune stimulation.

Conclusions: We found that TBK1 loss-of-function resulted in widespread cell autonomous microglial dysfunction, including global dysregulation of the microglial proteome and phosphoproteome, immune dysregulation and endocytic dysfunction, which were exacerbated or apparent only upon immune stimulation. Our data suggests that TBK1 loss-of-function mutations may contribute to ALS-FTD pathogenesis via cell autonomous microglial dysfunction. More broadly, our data adds to emerging evidence of a failure of microglia to mount an appropriate inflammatory response in ALS-FTD.

C04

Lysosomal Calcium Release through TRPML1 restores Astrocytic Calcium signaling in Early Alzheimer's Disease

Disha Shah[1], Millie Thackray[1], Casey Chertavian[1], Perlina Desai[1], Marie Dumoulin[2], Verity Mitchener[3], Molly Strom[4], Lorena Arancibia Carcamo[1], Bart De Strooper[1]

[1]UK Dementia Research Institute, University College London, United Kingdom, [2] VIB-UGent Center for Inflammation Research , Belgium, [3]UK Dementia Research Institute at at Edinburgh, United Kingdom, [4]Francis Crick Institute, United Kingdom

Introduction
Astrocytic calcium signaling is disrupted at early stages of Alzheimer's disease (AD), before amyloid plaque deposition. This early dysfunction mediates neuronal and network disturbances, positioning astrocytes in a central role in disease progression[1]. Because astrocytic calcium dynamics support key regulatory functions in the brain, identifying the mechanisms that initiate this disruption is critical. Lysosomes, in addition to their degradative role, serve as intracellular calcium stores that regulate organelle crosstalk, positioning them to shape astrocytic signaling and rendering them vulnerable to AD-related stress. Here, we investigate whether lysosomal dysfunction emerges before amyloid deposition and whether it drives early astrocytic calcium signaling deficits in vivo.

Methods

In-vivo two-photon calcium imaging was performed in heterozygous App^{NL-G-F} knock-in mice[2] and wild-type littermates at pre- and post-plaque stages following astrocyte-specific AAV expression of cytosolic or lysosome-targeted calcium indicators. Imaging experiments typically included N=4 mice per group, with 25-48 astrocytes analyzed per mouse. Lysosomal acidity and protein expression were assessed using the ratiometric pH-sensitive FIREpHLy probe and immunohistochemistry, respectively, while amyloid pathology was quantified using 82E1 staining. Statistical analyses employed ART ANOVA or nested t-tests with multiple-comparison correction as appropriate. All experimental procedures complied with the UK Animals (Scientific Procedures) Act and were approved by the relevant local ethical review board.

Results

In-vivo two-photon imaging revealed a pronounced suppression of astrocytic cytosolic calcium signaling at pre-plaque stages of AD, coinciding with early abnormalities in lysosomal function. Astrocytic lysosomes exhibited reduced luminal acidity, altered expression of lysosomal markers, and a marked loss of spontaneous lysosomal calcium release. This deficit was associated with reduced expression of the lysosomal calcium channel TRPML1. Astrocyte-specific restoration of TRPML1 rescued lysosomal calcium release and normalized cytosolic calcium signaling at early stages. Importantly, early TRPML1 expression prevented the later emergence of astrocytic calcium hyperactivity, reduced astrocyte reactivity, and markedly attenuated amyloid plaque accumulation in vivo.

Discussion and conclusion

These findings identify impaired lysosomal calcium release as an early upstream driver of astrocytic calcium signaling deficits in Alzheimer's disease. Our data link early lysosomal dysfunction to a broader collapse of astrocytic calcium signaling and subsequent disease progression. Restoring lysosomal calcium release stabilized astrocytic calcium dynamics, prevented later astrocytic hyperactivity, and

reduced amyloid pathology. Together, this work positions astrocytic lysosomal calcium signaling as a central and tractable mechanism in early AD.

C05

Glial-mediated mechanisms in the gut–brain axis: a scoping review of microbiota-driven neuroimmune communication

Blessing Rachael Adeyeye[1]

[1]Redeemer's University, Ede, Osun State, Nigeria., Nigeria

Abstract

Introduction:

The gut–brain axis represents a bidirectional communication network linking the gastrointestinal tract and central nervous system through neural, immune, and metabolic pathways. Increasing evidence implicates glial cells, including enteric glia, astrocytes, and microglia, as key mediators of microbiota-driven neuroimmune signaling. However, the extent and characteristics of glial involvement across gut–brain axis studies remain incompletely synthesized.

Aim:

This scoping review aimed to systematically map and summarize existing evidence on the roles of glial cells in microbiota-mediated gut–brain communication and neuroimmune regulation.

Methods:

A scoping review was conducted following PRISMA-ScR guidelines. Peer-reviewed articles published between 2005 and 2025 were identified from PubMed, Scopus, and Web of Science using predefined search terms related to gut microbiota, glial cells, and neuroimmune signaling. Inclusion criteria comprised experimental and observational studies involving enteric glia, astrocytes, or microglia in gut–brain axis contexts. Data extracted included study design, model organism, glial cell type, microbiota-related exposure, and neuroimmune outcomes. Descriptive statistical analyses were performed.

Results:

A total of n = 68 studies met the inclusion criteria, including 42 animal studies, 18 in vitro studies, and 8 human observational studies. Enteric glia were examined in 31% of studies, microglia in 46%, and astrocytes in 38% (some studies assessed multiple glial types). Microbial metabolites, particularly short-chain fatty acids, were reported to modulate glial inflammatory signaling in 54% of studies. Evidence of glial-mediated immune modulation influencing neuroinflammation or behavioral outcomes was reported in 61% of included studies.

Conclusion:

Current evidence supports a central role for glial cells as intermediaries in microbiota-driven gut–brain communication, particularly through immune and metabolic signaling pathways. This synthesis highlights glial cells as critical, yet under-integrated, components of the gut–brain axis and underscores their relevance as potential therapeutic targets in neuroinflammatory and neurodegenerative conditions.

Ethical considerations:

This study analyzed published data only and did not involve new experiments or human/animal participants.

C06

Role of microglia in concomitant pathology in Alzheimer's disease

Ishan Agrawal[1], Melisa Ho[2], Sanjay Kumar[2], Laura Parkkinen[3], Sally A. Cowley[1]

[1]1. James and Lillian Martin Centre for Stem Cell Research, Sir William Dunn School of Pathology, University of Oxford, United Kingdom, [2]GlaxoSmithKline, USA, [3]3. Nuffield Department of Clinical Neurosciences, Oxford Parkinson's Disease Centre, University of Oxford, United Kingdom

Background

Neuroinflammation plays an important role in the development and progression of neurodegenerative disorders, particularly Alzheimer's disease (AD) and Parkinson's disease (PD). Genome-wide association studies indicate that many inflammatory genes associated with AD are exclusively expressed in microglia. AD is the leading cause of dementia and is characterized by the accumulation of extracellular amyloid-β plaques and intracellular neurofibrillary tau tangles. However, clinical evidence suggests that cases of 'pure' pathology involving only amyloid-β and tau are relatively rare. More than 50% of AD patients also show accumulation of TDP-43, while approximately 33% exhibit accumulation of α-synuclein. This highlights the importance of concomitant pathology in AD.

Aims

The project aims to investigate how concomitant pathology affects microglia. It focuses on the development of methods to enrich pathogenic aggregated proteins from patients' brain samples, treating Induced pluripotent stem cell (iPSC) – derived microglia with the aggregates, and investigating the mechanisms behind neuroinflammation leading to neurodegeneration in AD.

Methods

We have standardized the isolation of amyloid-β, tau, TDP-43, and α-synuclein from brain tissue of patients in the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort. We have characterized the isolated aggregates using Western blotting, transmission electron microscopy (TEM), and seeding bioassays. Isolated aggregates were quantified using ELISA and mass-spectrometry. iPSC-derived microglia are being treated with combinations of the isolated aggregates to explore how this affects the genetic and proteomic landscape of these cells. Human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents under an IRB/REC (number 23/SC/024) approved protocol.

Results

Western blot and TEM (Figure 1) demonstrate isolation of pathogenic aggregates. Using a seeding bioassay, we have been able to confirm that isolated aggregates are seed competent. Comparison of several suppliers' ELISA kits shows them to give widely divergent results for the same samples.

Conclusions

We have standardized the isolation of seed competent aggregates from small-scale tissue samples. Treating microglia with different combinations of these aggregates will enhance our understanding of how mixed pathology influences the development and progression of AD and may help identify new targets for the potential treatment and management of the disease.

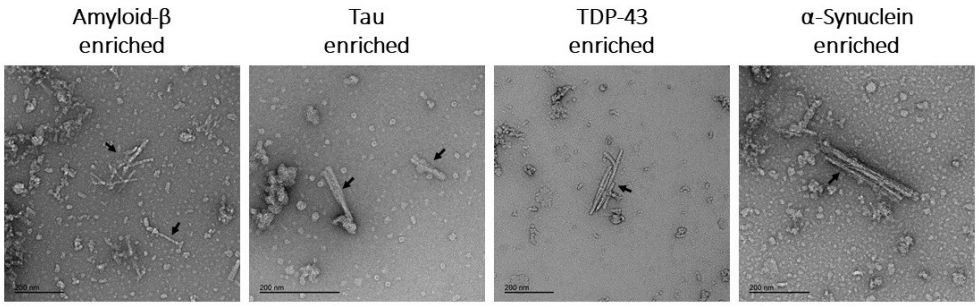


Figure 1. TEM-images of the enriched protein aggregates. Amyloid-β and tau were enriched from Alzheimer's disease patients', TDP-43 from frontotemporal lobar degeneration patient's and α-synuclein from dementia with Lewy bodies patient's brain tissue samples.

C07

Alpha-synuclein dynamics in reactive astrocytes

David Alliband[1], Jon Lane[2], Lucy Crompton[3]

[1]University of Bristol & UWE Bristol, United Kingdom, [2]University of Bristol, United Kingdom, [3]UWE Bristol, United Kingdom

Background:

Parkinson's is a neurodegenerative disease characterised by the formation of α-synuclein aggregates and dopaminergic neuron loss in the substantia nigra, leading to debilitating movement symptoms (Morris et al, 2024). Chronic inflammation in the brain is present in a wide range of neurodegenerative diseases, including Parkinson's. While research has traditionally focussed on the neurons affected by degeneration, our work explores the role of astrocytes, mediators of inflammation in the brain. In response to inflammation, astrocytes become "reactive", switching from a neurosupportive to neurotoxic phenotype. Although animal models of Parkinson's have shown that these reactive astrocytes cause neurodegeneration, the functional alterations elicited by inflammation that lead to neuronal loss are yet to be fully understood (Liddelow and Barres, 2017).

Results:

Using hiPSC-derived ventral-midbrain astrocytes (Crompton et al, 2021), we have identified a novel response in reactive astrocytes wherein vitro conditions recapitulating pro-inflammatory signals from activated microglia causes astrocytes to upregulate release of α-synuclein (figure 1). α-synuclein in the astrocyte conditioned media was measured by ELISA, and when comparing the untreated control (mean = 0.439 ng/ml, SD = 0.291 ng/ml) against the reactive astrocytes (mean = 0.835 ng/ml, SD = 0.333 ng/ml) there is a significant difference between the two conditions ($n = 7$, $p = <0.05$). To identify the mechanisms governing this response we have conducted a targeted siRNA screen of membrane trafficking genes which has revealed a number of exciting candidates including a component of the lysosomal V-ATPase (figure 2). To validate this we further targeted this complex with a shRNA and we found that the α-synuclein release associated with astrocyte reactivity was further increased (scramble shRNA mean = 1.475, SD= 0.257 vs V-ATPase shRNA) mean = 2.582 ng/ml, SD = 0.477 ng/ml; two-way ANOVA with Tukey's post-hoc test (figure 3, $n = 3$, $p = <0.01$).

Conclusions:

Ventral-midbrain astrocytes release α-synuclein when reactive in response to neuroinflammation contributing to pathology in a previously unidentified way. This suggests that astrocyte involvement in synucleinopathy may extend beyond clearance of neuronally secreted protein. This release of endogenous α-synuclein is upregulated by inhibition of proper lysosomal acidification, pointing to autolysosomal dysfunction as a possible causative mechanism behind this response.

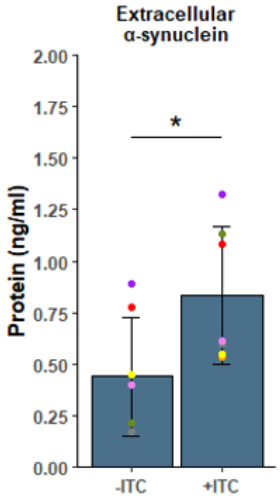


Figure 1: hiPSC-derived ventral-midbrain astrocytes treated for 24 hours with IL-1α (3ng/ml), TNF-α (30ng/ml), and C1q (400ng/ml) (ITC). Extracellular α-synuclein measured using ELISA (n = 3, t(2) = -2.994, p = 0.094).

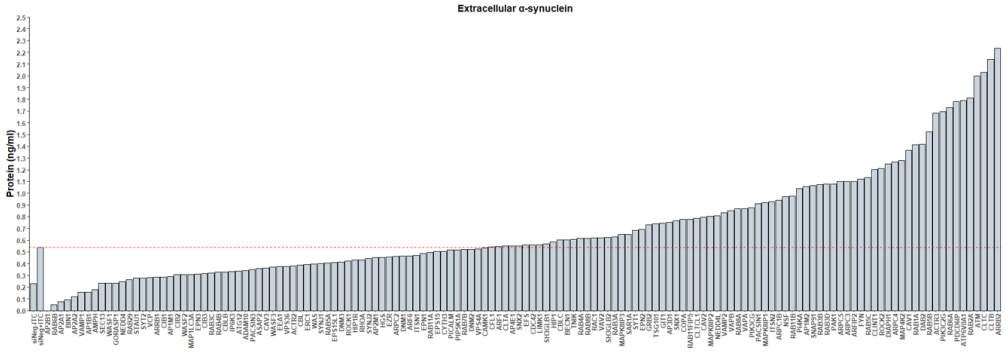


Figure 2: Extracellular α-synuclein from siRNA screen of 140 different membrane trafficking genes (50nM siRNA) in hiPSC-derived ventral-midbrain astrocytes measured via ELISA (n = 1). Cells were treated with IL-1α (3ng/ml), TNF-α (30ng/ml), and C1q (400ng/ml) (ITC). Controls included cells subjected to non-targeting siRNA in both ITC and untreated conditions. The red line indicates the level of extracellular α-synuclein from the ITC treated control.

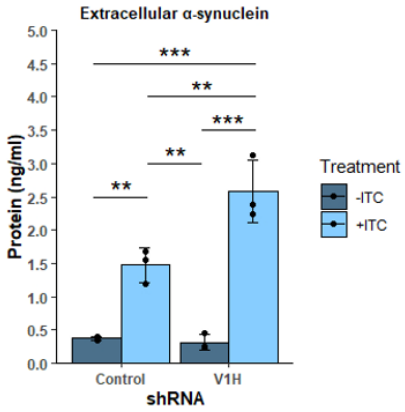


Figure 3: Extracellular α-synuclein from hiPSC-derived ventral-midbrain astrocytes treated with either control or V1H targeting shRNA and with or without IL-1α (3ng/ml), TNF-α (30ng/ml), and C1q (400ng/ml) (ITC). Two-way ANOVA (n = 3, F(1, 8) = 13.216, p = 0.006).

C08

Acute Microvascular Effects of Cilostazol and Isosorbide Mononitrate Reveal Redistribution of Cerebral Blood Supply in Wild-Type MiceBackground: White matter (WM) is specifically damaged by cerebral small vessel disease (cSVD), likely due to its susceptibility to chronic hypoperfusion. Cilostazol (CIL) and isosorbide mononitrate (ISMN) have been shown in clinical LACI trials to improve memory and reduce stroke risk; however, the acute cerebrovascular mechanisms and vascular level at which these drugs act remain unclear. We investigated whether CIL and ISMN induce age- and region-dependent acute changes in microvascular haemodynamics. Methods: Wild-type C57BL/6J mice of both sexes underwent cranial window implantation at approximately 12 weeks of age over WM or cortical grey matter (GM). For WM windows, ~800 µm of cortex was aspirated over the corpus callosum and a custom 3D-printed window implanted. GM windows were placed over the visual cortex without aspiration, preserving pial arteries. Mice were first recorded at 4 months of age; the same WM mice were re-recorded after 8 months of ageing (1 year old). Using a blinded crossover design, mice received vehicle or combined CIL+ISMN with ≥6-day washout. Haemodynamics were recorded at baseline, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h using combined laser Doppler flowmetry and haemoglobin spectroscopy. Measures included red blood cell (RBC) flux, RBC speed, concentration of moving RBCs, oxygen saturation (sO_2), total haemoglobin (HbT), and derived cerebral metabolic rate of oxygen ($CMRO_2$). Awake, head-fixed recordings were obtained during resting periods. Linear mixed-effects models were used with drug and timepoint as fixed effects and animal ID as a random effect, with Dunnett post-hoc tests. Drug doses were scaled from the LACI-2 trial using body-surface-area conversion. Results: In deep tissue containing WM of young mice (n = 8), CIL+ISMN significantly reduced RBC flux (treatment effect p = 0.022; timepoint × treatment p = 0.032), driven by a marked reduction at 6 h (estimate −0.22 ± 0.06, p = 0.0003). RBC concentration was also reduced (p = 0.0072), while RBC speed was unchanged. $CMRO_2$ decreased significantly (p = 0.016), most prominently at 6 h (p = 0.0007), without significant changes in sO_2. In the same mice re-examined longitudinally after 8 months of ageing (n = 4), WM responses to CIL+ISMN were preserved, with significant reductions in RBC flux (p =

0.013), RBC concentration (p = 0.041), and $CMRO_2$ (p = 0.007). In contrast, GM (n=4) showed a significant increase in moving RBC concentration (p = 0.003), a modest reduction in HbT (p = 0.044), and a trend toward increased sO_2 (p = 0.082), with no change in flux or speed. Blood pressure under sedation did not differ between baseline, vehicle, or drug conditions. Conclusions: Our data indicate region-specific microvascular responses to cilostazol and isosorbide mononitrate, with preserved cortical grey matter microcirculation and reduced red blood cell delivery and oxygen consumption in deep tissue containing white matter. These findings support an acute redistribution of microvascular resources rather than a uniform microvascular response. Ongoing two-photon imaging and chronic hypoperfusion models will determine the vascular origin and pathological relevance of this redistribution under chronic treatment conditions.

ALBESHR ALMASRI[1], Harry Trewhitt[1], Letitia McMullan[1], Catherine Hall[1]

[1]University of Sussex, United Kingdom

Background:

White matter (WM) is preferentially affected in cerebral small vessel disease (cSVD), likely due to chronic hypoperfusion. Although cilostazol (CIL) and isosorbide mononitrate (ISMN) improve outcomes in LACI trials, their acute cerebrovascular actions remain unclear. We examined whether combined CIL and ISMN cause region- and age-dependent acute changes in microvascular haemodynamics.

Methods:

Male and female wild-type C57BL/6J mice underwent cranial window implantation at approximately 12 weeks of age over either WM or cortical grey matter (GM). For WM windows, approximately 800 µm of cortex was aspirated to expose the corpus callosum and fitted with a custom 3D-printed window. GM windows were implanted over visual cortex without aspiration, preserving pial vasculature. Initial recordings were obtained at 4 months of age, with the same WM cohort reassessed following 8 months of ageing (1 year old).
Using a blinded crossover design, animals received vehicle or combined CIL+ISMN with a minimum 6-day washout. Haemodynamic recordings were acquired at baseline, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h using combined laser Doppler flowmetry and haemoglobin spectroscopy. Outcomes included red blood cell (RBC) flux, RBC speed, moving RBC concentration, oxygen saturation (sO_2), total haemoglobin (HbT), and derived cerebral metabolic rate of oxygen ($CMRO_2$). Measurements were performed in awake, head-fixed mice during resting periods. Linear mixed-effects models included drug and timepoint as fixed effects and animal ID as a random effect, with Dunnett post-hoc comparisons. Drug doses were scaled from the LACI-2 trial using body-surface-area conversion.

Results:

In deep tissue containing WM from young mice (n = 8), CIL+ISMN significantly decreased RBC flux (treatment effect p = 0.022; timepoint × treatment p = 0.032), with the strongest reduction observed at 6 h (estimate −0.22 ± 0.06, p = 0.0003). Moving RBC concentration was similarly reduced (p = 0.0072), while RBC speed was unaffected. $CMRO_2$ was significantly lowered (p = 0.016), most notably at 6 h (p = 0.0007), without accompanying changes in sO_2.
When the same mice were examined longitudinally after 8 months of ageing (n = 4), WM responses to CIL+ISMN were maintained, with significant reductions in RBC flux (p = 0.013), RBC concentration (p = 0.041), and $CMRO_2$ (p = 0.007).
In contrast, GM (n = 4) exhibited a significant increase in moving RBC concentration (p = 0.003), a modest decrease in HbT (p = 0.044), and a trend toward elevated sO_2 (p = 0.082), with no detectable changes in RBC flux or speed. Under sedation, arterial blood pressure did not differ between baseline, vehicle, or drug conditions.

Conclusions:

These findings demonstrate region-specific microvascular effects of cilostazol and isosorbide mononitrate, characterised by preserved cortical grey matter microcirculation alongside reduced red blood cell delivery and oxygen utilisation in deep tissue containing white matter. The data support an acute redistribution of microvascular resources rather than a uniform haemodynamic response. Ongoing two-photon imaging and chronic hypoperfusion studies will identify the vascular substrates and disease relevance of this redistribution under sustained treatment.

C09

Effects of Probiotics on Dysregulated Complement Mediated Synaptic Pruning Mechanisms relevant to complement C4 risk alleles for Schizophrenia

Ashmee Almeida[1], Wioleta Zelek[1], Sarah Carpanini[1], Daryn Michael[2], Josh Kerry-Smith[2], Timothy Hughes[1]

[1]Cardiff University, Wales, [2]Cultech, Wales

Introduction: Recent genome wide association studies have identified the C4 locus, specifically *C4A* in Schizophrenia (SCZ) risk[1]. *in vivo* and *in vitro* models of SCZ have highlighted that disease onset is at least in part due to dysfunctional complement mediated synaptic pruning[2]. Astrocytes are the predominant cellular source of C4 in the brain and influence neuronal C4 secretion. Further work is needed to clarify how astrocytes contribute to excessive neuronal synaptic pruning in SCZ and whether this can be modulated. Emerging evidence suggests individuals with SCZ carrying certain *C4* haplotypes have dysbiosis of the gut microbiome[3]. Futhermore, dietary probiotic supplementation has shown promising protective effects in clinical trials and in models of SCZ[4,5]. Such revelations highlight the potential of probiotic use as a tool to modulate complement driven synaptic pruning and as an adjunctive treatment for SCZ.

Objectives: The key aims of this project are to investigate the effects of probiotic conditioned media on modulating C4 expression first in relevant immortalised cells lines. This investigation will be furthered by utilising an IPSC model of neurons and astrocytes that models this SCZ specific genetic risk. We will probe the efficacy of probiotic metabolites on ameliorating this aberrant C4 production in these cells and thereby, aberrant synaptic pruning, and investigate the underlying mechanism.

Methods: qPCR, ELISA and immunocytochemistry were carried out to confirm *C4* expression and production in immortalised human derived neural and glial cell lines. Cell lines used were differentiated SH-SY5Y (neuronal), 1321N1(astrocytic), HMC3 (microglial). Immortalised cell lines were exposed to either lipopolysaccharide (LPS), interferon gamma (IFN-y) or interleukin-6 (IL-6), inflammatory cytokines shown to be increased in SCZ patients, for 48 hours. RNA was extracted and RT-qPCR performed to assess levels of *C4* expression. Three consortia of probiotic metabolite containing conditioned media were added to cell lines for 48 hours with and without inflammatory stimuli to assess the potential anit-inflammatory impact on *C4* expression.

Results: Astrocytic and neuronal cell lines were shown to produce and secrete C4. Exposure to all inflammatory stimuli significantly increased *C4* expression in these cell types. Exposure to probiotic conditioned media, resulted in a down regulation of basal C4 levels compared to untreated cells. Additionally, the increased expression of *C4* seen upon exposure to inflammatory stimuli was blocked by supplementing conditioned media. *C4* expression was significantly reduced across all conditioned when cells were exposed to conditioned media, with both SH-SY5Y and 1321N1 cells showing consistently lower expression levels of *C4* compared to stimulated cells alone. The same protective effects were seen

across almost three consortia of conditioned media. All experiments were carried out with an n=3 and Two way ANOVA was used for statistical analysis in all experiments.

Discussion: Confirming that astrocytes are the source of C4 in the brain highlights their importance as a target for mediating C4 production through probiotic supplementation. These results demonstrate the potential neuromodulatory effects of probiotic metabolites have when challenged with multiple inflammatory stimuli at reducing C4 expression and production; further strengthening their potential use as supplementary treatments for SCZ.

C10

RELN-DAB1: a novel pathway that interacts with APOE to modulate microglial activation and Alzheimer's disease-risk

Melissa Barber[1], Naciye Magusali[1], Umran Yaman[1], Xinran Hao[1], Tim Birkle[1], Aleksej Kubelka[1], Heleni Singer[1], Silvia LaPenna[1], William D Andrews[2], Modesta Blunskyte[1], Daria Gabriouchkina[1], John G Parnavelas[2], John Hardy[1], Dervis Salih[1]

[1]UCL UK DRI, UK, [2]UCL , UK

Introduction: Recent genetic studies have highlighted that RELN-DAB1 is a novel pathway that significantly modifies disease-risk in both familial and late-onset cases of Alzheimer's disease (AD)(Bracher-Smith, Leonenko et al. 2022, Lopera, Marino et al. 2023). While preclinical studies have shown that RELN counteracts amyloid and Tau pathology in mice (Lane-Donovan, Philips et al. 2015, Rossi, Gruart et al. 2020), how protection is conferred in the human brain remains unclear. A recent GWAS from the lab predicts that RELN-DAB1 interacts with APOE4 to modify disease-risk(Bracher-Smith, Leonenko et al. 2022).

Aim Here, we aim to investigate how RELN-DAB1/DAB2 and APOE signalling interact to modify microglia and the vasculature.

Methods Publicly available transcriptomic datasets of single-cell RNA sequences from human AD brains and controls were first mined to assess for changes in RELN-DAB1 pathways in AD. We next characterised the microglia in a germline N-*Reln* knock-down mouse model using immunocytochemistry. Finally, we cultured human APOE iPSC-differentiated microglia *in vitro* and knocked down DAB1 and DAB2 using siRNA, and characterised changes to microglia in bulk RNA transcriptomic studies and through automated analysis of microglial morphologies and activation states using Opera-Phenix imaging and Harmony analysis.

Results Our analysis of publicly available transcriptomic databases suggests that RELN-DAB1 pathways may alter neuroinflammation and the vascular system in AD, and that DAB1 is down regulated in response to amyloid. In addition, we observe that a *Reln* deficient environment results in the hypotrophying of microglial processes with indications of their increased phagocytosis within the adolescent brain (n=3-4 Reln wildtype or knock-down mice). We next study how siRNA *DAB1* knock-down in human iPSC-differentiated microglial models (n=3 MG differentiations per genotype and siRNA) and upon exposure to AD-relevant stressors *in vitro* (n=3-4 MG differentiations per genotype and siRNA) alters microglial morphologies, their activation states and phagocytosis.

Conclusion Together, we aim to understand how RELN-DAB1 and APOE genetic status may sensitize microglial responses to stressors in AD. We propose that RELN-DAB1 is a novel immune-modulatory pathway that interacts with APOE to alter AD-risk through its effects on microglial function in the human brain.

C11

Proliferation status impacts a temporally-evolving reactive microglial landscape during disease

Jack Barrington[1], Steaphan Connell[1], Vanessza Fentor[1], Sarah Choi[1], Mila Redzic[1], David Munro[1], Gaia Brezzo[1], Colin Crawford[1], Barry McColl[1]

[1]UK DRI, Institute for Neuroscience and Cardiovascular Research, University of Edinburgh, Scotland

Introduction

Microglia support brain health and resilience during various diseases. Compelling evidence supports a role for a reactive cell state often referred to as disease-associated microglia (DAM) in disease resilience, notably in Alzheimer's disease and in brain repair after injury. DAMs downregulate homeostatic features and upregulate markers of lysosomal, lipid metabolic and phagocytic pathways. These cells are observed alongside proliferating microglia in many brain pathologies, including acute injuries (e.g., stroke) where synchronised microglial activation facilitates better temporal study of cell state emergence and transitions. Although specific reactive microglial states are clinical targets, it is unclear how they emerge and evolve during disease. Addressing this knowledge gap by temporally tracking transcriptional/functional phenotypes of microglia is crucial for identifying and timing optimal therapeutic approaches.

Aims

To determine if cell-cycle influences emergence of reactive microglial states and their temporal evolution.

Methods

To determine the spatiotemporal distribution of microglial proliferation, photothrombotic stroke was induced on *Mki67*^{RFP} mice. Tissue was processed for flow cytometry (1-7d post-stroke, *n*=4) or histology (2-3d post-stroke, *n*=4). To fate-map proliferating cells, *Mki67*$^{IRES\text{-}CreERT2}$;*Rosa26*^{tdTomato} mice underwent sham or stroke surgeries and tamoxifen administered 1-3d post-surgery. TdTomato positive and negative microglial populations were separately sorted at 5d or 28d post-surgery for scRNAseq using 10x Genomics with feature barcoding technology (*n*=4/group). Brain tissue was taken from a separate cohort to determine the spatial distribution of tdTomato+ microglia at 5d, 14d and 28d post-sham/stroke (*n*=4). Sex-balanced cohorts were used throughout. All procedures complied with institutional and UK Home Office ethical regulations.

Results

We demonstrate microglial proliferation peaks between 2-3d in a mouse model of stroke. Using our fate-mapping model to time-stamp emerging proliferating microglia, we show these cells exit cell-cycle and daughter cells remain detectable up to 28d post-injury, revealing their chronic persistence. By combining our fate-mapping model with scRNAseq at early (5d) and late (28d) timepoints, we show cell-cycle favours emergence of metabolically active DAM-like cells among other states in the reactive microglial landscape at 5d. By 28d, many of these previously reactive cells return to a homeostatic state, whereas others transition to a chronic DAM-like phenotype marked by expression of antigen presentation pathways. The latter supports that microglial states have plasticity and may functionally (mal)adapt over the course of disease. We are currently applying *ex-vivo* functional assays to compare the temporal

association of transcriptional and functional phenotypes and understand if states are governed by environmental or cell-autonomous signals.

Conclusion

Our work supports that cell-cycle influences reactive microglial diversity by promoting adoption of restricted cell states. Assessing whether cell-cycle is necessary and/or sufficient to become DAM-like is now important for developing new therapies modulating DAMs in human brain disease.

C12

ABI3 inhibition has therapeutic potential in Alzheimer's disease

Ryan Bevan[1], Elena Simonazzi[1], David Harrison[1], Carolina Toste[1], Fang Li Chen[2], Adam Ranson, Joshua Stevenson-Hoare[2], Valentina Escott-Price[1], Zhen Zhao[3], B. Paul Morgan[1], Frank Sengpiel[2], Mark Good[2], Philip Taylor[1]

[1]UK Dementia Research Institute at Cardiff University, United Kingdom, [2]Cardiff University, United Kingdom, [3]University of Southern California, USA

Introduction

A rare coding variant (S209F) in *ABI3* (Abelson interactor family member 3), a core microglial component of the WAVE2 actin-regulatory complex, increases the risk of late-onset Alzheimer's disease (AD). However, the mechanistic contribution of *ABI3* to AD pathology remains poorly defined.

Aim

To determine whether loss of *Abi3* function opposes the effects of the AD-associated *Abi3* risk variant and to assess the therapeutic potential of Abi3 inhibition *in vivo*.

Methods

AD-related pathology was examined in the physiologically relevant $App^{NL\text{-}G\text{-}F}$ knock-in mouse model under conditions of *Abi3* deficiency or expression of $Abi3^{F212}$ (murine equivalent of the human S209F variant). Microglial morphology, lysosomal activation, amyloid pathology, synaptic integrity, and cognition were assessed using established histological, imaging, and behavioural approaches (n = 16 mice per genotype across assays, sex-balanced, aged to 2 and 6 months). Statistical comparisons were performed using mixed model regression. All animal procedures were conducted in accordance with institutional ethical guidelines.

Results

In the absence of disease, *Abi3* deficiency resulted in a marked reduction in microglial ramification and territory, accompanied by increased microglial density and elevated CD68 immunoreactivity, whereas $Abi3^{F212}$ expression produced modest increases in microglial arborisation. On the $App^{NL\text{-}G\text{-}F}$ background, *Abi3* deficiency directionally opposed the effects of the *Abi3* risk variant. *Abi3*-deficient mice exhibited a pronounced reduction in amyloid burden compared with $App^{NL\text{-}G\text{-}F}$ controls, while amyloid pathology was increased in $Abi3^{F212}$-expressing mice. Behaviourally, *Abi3* deficiency was associated with selective improvements in cognitive performance, with corresponding impairments observed following expression of $Abi3^{F212}$. Mechanistically, the beneficial effects of *Abi3* deficiency were associated with preservation of synaptic integrity in amyloid-associated regions and a substantial reduction in dynamic microglial parenchymal surveillance.

Conclusion

Loss of *Abi3* function directionally opposes the pathogenic effects of the AD-associated *Abi3* risk variant, resulting in reduced amyloid burden, improved cognitive outcomes, and protection against disease-associated synapse loss. These findings support *ABI3* inhibition as a potential therapeutic strategy for Alzheimer's disease.

C13

Astrocytic epigenetic & molecular mechanisms regulating cognitive impairment in epileptic mice

Bíborka Balázs[1], Dylan Dunkwu[1], Carlos Lee[1], Yossra Serroukh[1], Oliver Fetherston-Dilke[1], Vishnu Pilakkal[1], Jose Gutierrez-Marcos[1], Amol Bhandare[1]

[1]School of Life Sciences, University of Warwick, Coventry, UK, UK

Epilepsy is underpinned by complex disruptions in neural circuitry and glial function, where astrocytes play a pivotal role in maintaining homeostasis and neuroinflammation. Worldwide 65 million people live with epilepsy, and nearly 30% exhibit resistance to conventional anti-seizure treatments. Cognitive comorbidities are a major concern in people with epilepsy, with nearly 50% experiencing working memory deficits and accelerated long-term forgetting of verbal, non-verbal, and autobiographical information, significantly impairing their quality of life. Seizure-induced astrocytic dysregulation may contribute to cognitive deficits in people with epilepsy. Astrocytic adenosine A2A receptor (A2AR) influence memory processes and astrogliosis, and their expression is subject to epigenetic regulation. Seizures trigger adenosine surge capable of inducing epigenetic modifications, yet the molecular and epigenetic landscape of astrocytes in epilepsy-related memory impairment remains unclear. In this project, we aim to assess the impact of chronic seizures on short-term memory in mice using the novel object recognition test and to characterize associated astrocyte-specific epigenetic and molecular changes.

All animal experiments were compliant with the European Commission Directive 2010/63/EU (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes) and the United Kingdom Home office (Scientific Procedures) Act (1986). The project was approved by the University of Warwick's AWERB (PP1674884) committee and carried out in accordance with local legislation and institutional requirements. Initially, adult (8-10 weeks) C57BL/6 male mice were implanted with hippocampal cannula and EEG electrodes under isoflurane anesthesia. Two weeks after the surgical recovery, we induced epilepsy in mice through intrahippocampal kainic acid injection through pre-installed intrahippocampal cannula. Five weeks after epilepsy induction epileptic (n=7) and sham control (n=6) mice were tested for short-term memory using the novel object recognition test. To evaluate epigenetic contributions, we performed assay for transposase-accessible chromatin (ATAC)-sequencing and epigenomic profiling of hippocampal astrocytes five weeks post-epilepsy induction, and few days after short term memory testing. Immunohistochemistry assessed hippocampal expression of neuronal activity-regulated cytoskeleton-associated protein (Arc), astrocytic A2AR, connexin-43 (Cx43), and astrocyte morphology in epileptic versus control mice and analysed using Mann-Witney unpaired t-test.

Novel object recognition test revealed epileptic mice exhibit short-term memory impairment. Epileptic mice also exhibit elevated astrocytic A2AR and Cx43 expression, reduced neuronal Arc levels, and pronounced astrocytic morphological changes in memory-related hippocampal regions. Epigenomic analysis corroborated these findings and identified key genes with distinct patterns of chromatin accessibility in astrocytes from epileptic mice. Collectively, our results suggest that seizure-driven alterations in astrocytic morphology and receptor expression, possibly through epigenetic regulation

contribute to cognitive dysfunction in epilepsy. Targeting astrocyte-specific epigenetic mechanisms may represent a promising therapeutic strategy for mitigating memory impairment in epilepsy.

C14

Altered complement expression in AD contributes to immune dysregulation in microglia and astrocytes

Matthew Bright[1], B. Paul Morgan[1], Wioleta M. Zelek[1]

[1]Cardiff University, Department of Infection and Immunity, UK Dementia Institute at Cardiff, United Kingdom

Intro: Complement contributes significantly to pathophysiology in many neurodegenerative diseases (NDDs) including Alzheimer's Disease (AD); yet, the source of complement in the brain is poorly understood. Targeting complement effectively in NDDs requires understanding complement expression in the healthy brain, how it changes and the effect of altered expression on the cell populations in the brain.

Objectives: We aimed to characterise which cell types in the brain express complement in the healthy brain, how expression is altered in AD, and the functional implications of any alterations.

Methods: We integrated single-nucleus RNA sequencing datasets including 606,265 cells across 97 donor frontal cortices (60 AD: 36 male, 24 female; 37 controls: 23 male, 14 female). Differential expression analysis (DEA) using the Wilcoxon rank sum test (Bonferroni adjusted) was performed to assess the effects of AD pathology, sex, and age on complement gene expression across nine cell types. Protein-protein interaction networks were inferred with STRING, and cell-cell communication (CCC) analyses using CellChat examined complement-related signalling changes in AD.

Results: Complement genes displayed cell-type–specific expression in healthy and AD cortices. DEA identified significant AD-associated changes in complement gene expression, most prominently in microglia, where *C1QA/B/C*, *CFD*, *C3*, *C5*, *ITGAX*, *ITGB2* and *VSIG4* were strongly upregulated. STRING placed these genes in an interacting protein cluster enriched for synaptic pruning and immune activation. Additional complement genes (*C1R*, *C1S*, *C7*, *SERPING1*, *SRPX2*) were upregulated in endothelial cells, fibroblasts, and pericytes – cells linked to vasculature. CCC revealed widespread alterations in immune signalling mediated by complement proteins especially in astrocytes and microglia – including CD23, CD40, ICAM and vitronectin signalling - highlighting complement dysregulation affects multiple immune pathways within glial populations. DEA also showed greater loss of complement regulator expression in females in AD, and that dysregulation is most severe in younger AD cases.

Conclusion: Our comprehensive atlas of complement expression in AD and control frontal cortex demonstrates that AD profoundly disrupts complement homeostasis. Dysregulation is strongest in microglia and linked to enhanced complement-mediated synaptic pruning and immune responses. Widespread alterations in complement-mediated intercellular signalling in glial cells indicates glia are key to understanding how complement contributes to AD.

C15

Interstitial [K+]o oscillations in mouse optic nerve induced by metabolic stress

Angus Brown[1], Amy Hopper[1]

[1]University of Nottingham, UK

Interstitial K^+ ($[K^+]o$) regulation is required to counteract activity-induced increases in $[K^+]o$ that result from release of K^+ from neural compartments. Such buffering has been assumed as the sole responsibility of astrocytes in central tissue. Astrocytes display a range of transporters, pumps, and ion channels that facilitate this buffering. Evolution has taken advantage of such transient $[K^+]o$ elevations which act as a molecule signalling astrocytes a neuronal need for energy substrate, provided by lactate release from astrocytes.

The *ex vivo* optic nerves from 74 adult female CD-1 mice were sustained in a superfusion chamber bathed with artificial cerebrospinal fluid (aCSF) containing 3 mM $[K^+]$ and 95% O2 to support conduction over a period of many hours. All experiments were approved by the University of Nottingham Animal Care and Ethics Committee and were carried out in accordance with the Animals (Scientific Procedures) Act 1986 under the appropriate authority of establishment (NON ASPA 2321). The stimulus evoked compound action potential (CAP) and $[K^+]o$ were simultaneously recorded with suction electrodes, and ion sensitive micro-electrodes, respectively.

When the *ex vivo* mouse optic nerve was exposed to a stress aCSF that contained elevated $[K^+]$ (9 mM) with glucose substituted by lactate, unique spontaneous recurrent reciprocal $[K^+]o$ and CAP oscillations occurred. The oscillations started within 9.1 ± 5.6 min (n = 11) of exposure to stress aCSF, at a frequency of 0.34 ± 0.06 min^{-1}, and lasted for several hours. Powerful buffering ensured that $[K^+]o$ did not match aCSF $[K^+]$, e.g. in 9 mM aCSF $[K^+]$ the $[K^+]o$ was 4.44 ± 1.3 mM (n = 4). The mechanism of the oscillations was deduced via application of metabolic inhibitors, and transporter and ion channel blockers. The onset of the oscillations coincided with cessation of astrocytic metabolism, where the CAP fell coincident with an elevation in interstitial $[K^+]o$ at a rate of 2.72 ± 1.31mM min^{-1} (n = 5) towards aCSF $[K^+]$. This elevated $[K^+]o$ activated voltage gated Na^+ channels, leading to axonal Na^+ influx and activation of the axonal Na-K ATPase. The resulting Na^+ efflux from the axon to the interstitial space was matched with an influx of K^+ from the interstitial space into the axon, reflected in a fall in $[K^+]o$ at a rate of 1.40 ± 0.71mM min^{-1} (n = 5). This was a transient effect, which stopped when $[K^+]o$ fell below the threshold required for Na^+ channel activation.

This cyclical oscillatory behaviour continued indefinitely until either glucose (n = 6) or 3 mM K^+ (n = 5) were restored to the aCSF. These oscillations exposed the inability of astrocytes to utilise interstitial lactate as a energy substrate, and also highlighted the previously unrecognised extremely powerful axonal $[K^+]o$ buffering.

C16

Microglial pathology in response to IL-6 is driven by cis, rather than trans, signalling

Amelia Brown[1], Andreea-Ingrid Baloc[1], Catarina De Brito E Raimundo[1], Katherine Long[1], Anthony Vernon[1]

[1]King's College London, United Kingdom

Introduction

Interleukin-6 (IL-6) is a key cytokine upregulated by infection in pregnant and non-pregnant individuals. IL-6 can signal through two signalling pathways - cis and trans. Trans signalling is associated with a pathogenic response[1], and implicated in peripheral disease[2,3]. However, we lack evidence about trans signalling in human brain. As IL-6 can cross human placenta, and mediates induction of behavioural phenotypes in animal maternal immune activation models, differential mechanisms of signalling may hold relevance for aetiology of neurodevelopmental disorders. Microglia express both membrane-bound IL-6 receptor (IL-6R), and signal transducer gp130[4], enabling response through both pathways. We sought to explore whether microglia exhibit differential phenotypes when either cis- or trans IL-6 signalling is activated.

Methods

Human iPSC-derived macrophage precursors were differentiated into microglia-like cells as described previously [4]. On day 14, N=4 neurotypical donors were exposed to i) IL-6, ii) IL-6 and soluble gp130, (cis signalling), or iii) hyper IL-6, a fusion construct of IL-6/soluble IL-6Ra, (trans signalling), (or iv) vehicle,) for 3 and 24 hours. Transcriptomic changes were assessed via bulk RNA sequencing using the DESeq2 package in R (version 4.4.2). Pooled media samples from 24hr-treated microglia were incubated with proteome profiler array membranes, to provide a qualitative read-out of cytokine secretion. In addition, a FACS-based measure of phagocytosis of E. coli particles was performed.

Results

Microglia exhibited a robust transcriptional response to IL-6 (>2200 DEGs, 5% FDR), unaffected by blockade of trans signalling, at 3hrs. In contrast, trans signalling led to differential expression of 15 genes at 3hrs. Chiefly among these was STAT3, indicative of canonical IL-6 activation, which was increased to a similar level in all IL-6-treatment conditions. These patterns were conserved at 24hrs, though DEGs were relatively lower (~1300) in IL-6 and cis-treated cells. GO enrichment analysis identified generic inflammation-related pathways, including regulation of inflammatory response, and cellular response to interleukin-6, after IL-6 and trans-treatment, respectively. Most DEGs produced by trans signalling were also altered in IL-6 and cis-treated microglia. Uniquely, the gene IFI16 was upregulated by trans signalling only at both 3 and 24hrs. Of 34 cytokines measured, 10 were detectable, and seven, including MIP-1a, RANTES, MCP-1, and CCL1, were increased by IL-6 and cis-treatment. Trans-treated microglia upregulated MCP-1 and CCL1 alone. At 3 hours, two of four cell lines exhibited higher mean fluorescence intensity (MFI) in all IL-6-treated conditions, compared with vehicle, indicative of increased phagocytic behaviour. At 24 hours, MFI in the remaining two lines was consistently increased across IL-6-treatments, whilst the other two lines displayed more stochastic patterns. Given variability over

time, group-wise changes were not statistically significant ($\chi2(3)>1.72$, $p>0.32$), and suggest high donor-driven variability.

Conclusions

Results suggest microglia exhibit a muted transcriptional response to IL-6 trans signalling, compared to cis signalling. This occurs despite indications of a standard cellular response to IL-6, including cytokine secretion and transcriptional activation of relevant pathways. Future studies will examine the role of genes, particularly IFI16, uniquely upregulated by IL-6 trans signalling. Further studies to determine functional consequences of these pathways on cell motility and morphology are currently underway.

C17

Snap25-dependent neuronal activity governs glia-mediated corticospinal tract pruning

Beatriz Campos Codo[1], Luana Campos Soares[1], Anna Hoerder-Suabedissen[1], Zoltan Molnar[1], Auguste Vadisiute[1]

[1]University of Oxford, United Kingdom

During postnatal development, neural circuits undergo extensive refinement to establish efficient and mature connectivity. A key mechanism underlying this process is axonal pruning, whereby redundant projections are selectively eliminated in an activity-dependent manner. The corticospinal tract (CST), essential for voluntary motor control, undergoes large-scale pruning during early postnatal life, a process in which glia—particularly microglia, the brain's resident immune cells—play an active role through the recognition and phagocytosis of supernumerary axons and synapses.

Neuronal activity is a critical signal for glial engagement during circuit refinement. Snap25, a presynaptic protein required for synaptic vesicle fusion and regulated neurotransmitter release, is a central regulator of activity-dependent signalling. Previous work from our laboratory demonstrated that selective loss of Snap25 in cortical layer 5 neurons reduce synaptic transmission, alters glia–neuron interactions, and leads to sex-specific differences in microglial activity.

Here, we investigate whether Snap25-dependent neuronal activity is required for CST axonal pruning and whether glia contribute to this process. Using Cre-dependent AAV-mediated labelling, we targeted primary motor cortex (M1) neurons in male and female Snap25 conditional knockout and wild-type mice at postnatal day 8. Brains were collected four weeks later and analysed using fluorescence imaging to assess CST projections. All animal experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986 (ASPA), and approved by the university's ethical review committee.

Preliminary findings indicate that, in the absence of Snap25, the normally eliminated M1 projection to the superior colliculus persists, suggesting a failure of large-scale CST pruning. These results support a role for Snap25-dependent neuronal activity in driving glia-mediated circuit refinement. Ongoing quantitative and glial analyses will further elucidate the mechanisms by which activity-dependent signals shape CST development and whether these processes differ between sexes.

C18

Can Probiotics Mitigate Neuroinflammation and Improve Early Neurodevelopmental Outcome Caused by Gut Dysbiosis in Rat Offspring?

Ana Maria Catrina [1], Mara Ioana Ionescu[2], Vlad Morozan[2], Cerasela Haidoiu[1], Vladimir Suhaianu[1], Cristian Ciotei[2], Mara Belcin[2], Teodora Chirila[2], Ana-Maria Zagrean[2]

[1]1. „Cantacuzino" National Military Medical Institute for Research and Development, Bucharest, Romania , Romania, [2]2. Department of Physiology -Neuroscience, Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; , Romania

Introduction

Neuroinflammation is regulated by dynamic interactions between microglia and astrocytes and plays a crucial role in brain development. Increasing evidence highlights the microbiota–gut–brain axis as a key modulator of neuroinflammatory processes and neurodevelopment. Modulation of maternal gut microbiota during pregnancy may therefore represent a promising therapeutic strategy for improving offspring brain health.

Aims/objectives

This study investigated the impact of maternal gut dysbiosis on offspring neuroinflammation and neurodevelopment and evaluated the potential protective effects of probiotic supplementation.

Methods

Experiments were conducted on Wistar rat offspring (n=100) derived from dams assigned to four gestational treatment groups: control, antibiotics, probiotics, and antibiotics+probiotics. Animals were housed in the Biological Service Unit under standard conditions, with *ad libitum* access to food and water. All procedures were approved by the Ethics Committee of the Cantacuzino National Military Medical Institute for Research and Development (No. 319/2023) and by the veterinary health authority (No. 37/23.08.2023).

Maternal gut dysbiosis was induced by administering an antibiotic cocktail (ampicillin, vancomycin, neomycin, and meropenem) from embryonic day E11. A multi-strain probiotic was administered throughout pregnancy.

On postnatal day 6, pups were exposed to either perinatal asphyxia (PA) or normoxia (N), resulting in eight experimental groups: control (C-N, C-PA), antibiotics (AB-N, AB-PA), probiotics (P-N, P-PA), and antibiotics+probiotics (AB-P-N, AB-P-PA).

Early neurodevelopmental assessment was performed between postnatal days 7 and 9 using the righting reflex, negative geotaxis, and grip strength tests. The day following PA/normoxia, hippocampal tissue was collected for TNF-α quantification using ELISA.

Between postnatal days 45 and 65, offspring underwent late behavioral testing and were sacrificed on postnatal day 75. Brain tissue was harvested, fixed, paraffin-embedded, and processed for immunohistochemistry. Sections were immunostained for microglial (IBA-1) and astrocytic (GFAP) markers using a Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab64264) and analyzed using a Zeiss Axiolab 5 microscope.

Results

Offspring exposed to maternal antibiotics and/or PA exhibited impaired early neurodevelopmental reflexes and increased hippocampal inflammatory markers.

In the control group, exposure to PA induced marked activation of both microglia and astrocytes compared with normoxic conditions. IBA-1 immunostaining revealed a shift in microglial morphology from a ramified, surveillant phenotype to an amoeboid, activated state. GFAP immunolabeling demonstrated reactive astrocytosis in PA-exposed offspring.

Maternal antibiotic exposure induced microglial activation even under normoxic conditions, an effect further exacerbated by PA. Probiotic supplementation significantly attenuated antibiotic-induced microglial activation in both conditions.

Similarly, antibiotic-induced astrocytic activation was observed in normoxic offspring and intensified following PA. Probiotic administration mitigated astrocytic reactivity associated with both antibiotic exposure and perinatal asphyxia.

Conclusions

These findings indicate that probiotics modulate the microbiota–gut–brain axis, reduce neuroinflammation, and improve neurodevelopmental trajectories in offspring, supporting their potential as a preventive strategy against dysbiosis-related neurodevelopmental disturbances.

C19

Integrative bulk- and single-cell transcriptomic analysis reveals conserved astrocytic metabolic pathways across stress, antidepressant, and immune paradigms.

Malcolm Charangwa[1], Valentina Mosienko[1]

[1]University of Bristol, United Kingdom

Title:

Integrative bulk- and single-cell transcriptomic analysis reveals conserved astrocytic metabolic pathways across stress, antidepressant, and immune paradigms.

Authors:

Malcolm Charangwa, Valentina Mosienko

Abstract:

Major depressive disorder is a one of the leading contributors to global disability, accounting for around 56 million disability adjusted life years (DALYs) globally (2% of all DALYs) (Rong et al., 2025). Despite this burden, current first-line antidepressants, including selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, remain ineffective in 10-30% of all patients, who are deemed treatment resistant. Emerging evidence suggests that the antidepressant efficacy of fluoxetine is not solely mediated by serotonergic mechanisms but instead involves broader effects on brain energy metabolism (Pan et al., 2022). Astrocytes, the most abundant cell type in the central nervous system (CNS), accounts for 20-40% of all cells in the brain (Akdemir et al. 2020) and play a central role in neuronal energetic support through metabolic coupling mechanisms such as the astrocyte-neuron lactate shuttle (Kim et al., 2025). However, the molecular pathways by which antidepressants modulate astrocytic metabolism remain poorly defined.

Previous work from our group has shown that fluoxetine alters astrocytic lactate release in vitro, suggesting a metabolic mechanism contributing to its antidepressant effects. To contextualise and extend these findings, we conducted a large-scale, quantitative systematic review and integrative transcriptomic analysis to identify conserved astrocytic genes, regulators, and pathways across three biologically relevant paradigms: stress exposure, antidepressant treatment, and immune challenge. These paradigms capture convergent pathophysiological processes implicated in depression while allowing identification of shared astrocytic responses that may underlie treatment efficacy or resistance.

A systematic literature search was performed using PubMed due to its comprehensive coverage of biomedical and neuroscience research and its suitability for reproducible systematic review workflows.

Three structured search strategies were designed to capture astrocyte-specific transcriptomic studies within each paradigm, yielding a total of 710 citations. A predefined systematic review protocol was applied, with large-language-model–assisted screening used to triage studies based on relevance, experimental design, and astrocytic resolution. All automated decisions were manually reviewed prior to final inclusion. Eligible studies included bulk and single-cell RNA-sequencing datasets with sufficient methodological transparency for downstream integration.

Differentially expressed gene lists from included studies were harmonised and integrated using custom R pipelines, alongside our previously generated RNA-sequencing data from primary rat astrocytes treated with fluoxetine for 4 and 24 hours. Comparative analyses were performed to identify genes shared across all paradigms, genes specific to subsets of paradigms, and genes unique to fluoxetine exposure. Network-level analyses were used to infer potential upstream regulators and enriched biological pathways, with visualisation of overlap patterns generated to assess commonality across experimental contexts.

This integrative approach is expected to reveal conserved astrocytic metabolic pathways that are consistently altered across stress, immune, and antidepressant conditions. Identifying shared hub genes and regulatory networks provides mechanistic insight into how astrocytic metabolism may contribute to antidepressant efficacy and treatment resistance. More broadly, this work highlights astrocytes as active modulators of antidepressant response and offers a data-driven framework to inform the development of novel, metabolism-targeted therapeutic strategies for depression.

C20

Derivation of perineuronal oligodendrocytes from induced pluripotent stem cells to model their role in Parkinson's disease pathogenesis.

Kimberly Ai Xian Cheam[1], Caleb Webber[2]

[1]Dementia Research Institute, Cardiff University, United Kingdon, [2]Dementia Research Institute, Cardiff University, United Kingdom

Parkinson's disease (PD) is a neurodegenerative disorder with incompletely understood cellular and genetic mechanisms. We have created a deep cellular atlas of the human ventral substantia nigra across PD stages by integrating full-length single-nuclei sequencing with genome-wide association studies (GWAS). This atlas revealed that genetic risk converges on AGTR1^{+} dopaminergic neurons and perineuronal oligodendrocytes (pODCs), both of which are reduced in PD, as well as oligodendrocyte precursor cells (OPCs), with risk enriched among disease-disrupted cellular interactions. The atlas also highlighted convergence of PD genetic risk loci with insulin resistance pathways, particularly within pODCs, which showed reduced expression of PI3K/AKT/mTOR pathway genes. Perineuronal oligodendrocytes are a distinct oligodendrocyte population in grey matter that establish direct somatic contact with neuronal cell bodies. Unlike classical oligodendrocytes, these cells do not myelinate, suggesting they function to provide trophic and metabolic support to neurons and modulate neuronal activity. Perineuronal oligodendrocytes are highly understudied.

Given our limited understanding of perineuronal oligodendrocytes, we are using cellular atlas insights to guide pODC derivation from induced pluripotent stem cells (iPSCs). We are testing established directed-differentiation protocols to derive OPCs from iPSCs using either neurosphere-based or adherent culture approaches, then differentiate these OPCs into pODCs. We are also testing direct reprogramming methods through overexpression of transcription factor combinations essential for oligodendrocyte fate specification. After establishing robust OPC and oligodendrocyte differentiation, we will introduce modifications to small molecule concentrations, timing, and transcription factor expression patterns obtained from our atlas data and other *in silico* analysis to promote perineuronal oligodendrocyte fate. A key approach will be to characterise the cells at key differentiation timepoints using scRNA sequencing to track lineage specification. This will be supported by other characterisation methods such as qPCR and immunofluorescence.

Once we establish a robust protocol to derive pODCs, we will increase culture complexity by incorporating pODCs into 3D myelinating organoids (myelinoids) and co-culturing pODCs with iPSC-derived dopaminergic neurons to recapitulate the *in vivo* perineuronal architecture. This culture system will enable us to investigate PD genetic risk using patient-derived cells and elucidate the metabolic and functional consequences of pODC dysfunction in disease pathogenesis.

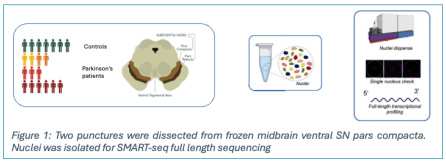


Figure 1: Two punctures were dissected from frozen midbrain ventral SN pars compacta. Nuclei was isolated for SMART-seq full length sequencing

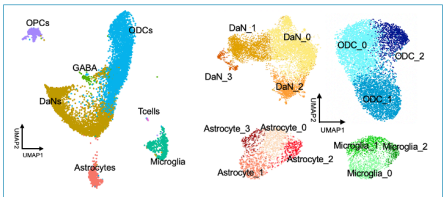


Figure 2: (A) A total of 7 major cell types were identified with more than 8000 expressed genes detected per nuclei. (B) Different cellular subtypes of the dopaminergic neurons (DaN), oligodendrocyte (ODC), astrocyte and microglia were also identified

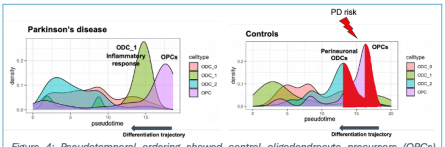


Figure 4: Pseudotemporal ordering showed control oligodendrocyte precursors (OPCs) differentiate into perineuronal. In PD, OPCs differentiate into a subtype of ODCs characterized by genes involved in immune response

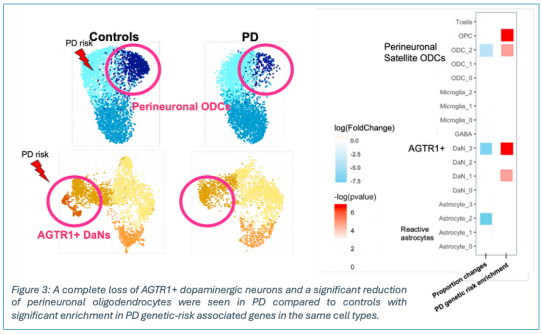


Figure 3: A complete loss of AGTR1+ dopaminergic neurons and a significant reduction of perineuronal oligodendrocytes were seen in PD compared to controls with significant enrichment in PD genetic-risk associated genes in the same cell types.

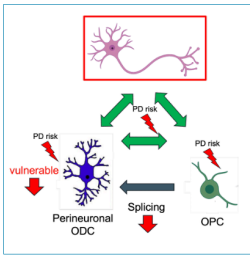


Figure 5: The overview of the possible cellular mechanisms identified from the sn-atlas. Our data highlight that AGTR1+ DaNs and ODCs are lost and reduced in PD patients. PD genetic risk variants act across AGTR1+ DaNs, ODCs and OPCs, disrupting the neuroprotective crosstalk between these cell types and eroding the supportive environment making the AGTR1 neurons more vulnerable given that they are already under high metabolic and oxidative stress. The differential splicing also identified dysregulation in the differentiation of OPC into perineuronal ODC, suggesting a selective reduction of this ODC subtype that consequently affects the neurons. A multi-cellular vulnerability exists in which the loss of perineuronal ODCs and disrupted OPC differentiation act together to drive selective degradation of AGTR1 DaNs in PD. These findings provide the rationale for developing iPSC-derived perineuronal ODC models that can recapitulate this vulnerability axis in vitro and serve as a platform for therapeutic interrogation

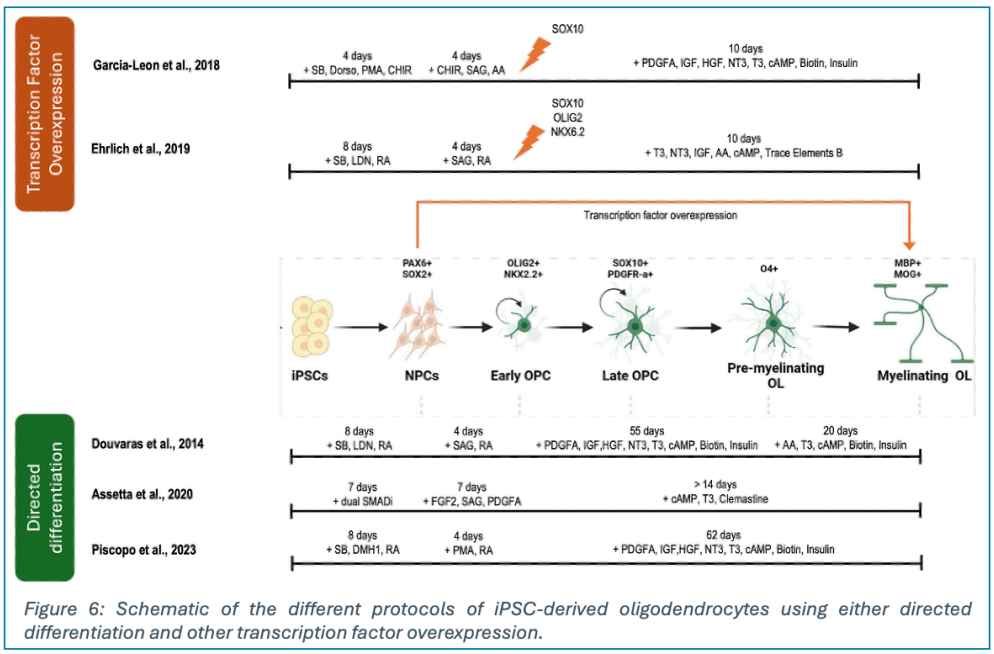


Figure 6: Schematic of the different protocols of iPSC-derived oligodendrocytes using either directed differentiation and other transcription factor overexpression.

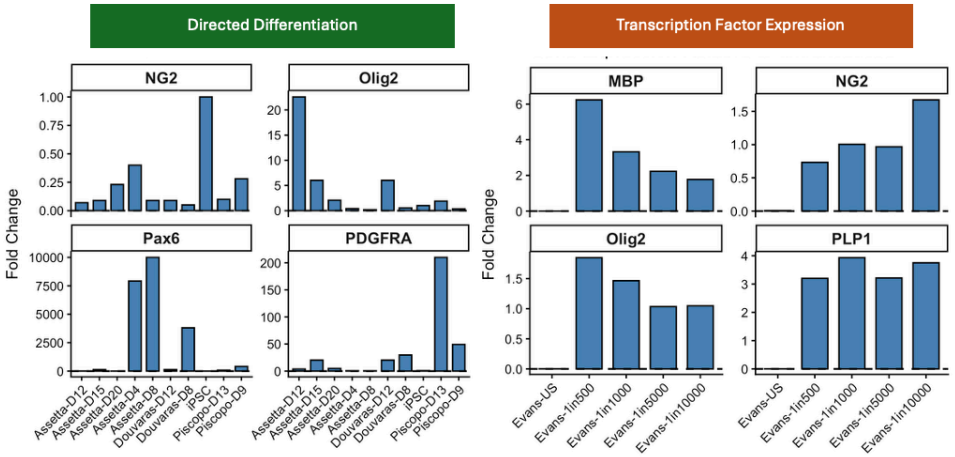


Figure 6: Pseudotemporal ordering showed control oligodendrocyte precursors (OPCs) differentiate into perineuronal.

C21

Hypoxia Suppresses Microglial Inflammatory Responses While HIF1α Differentially Modulates Activation Across Microglial Models

John Bennett[1], Stuart Jenkins[2], Rebecca Edwards[1], mark dallas[3], Ruoli Chen[1]

[1]School of Allied Health Professions and Pharmacy, Keele University, United Kingdom, [2]School of Medicine, Keele University, United Kingdom, [3]School of Pharmacy, University of Reading, United Kingdom

Introduction
Microglia dynamically regulate their phenotype in response to brain microenvironment conditions, with this changing their mediation of inflammatory and reparative processes. Hypoxic signalling is a central driver of microglial reprogramming, particularly in the context of neurodegenerative disease. Key mediators of this response include hypoxia-inducible factor 1 alpha (HIF1α) and Toll-like receptor 4 (TLR4), which coordinate immune adaptation under low oxygen conditions. However, the molecular crosstalk between HIF1α, TLR4, and subsequent functional responses remains poorly defined.

Aim

This study aims to understand microglial behaviour in hypoxic niches and its implications for inflammation within the neural environment and tissue repair.

Methods
All animals for this research were sacrificed by pentobarbitol injection via (*Animals (Scientific Procedures) Act*, 1986) (ASPA) compliant Schedule 1 procedures. Work was performed with Keele University ethics committee approval under establishment licence X350251A8.

This study examined the inflammatory and functional responses of primary microglia from postnatal mouse brains (P1-P3) and BV-2 cells under normoxic (21% O_2) and hypoxic (1% O_2) conditions, following 24h stimulation with a range of LPS concentrations (0–10,000 ng/mL). To investigate HIF1α involvement, cultures were simultaneously treated with the prolyl hydroxylase inhibitor FG4592 (Roxadustat). Effects on TNF-α production, metabolic activity, and cytotoxicity were respectively assessed by ELISA, MTT, and LDH assays. Phagocytic function was analysed using fluorescent nanoparticle uptake, and gene expression of pro-inflammatory and anti-inflammatory markers including TNF-α, IL-6, Arg1, and TLR4 assessed by qPCR.

Results
Primary microglia displayed by mean ~49% higher TNF-α concentrations than BV-2 cells across all concentrations ≥100 ng/mL. Hypoxia suppressed TNF-α production in both cell types, and reduced microglial viability and phagocytosis in BV-2 cultures. FG4592 sensitised primary microglia to low-dose LPS (1–10 ng/mL); increasing TNF-α output at these doses without doing so at greater LPS concentrations. This sensitisation was not observed in BV-2 cells, where FG4592 reduced TNF-α release in a manner comparable to hypoxia. HIF1α stabilisation increased IL-6 and Arg1 expression in BV-2 cultures. Additionally, it did not significantly augment the phagocytotic activity of primary cells. Taken in their totality, these results suggest that while HIF1α contributes to microglial pro-inflammatory cytokine production, it does so though context-dependent regulation of microglial function.

Conclusions
This study demonstrates that hypoxia and HIF1α stabilisation do not universally enhance microglial pro-inflammatory responses. Instead, pro-inflammatory microglial function is suppressed by hypoxia, while

HIF1's effects are cell-line specific and sensitive to the intensity of inflammatory stimuli. BV-2 cells showed diminished capacity for inflammatory activation and adaptation under hypoxic stress, while primary microglia demonstrated increased sensitivity to low-level inflammatory cues when HIF1α was stabilised. These findings highlight the limitations of immortalised microglia as proxies for primary cell models. They suggest that therapeutic modalities targeting HIF1α are unlikely to exacerbate already highly inflammatory microenvironments, such as in in stroke, but may bias microglia towards greater TNF- α release overall.

C22

Tracing brain development one radial glia at a time

Giselle Cheung[1], Florian M. Pauler[2], Peter Koppensteiner[2], Thomas Krausgruber[3], Carmen Streicher[2], Martin Schrammel[2], Natalie Gutmann-Ozgen[2], Alexis E. Ivec[2], Christoph Bock[3], Ryuichi Shigemoto[2], Simon Hippenmeyer[2]

[1]University of Exeter, United Kingdom, [2]Institute of Science of Technology Austria, Austria, [3]Medical University of Vienna, Austria

Introduction: The evolutionarily conserved Superior Colliculus (SC) is a midbrain region essential for multisensory integration and motor responses[1]. It is a highly-organised layered structure receiving inputs from different sensory modalities and connected to multiple brain regions including cortex, midbrain and brainstem[2]. Its dysfunction has been implicated in neurodevelopmental disorders like autism and attention deficit hyperactivity disorders[3,4]. Research continues to discover unique functions and novel cell populations in the mouse SC, for example its role in a secondary visual pathway bypassing the visual cortex[5]. However, how cell-type diversity is generated throughout development is still unknown despite the characterisation of radial glia progenitors (RGPs) in 1992 in the analogous structure in the chick[6]. **Aim:** The aim of this study is to determine the precise lineage programme instructing radial glia progenitors (RGPs) to generate SC cell-type diversity. **Methods:** We combined in-silico transcriptomic analyses, Mosaic Analysis with Double Markers (MADM)-based clonal analysis with single-cell RNA-sequencing to define SC development at single RGP level. Statistical analyses were performed using ANOVA, t-test, and Chi-squared test. All experiments received ethical approval from Austrian authorities. **Results:** We have uncovered a precise map of RGP behaviour, division patterns and potential in generating neurons, astrocytes and oligodendrocytes throughout development at single-progenitor resolution. Our data showed that individual SC RGPs are exceptionally multipotent and have the capacity to generate the full spectrum of cell-type diversity including all excitatory and inhibitory neuronal cell-types, astrocytes and oligodendrocytes in the SC. We also found that cell-type diversity is regulated by *Pten*, a key autism risk gene[7]. **Conclusions:** The delineation of the concerted production of neurons and glia during SC development provides important insights into how sensory processing is established during development and how it can be disrupted leading to lasting neurological defects.

C23

Glial Contributions to Circadian Timekeeping in the Mouse Dorsal Vagal Complex and their Entrainment by Feeding

Charlotte Muir[1], Sama Al Tehami[1], Hugh D. Piggins[1], Lukasz Chrobok[1]

[1]University of Bristol, United Kingdom

Circadian timekeeping enables organisms to anticipate and adapt to daily environmental cycles. In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus is recognised as the primary circadian clock; however, it is now well established that additional, autonomous clocks are distributed throughout the brain and peripheral tissues. Importantly, circadian organisation is not solely a neuronal property. Glial cells express clock genes and actively contribute to circadian function, yet their roles outside the SCN remain poorly understood. Here, we focus on the dorsal vagal complex (DVC), a brainstem structure that plays key roles in satiety and metabolic regulation. Previously, we identified robust clock gene expression across both neuronal and non-neuronal cell populations of the DVC. This prompted us to ask how glial cells – specifically astrocytes and ependymocytes – contribute to circadian timekeeping and oscillator coupling within the DVC.

To address this, we combined ex vivo, molecular, and in vivo approaches. Circadian rhythms were monitored using PER2::LUC bioluminescence recordings from organotypic DVC slices cultured and imaged for up to one week on an EM-CCD equipped Olympus LV200 system. To probe glial–glial and glial–neuronal communication, we applied TAT-GAP19 to block connexin-43 (Cx43) hemichannels and fluoroacetate to inhibit astrocytic metabolism, and quantified rhythm robustness and phase relationships between putative oscillators. Complementary in vivo studies were performed by measuring spatial and temporal expression of clock genes and glial markers using RNAscope and immunohistochemistry across the 24-hour cycle. These were augmented by, NanoString transcriptomic profiling to determine how neuronal clock disruption (neuronal Bmal1 knockout) impacts glial and neuronal gene expression in the DVC. Finally, we employed time-restricted feeding paradigms at distinct phases of the day to assess how physiologically relevant feeding cues reshape neuronal and glial clocks in vivo, analysed across four circadian time points.

We identify robust clock gene expression in multiple DVC glial populations, including astrocytes within the nucleus tractus solitarius (NTS), glial cells forming a specialised area postrema–nucleus of the solitary tract glial border, and ependymal cells lining the wall of the central canal. PER2::LUC recordings reveal that, ex vivo, ependymal rhythms are stably anti-phased relative to putatively neuronal oscillators. In vivo, these oscillators are phase-aligned under ad libitum feeding but become differentially shifted by scheduled feeding. Under time-restricted feeding, a phase difference between ependymal and neuronal rhythms re-emerges, albeit smaller than that observed ex vivo. Notably, pharmacological blockade of Cx43 hemichannels reduces this phase disparity in culture, implicating glial communication pathways in oscillator coupling.

Together, our findings demonstrate that, analogous to the SCN, glial cells make a substantive contribution to circadian timekeeping within the DVC. Glial–neuronal communication is critical for phase alignment and likely involved in entrainment to feeding schedules. Given that clock genes act as transcriptional regulators, it is plausible that glial clock activity shapes downstream gene expression, glial physiology, and sensitivity to metabolic cues. These data support a more universal role for glia in mammalian circadian systems and motivate future studies to define the physiological consequences of selectively disrupting glial clocks in brainstem circuits controlling feeding and metabolism.

C24

Focal astrocytic Kir4.1 loss drives seizures, spreading depolarisations and post-ictal impairments

Neela K. Codadu[1], Yunan Gao[1], Olga Tiurikova[1], Eduard Masvidal-Codina[2], Anton Guimera-Brunet[2], Dmitri Rusakov[1], Rob C. Wykes[1]

[1]Research Department of Epilepsy, Institute of Neurology, UCL, United Kingdom, [2]Institut de Microelectrònica de Barcelona, IMB-CNM (CSIC), Spain

Introduction:

Astrocytes regulate neuronal excitability through potassium buffering mediated by Kir4.1 inwardly-rectifying potassium channels. Reduced astrocytic Kir4.1 expression has been reported in human temporal lobe epilepsy, yet its causal role in epileptogenesis remains unclear. In epilepsy, spreading depolarisations (SD), slowly propagating waves of neuronal and glial depolarisation, are temporally associated with seizures (seizures+SD) and have been associated with post-ictal period (period immediately following seizure termination) and Sudden Unexpected Death in Epilepsy. However, whether astrocytic Kir4.1 loss is sufficient to drive seizures and SD, and how SD shape post-ictal state, is unknown.

Methods:

We generated focal Kir4.1 knockout mice (Kir4.1-cKO) by deleting the Kcnj10 gene encoding Kir4.1 channels in hippocampal astrocytes of adult Kir4.1-floxed mice using viral Cre recombinase. Ex vivo validation of Kir4.1 loss was performed using western blotting. Network electrophysiological activity and extracellular potassium dynamics were examined in hippocampal slices using local field potential (LFP) and genetically-encoded potassium indicator (GINKO) fluorescence recordings, respectively. To test seizure and SD susceptibility in vivo, optogenetics and DC-coupled graphene micro-transistor arrays were used in awake head-fixed mice. Continuous AC-coupled, and video-DC-coupled ECoG telemetry recordings were employed to characterise the emergence of spontaneous seizures and SD.

Results:

Kir4.1-cKO showed significantly reduced Kir4.1 protein expression (Kir4.1 expression relative to GAPDH: Controls, 0.85±0.11, n=5 mice; cKO, 0.46±0.08, n=4 mice; p=0.03). Electrical stimulation of Schaffer collaterals evoked larger LFP and GINKO fluorescence responses in CA1 stratum radiatum, indicating impaired potassium buffering. Chronic AC-coupled telemetry in Kir4.1-cKO (n=10 mice) revealed the emergence of spontaneous seizures 7–10 days post-viral injection. In awake head-fixed mice, Kir4.1-cKO hippocampal networks show increased susceptibility to optogenetic stimulation-induced seizures and seizures+SD. Optogenetic trains (10 s of 5, 20, and 50 Hz) induced significantly larger DC shifts in Kir4.1-cKO (1.78±0.37 mV; n=15 trials; 5 mice) than in controls (0.30±0.09 mV; n=12 trials, 4 mice; $p<0.0001$). Larger DC shifts were associated with the occurrence of seizures (seizure: 2.03±0.43 mV, n=12 trials; no seizure: 0.39±0.09 mV, n=15 trials; p=0.003). At higher stimulation frequencies, seizures+SD occurred in the majority of Kir4.1-cKO mice (20 Hz: 60%; 50 Hz: 80%). Seizures+SD profoundly altered the post-ictal

state, producing significantly prolonged post-ictal depression and recovery period (seizures: 112.02±25.56s, n=4; seizure+SD: 360.10±75.84s, n=7; p=0.016).

Chronic video-DC-coupled telemetry (six mice monitored for three weeks) revealed spontaneous seizures alone (n=38) and seizures+SD (n=40). Compared with seizures alone, seizures+SD exhibited higher power (25-80 Hz: seizures, 8.91±0.82 a.u.; seizure+SD, 16.49±1.04 a.u.; $p<0.0001$) and longer intervals to the next seizure event (inter-event intervals: seizures, 113.70±34.29 mins; seizures+SD, 168.01±35.94 mins; p=0.002). Video-DC-coupled ECoG analysis of post-ictal behaviour (120-second period following seizure termination) revealed altered behaviour such as behavioural arrest, loss of posture, and body jerks for ~87% (104.83±5.54 s) of the post-ictal period after seizures+SD (n=12), compared with ~1.7% after seizures alone (2.00±1.13 s, n=12; $p<0.0001$), indicating severely delayed functional recovery.

Conclusion:

Focal hippocampal astrocytic Kir4.1 loss is sufficient to generate spontaneous seizures with a high incidence of SD, which exacerbate post-ictal impairments. This model provides mechanistic insight into how astrocytic potassium dysregulation drives epileptogenesis and seizure pathology.

C25

Revealing the molecular mechanisms of endocytic polygenic risk in late onset Alzheimer's disease in iPSC-derived Microglia

Srilakshmi Goberdhan[1], Mizuki Morisaki[1], Matilda Kakengi[1], Patricia Dos Santos Rodrigues[1], Atahualpa Castillo Morales[1], Julie Williams[1], Natalie Connor-Robson[1]

[1]Cardiff University, Dementia Research Institute, United Kingdom

Aims

Late onset Alzheimer's disease (LOAD) is genetically complex but GWAS studies and histopathological data consistently implicate endocytic dysfunction which is an early disease phenotype (1,2,3). Studying the sporadic disease is hard using conventional models where relatively few genetic manipulations can be made. It remains essential for us to understand how multiple LOAD risk genes come together to drive the disease.

Methods

We have developed a novel endocytic pathway specific polygenic risk score (PRS) and applied it to a genetically well characterised cohort of LOAD individuals. We have stratified the patient cohort and selected individuals with high endocytic PRS and reprogrammed 20 PBMC samples to induced pluripotent stem cell lines to compare to healthy age matched controls.

Results

Our endocytic PRS can be used to predict LOAD with >70% accuracy alone or increased to >90% when combined with biomarker data. We have demonstrated that the PRS is not impacted by APOE status and suggest there are further endocytic risk genes to be discovered in association with AD. We have used these lines to examine underlying disease mechanisms by differentiating 20 lines from individuals with high endocytic PRS and 12 from healthy age matched controls with low PRS to microglia and have carried out transcriptomics, proteomics, live imaging assays to assess endocytic and phagocytic behaviour including amyloid beta uptake and assessed microglial interactions with neurons in coculture. This body of data is currently being analysed but will provide the first insights into how complex genetic changes associated with LOAD drive disease mechanisms which is key to developing better therapeutic targets.

Conclusions

Our work demonstrates it is possible to stratify patient cohorts on the basis on underlying disease mechanisms. This is important for studying the molecular basis of disease and for identifying patients most likely to respond to specific treatment strategies. We have demonstrated our endocytic pathway specific PRS has high accuracy at predicting LOAD, is not impacted by APOE status and suggests further endocytic risk genes are yet to be identified. We have developed a novel iPSC resource to study

endocytic mechanisms of LOAD and have undertaken deep phenotypic characterisation of iPSC-derived microglia.

C26

Contribution of visual stimulus and locomotion to astrocytic calcium responses in primary visual cortex of awake mice

Sara de Leo[1], Fábio Ribeiro Rodrigues[1], Joanna Holeniewska[1], Samuel G. Solomon[1]

[1]University College London, UK

Introduction: Astrocytes in the sensory cortices have access to both sensory and internal (e.g. arousal) signals. The relative contribution of these inputs to astrocytic calcium signalling remains unclear. We therefore sought to establish how calcium signals of astrocytes in the primary visual cortex (V1) of awake mice respond to changes in visual stimulus, and changes in locomotion or arousal.

Methods: We measured population-level astrocytic calcium responses from V1 in 6–12-month-old mice (C57/BJ6 background with humanised MAPT gene), of either sex. Animals were anaesthetized with isoflurane (3% induction, 1.5% maintenance) and prepared for aseptic surgery, a craniotomy was made above V1, and a bar was attached to the skull for subsequent head-fixation. In 8 animals, AAV8-GFAP-GCaMP6f was injected, and a fibre-photometry cannula was implanted above the dura. In 13 animals an electrode was instead implanted in layer 4 to measure visually evoked local field potentials (VEPs). At least 3 weeks after recovery from anaesthesia, astrocytic calcium or neural VEPs were measured in awake head-fixed animals, free to locomote on a wheel, in the presence and absence of brief (0.5 - 1.5s) flashed visual stimuli. Calcium signals (ΔF/F) were calculated as the peak change in fluorescence in the 2-10s following onset of spontaneous locomotion bouts, or appearance of a visual stimulus, relative to that in the 1s prior. All procedures were performed under appropriate project and personal licenses from the UK Home Office.

Results: Astrocytic calcium increased rapidly at the onset of locomotion (mean±sem: 12.3%±3.8, n=8). A high-contrast visual stimulus also elicited an increase in calcium levels relative to pre-stimulus baseline, but response amplitude depended on the behavioural state of the animal. Response was larger if the animals were already locomoting (movement speed exceeding 1 cm/s; 8.0%±1.9, n=7) than if they were stationary (1.8%±0.4; p =0.016, Wilcoxon Signed Rank). Subsequent analyses were therefore confined to trials when the animal was locomoting. Astrocytic responses increased with stimulus contrast (12% contrast: 2.2%±0.8; 100% contrast: 7.8%±2.2, n=6). Astrocytic responses were visuotopic: small stimuli presented in ipsilateral visual field evoked much lower responses (1.1%±0.6, n=8) than those in an appropriate location in contralateral visual field (6.8%±3.0; p = 0.023, Wilcoxon Signed Rank). Responses to small, flashed squares in the contralateral hemifield suggested presence of receptive fields that were similar in size to those obtained for neural VEPs.

Conclusion: Astrocytes in mouse V1 respond with increase in calcium levels to the onset of locomotion, or the change in arousal that accompanies locomotion. Astrocytes also respond on presentation of visual stimuli, but that response depends on behavioural state. When an animal is locomoting, astrocytic population response increases with the strength of a visual stimulus and is tuned to the location of that stimulus in the visual field.

C27

Microcount: an automated pipeline for quantitative analysis of glial morphology in brain tissue

Vanessa Drevenakova[1], Albert Ugwudike[1], Ethan Qiyixing Liu[1], Lok Yin Nicholas Chan[1], Nga Yin Tam[1], Emilie Wielezynski[1], Valeria Dosso[1], Sarina Grewal[1], Laura Li Yu[1], Auguste Vadisiute[2], Clíona Farrell[3], Zoltán Molnár[2], Frances Wiseman[3], Sophie V. Morse[1]

[1]Imperial College London, United Kingdom, [2]University of Oxford, United Kingdom, [3]University College London, United Kingdom

Glial morphology is closely linked to cellular function, providing important insights into physiological and pathological processes in the central nervous system. Despite this, quantitative analysis of glial morphology remains challenging due to the time-intensive nature of manual segmentation, variability between annotators, the need for coding expertise, and reliance on costly proprietary software.

We developed Microcount, a fully integrated, open-source MATLAB-based pipeline for automated segmentation and morphological analysis of glial cells in microscopy images. Microcount enables atlas-based anatomical region-of-interest (ROI) selection using the Allen Mouse Brain Atlas with a custom segmentation strategy that enables physiologically accurate reconstruction of glial somas and processes in two-dimensional images. The pipeline provides quantitative measures of cell density, soma size, branching complexity, convexity, Sholl index, and co-marker co-localisation. Importantly, it is implemented through an intuitive user interface (UI), which removes the need for coding expertise.

We validated Microcount using mouse and human datasets spanning different glial populations, staining methods, and imaging modalities, including fluorescent and DAB-labelled tissue stained for ionised calcium-binding adaptor molecule 1 (Iba1), cluster of differentiation 68 (CD68), glial fibrillary acidic protein (GFAP), S100 calcium-binding protein B (S100B), and purinergic receptor P2Y12 (P2RY12). We also performed a comparison with manual segmentation on 16 fluorescence microscopy images (1000 × 1000 pixels) segmented independently by five trained annotators. Microcount's segmentations showed strong agreement with consensus manual annotations (mean F1 score = 0.68, accuracy = 0.87). Furthermore, morphological and activation-related measurements generated by Microcount correlated well with those generated from manual segmentations (Pearson's r range = 0.72-0.98, $p < 0.05$), whilst requiring orders of magnitude less time to compute. Validation of Microcount in a chronic mouse inflammation model using adult C57BL/6 mice treated with lipopolysaccharide (0.75 mg/kg, intraperitoneal injection, once daily for 7 days; n = 4 per group) detected significant increases in glial density, soma size, and CD68 expression (two-way ANOVA with Šidák's correction, $p < 0.05$) compared to saline control. Together, these findings establish Microcount as a robust, accessible, and scalable tool for high-throughput analysis of glial morphology and marker expression in health and disease.

C28

Dynamic Compensatory Interplay of Potassium Channels in Oligodendrocytes: Regulated by TRPA1

Xiangyi Du[1], Vincenzo Giacco[1], Matthew Russel[2], Xiaolan Wu[1], Jackson McLean[1], Mansil Yoon[1], Craig A. Sexton[1], Pingting Cao[1], Christlyn Espiritu[1], Navami Sangeeth[1], Priscilla Meccheri[1], Svenja Vorthmann[1], Laren Alphan[1], Janice Kamajaya[1], Anwen Bullen[3], Alejandra Carbajal[2], Leanne Allison[2], Angela Hodges[4], Nicola B. Hamilton[1]

[1]Wolfson Sensory, Pain and Regeneration Centre, King's College London, London, United Kingdom, [2]Centre for Ultrastructural Imaging, King's College London, London, United Kingdom, [3]Ear Institute, University College London, London, United Kingdom, [4]Maurice Wohl Clinical Neuroscience Institute, King's College London, London, United Kingdom

Introduction:

Potassium channels in oligodendrocytes, including inwardly rectifying (Kir), two-pore-domain (K2P), and voltage-gated (Kv) channels, are essential for potassium buffering, maintenance of myelin integrity, and central nervous system homeostasis (González et al., 2012). Disruption of oligodendroglial potassium conductance compromises potassium clearance, delays axonal recovery following repetitive firing, and is associated with seizures and motor dysfunction (Valerie et al., 2018). However, how distinct potassium channel populations are coordinated within oligodendrocytes, and whether this regulation differs between grey and white matter (GM, WM), remains poorly understood.

Methods:

Using whole-cell patch-clamp recordings in acute brain slices from C57BL/6 wild-type (WT) and TRPA1 global knockout (gKO) mice of both sexes, we assessed potassium conductance in mature oligodendrocytes (MOL) of the motor cortex and corpus callosum. Also, complementary immunohistochemical analyses across postnatal development in both sexes were used to examine associated changes in potassium channel and myelin protein expression.

Results:

We identify region-specific mechanisms of potassium channel regulation. Pharmacological blockade of Kir channels with barium (100 µM) significantly reduced potassium conductance in GM MOLs (ΔgK (%) = -30.7 ± 4.2, n = 11) but had minimal effects in WM MOLs (ΔgK (%) = -3.2 ± 3.0, n = 29; $P < 0.0001$), indicating differential reliance on Kir-mediated conductance across brain regions.

Previous studies suggested that the Transient Receptor Potential Ankyrin 1 (TRPA1) channel is expressed in oligodendrocytes and may influence potassium channel function (Hamilton et al., 2016). Consistent with this, activation of TRPA1 using multiple agonists, such as carvacrol, robustly inhibited oligodendroglial potassium conductance (caracrol evoked ΔgK (%) = -40.15 ± 2.16 %, n = 48).Moreover, TRPA1 global knockout mice exhibited a marked reduction in resting potassium conductance at postnatal days P12–P18 (WT gK (nS) = 6.77 ± 1.13, n = 8, gKO gK (nS) = 3.56 ± 0.48, n = 8; $P = 0.0070$), followed by an apparent compensation at P18–P25 (WT gK (nS) = 11.23 ± 1.36, n = 11, gKO gK (nS) = 10.77 ± 1.01, n = 7; $P = 0.8117$), when conductance returns to wild-type levels. Immunohistochemical analyses reveals a coincidental reduction in myelin basic protein (MBP) expression at P21 ($P = 0.01$, n = 5 each) that recovers by P30 ($P = 0.817$, n= 5 each). We found no significant sex differences.

Conclusion:

Ongoing work aims to determine how TRPA1 regulates oligodendroglial potassium channel expression and conductance in a region- and developmental stage-dependent manner, and how these mechanisms impact myelin structure and potassium siphoning. Together, these findings identify TRPA1 as a critical regulator of oligodendroglial potassium channel function and myelin stability, with potential relevance for activity-dependent myelin vulnerability in demyelinating disease.

C29

Cardiac glia as active regulators of autonomic heart control: defining glial contributions to cardiac physiology and stress-induced vulnerability

Rimma Dugarova[1], Shefeeq Theparambil[1], Svetlana Mastitskaya[1]

[1]University of Bristol, United Kingdom

Introduction

Cardiac function is tightly controlled by autonomic neural circuits, yet the contribution of associated glial cells to neuro-cardiac communication remains poorly understood. Converging evidence supports the existence of peripheral tripartite synapse-like structures within cardiac autonomic circuits, where glial cells form intimate contacts with autonomic neurons and pacemaker cells. Three populations of cardiac-associated glia are consistently identified: Schwann cells, satellite glial cells and cardiac nexus glia. Genetic ablation of cardiac nexus glia abolishes autonomic heart rate modulation and increases arrhythmia vulnerability (Kikel-Coury *et al.*, 2021), while spatial transcriptomics identifies glial populations expressing neurexins and glutamate-handling machinery adjacent to human pacemaker cells (Kanemaru *et al.*, 2023). However, the mechanisms by which cardiac glia regulate electrophysiology, contractile function and haemodynamics *in vivo* remain undefined. It is also unknown whether chronic stress induces cardiac astroglial reactivity similar to CNS glia and whether this contributes to adverse post-ischaemic outcomes.

Methods

Cardiac glia will be selectively manipulated using astrocyte-specific Cre driver mice (GFAP-Cre or Aldh1l1-CreERT2) crossed with floxed effector lines. The cardiovascular phenotype will be characterised across three domains. Electrophysiology: action potential duration (APD), conduction velocity and arrhythmia susceptibility via burst pacing and programmed electrical stimulation will be studied in Langendorff-perfused hearts. Echocardiography**:** ejection fraction (EF), fractional shortening and diastolic function (E/A ratio, tissue Doppler) will be assessed longitudinally. Haemodynamics**:** pressure-volume catheterisation will quantify contractility (dP/dt max), relaxation (dP/dt min) and pressure-volume relationships.

A chronic variable stress model (4 weeks) will be implemented in parallel cohorts using a 2×2 factorial design (glial manipulation × stress). Cardiac glial inflammatory state will be assessed by GFAP immunostaining, morphological indices of astroglial activation and pro-inflammatory cytokine expression (IL-1β, TNF-α) via RT-qPCR. Recovery after myocardial ischaemia/reperfusion will be assessed by LAD coronary artery ligation, with infarct size, peri-infarct fibrosis and functional recovery (echocardiography at 1, 7, 14 and 28 days post-infarction) as outcomes.

Approach for statistical analysis

Primary outcomes: (1) APD and arrhythmia inducibility; (2) EF and dP/dt max; (3) infarct size and GFAP expression intensity. Continuous outcomes will be analysed by two-way ANOVA (genotype × stress) with Tukey's post-hoc correction. Longitudinal echocardiographic data will use linear mixed-effects models. Arrhythmia incidence will be compared using Fisher's exact test. Sample sizes (n=8-12 per group) are based on published effect sizes in glial ablation models to achieve 80% power at $\alpha=0.05$.

Expected value of results

These studies will establish whether cardiac glia are active participants in neuro-cardiac signalling or passive bystanders. If chronic stress drives cardiac astroglial inflammation and worsens post-ischaemic outcomes, this would identify cardiac glia as a cellular link between psychosocial stress and adverse cardiac events. This could reveal peripheral glial signalling pathways as novel therapeutic targets for arrhythmias and stress-related cardiac disease.

C30

Astrocytes regulate neuroendocrine functions in a sex-dependent manner through Cdk4 signalization

Mélanie C Faure[1], Nicolas Gurtner[1], Alex Kanmacher [1], Melvin Poirier [1], Lluis Fajas[1], Sarah Geller [1]

[1]Center of Integrative Genomic, University of Lausanne, Suisse

Astrocytes regulate neuroendocrine functions in a sex-dependent manner through Cdk4 signalization.

Astrocytes are key regulators of hypothalamic neuronal circuits controlling neuroendocrine functions. They also display sexually dimorphic features across several hypothalamic regions. However, the astrocytic factors contributing to the sex-specific regulation of these functions remain poorly understood. We recently identified the kinase Cdk4 as a potential regulator of neuroendocrine functions related to reproduction, stress, and metabolism, in a sex-dependent manner, using Cdk4 Neo-RIP mice (a full body KO). While Cdk4 is classically known for its role in cell cycle control, it has also been implicated in regulating cellular plasticity and metabolism in cancer and peripheral tissues. In the brain, Cdk4 is predominantly expressed in glial cells, particularly hypothalamic astrocytes, where its expression is modulated by both diet and sex. Based on these findings, we hypothesize that astrocytic Cdk4 modulates adult neuroendocrine functions through sex-specific effects on astrocyte metabolism and/or plasticity. To test this, we generated an inducible astrocyte-specific Cdk4 knockout model using the Cre-LoxP system ($Cdk4^{flox/flox}$ hGFAP-Cre ERT2 mice). Mice (Cre ERT2 $^{+/+}$ or $^{Tg/+}$) received intraperitoneal injections of tamoxifen (100 mg/kg/day) for four consecutive days.

Female KO mice exhibited disrupted estrous cycles, though fertility remained intact in both sexes. Under chow diet, increased weight gain and adiposity were observed only in male KO mice, with no differences in females. In contrast, under a 60% high-fat-diet, these differences disappeared in males but emerged in females.

Under chow diet and mildly stressful novel condition (e.g., calorimetric cages), both sexes displayed reduced food intake and body weight, with males also exhibiting elevated circulating corticosterone. Behavioral assessments of novelty responses revealed sex-specific differences: male KO mice entered novel zones more rapidly, reflecting increased exploratory behavior, while female KO mice showed decreased entries into the light zone and central food area, indicative of heightened anxiety or stress-related avoidance.

Altogether, our findings indicate that astrocytic Cdk4 regulates energy homeostasis in a sex and diet-specific manner and contributes to behavioral adaptation to novel or mildly stressful environments. Ongoing studies aim to identify hypothalamic regions exhibiting altered astrocyte number or morphology (immunofluorescence) and characterizing molecular pathways affected by Cdk4 loss (single-cell RNA sequencing). Future studies will selectively delete Cdk4 in astrocytes of specific hypothalamic nuclei using viral vectors and investigate the underlying mechanisms in vitro through primary hypothalamic astrocyte cultures.

C31

NTS astrocytic endozepines link insulin signalling to glucose metabolism in male rats

Lauryn E New[1], Niannian Wanng[2], Holly E. Smith[1], Ross Birks[1], Shabbir Khan Afridi[3], Joanne C. Griffiths[1], Ryan Hains[1], Jamie Johnston[1], Beatrice M Filippi[1]

[1]University of Leeds, United Kingdom, [2]Queen Mary Yniversity, United Kingdom, [3]University College of London, United Kingdom

Introduction and aims:

The central nervous system contributes to glucose homeostasis by integrating hormonal signals such as insulin to regulate hepatic glucose production (HGP). The nucleus of the solitary tract (NTS) within the dorsal vagal complex is an important site of central insulin action, yet the specific cellular mechanisms mediating this effect remain unclear. Astrocytes are increasingly recognized as active metabolic sensors in the brain. This study investigated whether insulin acts on NTS astrocytes to regulate glucose metabolism and explored the downstream signalling pathways involved.

Methods:

Male Sprague–Dawley rats were used to examine insulin signalling in the NTS. Cell-type–specific insulin receptor expression and activation were assessed using RNAscope, FITC-labelled insulin, and c-Fos immunohistochemistry. Astrocytic insulin signalling was selectively disrupted using a GFAP-Cre–dependent insulin receptor knockdown. Hepatic glucose production and glucose uptake were measured using pancreatic euglycemic and hyperinsulinemic clamp techniques. Endozepine release from primary astrocytes was quantified by ELISA, and GABAa receptor signalling was modulated pharmacologically via targeted NTS infusions.

Statistical analysis: Data are presented as mean ± SEM. Sample sizes ranged from n = 3–4 animals for histological and RNAscope analyses to n = 5–12 animals per group for in vivo clamp and pharmacological studies. In vitro experiments using primary astrocyte cultures were performed with n = 3–6 independent preparations. Statistical significance was assessed using unpaired Student's t-tests or one- and two-way ANOVA with appropriate post hoc comparisons. A p value < 0.05 was considered statistically significant.

Ethical approval: All animal experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986, approved by the University of Leeds Ethical Review Committee, and are reported in compliance with ARRIVE guidelines.

Results:

Insulin receptors were predominantly expressed in NTS astrocytes, with fewer receptors detected in neuronal populations. Insulin administration in the NTS induced widespread neuronal activation, while direct insulin signalling occurred mainly in astrocytes. Astrocyte-specific insulin receptor knockdown abolished insulin-dependent suppression of hepatic glucose production without affecting glucose uptake. Insulin stimulated the release of endozepines from astrocytes, and direct NTS infusion of

endozepines mimicked insulin's metabolic effects. Blockade of the benzodiazepine binding site of GABAa receptors prevented insulin- and endozepine-mediated suppression of HGP, while GABAa receptor antagonists reproduced the effect of insulin. Importantly, endozepine or GABA antagonist administration restored NTS control of glucose production in high-fat-diet–induced insulin-resistant rats.

Conclusion:

These findings identify NTS astrocytes as key mediators of central insulin action on glucose metabolism. Insulin signalling in astrocytes promotes endozepine release, which reduces GABAergic inhibition to suppress hepatic glucose production. This astrocyte-dependent pathway provides new insight into brain–liver communication and suggests potential therapeutic targets for insulin resistance and type 2 diabetes.

C32

Astrocytes as drivers of pathogenicity in Leber's Hereditary Optic Neuropathy (LHON)

Wyn Firth[1], Marcela Votruba[1]

[1]Cardiff University, United Kingdom

Introduction:

Astrocytes play key roles in maintaining homeostasis within the central nervous system, with metabolism and cellular bioenergetics emerging as key mediators of this role. Moreover, recent evidence suggests that perturbation of astrocyte metabolism is a feature of many neurodegenerative conditions. LHON is an inherited neurodegenerative condition which typically manifests as sudden onset bilateral loss of vision and retinal ganglion cells. LHON is driven by deficiencies in respiratory Complex I, with consequent neurodegeneration explained by insufficient adenosine triphosphate (ATP) and aberrant reactive oxygen species (ROS) production. Curiously, spontaneous visual recovery has been noted, for reasons that remain unclear. Moreover, the contribution of astroglia to LHON pathology remains poorly understood though post-mortem evidence indicates a degree of gliosis in affected retinae, indicating astroglial involvement. Given the growing body of evidence supporting a role for aberrant mitochondrial respiration as a key contributor to astrocyte reactivity in various conditions, here we tested the hypothesis that LHON-affected astrocytes will exhibit morphological and functional changes indicative of astrocyte reactivity.

Aims:

Our aims were to determine whether LHON-affected astrocytes demonstrated bioenergetic or morphological shifts indicative of astrocyte reactivity, and to interrogate whether these changes were related to LHON phenotype severity and visual recovery status.

Methods:

Induced pluripotent stem cells derived from either a healthy control donor (WT) or LHON-affected patients ± visual recovery (recovery, R; no recovery, NR) were differentiated into astrocytes. Immunocytochemistry was used to confirm astrocyte identity. Mean fluorescence intensity (MFI) was measured and corrected for cell area using an automated pipeline. Live extracellular metabolic flux analyses were performed to interrogate changes to astrocyte respiration.

Results:

≥99% of cells were immunopositive for the astrocyte markers glial fibrillary acidic protein (GFAP) and S100B, indicating astrocyte fate specification across all genotypes. GFAP MFI was significantly reduced in LHON-NR astrocytes relative to WT, whereas LHON-R did not differ significantly from either WT or LHON-NR. S100B MFI was significantly reduced in both LHON phenotypes relative to WT with no significant difference between LHON phenotypes. Bioenergetic analyses indicated significantly reduced mitochondrial respiration in LHON-NR astrocytes and reduced coupling of oxygen consumption to ATP production relative to both WT and LHON-R astrocytes. Oxygen-ATP coupling was also significantly reduced in LHON-R astrocytes relative to WT. Both LHON phenotypes exhibited a compensatory increase in extracellular acidification, a proxy of glycolysis, relative to WT.

Conclusions:

Preliminary data indicate significant perturbation of mitochondrial respiration in LHON-affected astrocytes, with concomitant upregulation of glycolysis. These data are indicative of a reactive astrocyte phenotype and suggest a pro-inflammatory response. LHON-NR astrocytes exhibited greater deviation from WT astrocytes than LHON-R. How this contributes to LHON severity or recovery capacity remains unclear. The reduced GFAP and S100B MFIs may be explained by secretion of these factors or metabolic impairment impinging protein synthesis. Future work will focus on elucidating the metabolic phenotype of LHON-affected astrocytes, assessing cell motility, and characterising cytokine and ROS release profiles. Coculture assays will be used to explore how LHON-affected astrocytes affect neuronal health. Differences between LHON phenotypes will form a key part of our analyses.

C33

Astrocyte proteome dynamics in ageing and partial reprogramming in mice

Katrine Gaasdal-Bech[1], Aida Platero-Luengo[2], Robert Illingworth[3], Blanca Díaz Castro[4]

[1]Institute for Neuroscience and Cardiovascular Research, College of Medicine and Veterinary Medicine, The University of Edinburgh, Chancellor's Building, EH16 4SB Edinburgh, United Kingdom, United Kingdom, [2]The Institute of Biomedicine of Seville (IBiS), University of Seville, 41013 Seville, Spain, [3]Centre for Regenerative Medicine, Institute for Regeneration and Repair, The University of Edinburgh, Edinburgh BioQuarter, EH16 4UU Edinburgh, United Kingdom, [4]UK Dementia Research Institute at The University of Edinburgh, Chancellor's Building, EH16 4SB Edinburgh, United Kingdom, United Kingdom

Ageing is the primary risk factor for multiple neurodegenerative disorders. Cellular ageing is, however, malleable: When combining the Yamanaka factors (Oct4, Sox2, Klf4, c-Myc; OSKM) in careful moderation through a technique called partial reprogramming, cells can be reprogrammed into an epigenetically younger state without losing their initial identity and function. In mice, partial reprogramming improves age-related memory decline, although the mechanisms behind this improvement are still unknown. Although partial reprogramming is likely too invasive for human use, the technique can still be used to study rejuvenation mechanisms. Astrocytes are among the earliest and most affected brain cells by ageing and are integral in maintaining brain homeostasis. With ageing, astrocyte functions become impaired, contributing to neurological decline, and make the brain vulnerable to pathology. However, if rejuvenated, this ageing astrocyte phenotype may be reversed and may potentially improve the health of other neural cells. Here, we use mass spectrometry to study protein changes following partial reprogramming in 5- and 15-months-old mice. Immunohistochemistry was performed to confirm cell-specificity of the astrocyte-specific Gfap TurboID adeno-associated virus (AAV), used to perform astrocyte-specific proteomics, with linear mixed effects models used for immunohistochemical quantification statistics (Gfap TurboID AAV, N = 7-8 mice; no-AAV control, N = 3 mice). Using differential expression and Rank–Rank Hypergeometric Overlap analyses, we compared proteomic changes happening with ageing (5-months (N = 6) vs 15-months (N = 8) and with partial reprogramming (15-month-old partially reprogramed mice (N = 7) vs age-matched controls (N = 8)). Preliminary results indicate that several proteins upregulated with ageing show reduced expression following partial reprogramming, suggesting a shift in the astrocyte proteome consistent with a more youthful state. Further analyses and experiments will aim to identify the pathways and mechanisms involved which may inform future strategies for neuroprotective therapies. All animal work was carried out in accordance with UK Home Office regulations.

C34

Astrocyte gene therapy reduces seizure frequency in rodent models of acute seizures and chronic epilepsy

Yunan Gao[1], Neela K Codadu[1], Xueting Ban[2], Yuyan Weng[2], Mengyue Niu[2], Kagan Agan[1], Nicholas D Mazarakis[2], Robert C Wykes[1]

[1]WykesNeuroLab, Research Department of Epilepsy, UCL Queen Square Institute of Neurology, United Kingdom, [2]Gene Therapy, Department of Brain Sciences, Imperial College London, United Kingdom

Introduction

Kir4.1 is an ATP-sensitive inwardly-rectifying potassium ion channel encoded by the *KCNJ10* gene and expressed exclusively in glial cells. It plays a key role in regulating spatial K^+ buffering of astrocytes. Kir4.1 loss-of-function results in increased extracellular K^+ level and impaired glutamate uptake that cause neuronal hyperexcitability and seizures. A functional decrease in Kir4.1 expression has been reported in human acquired and genetic epilepsies, and in rodent models of epilepsy (1-3). Thus, it is hypothesized that upregulation of Kir4.1 in astrocytes will counteract pathogenic rises in extracellular K^+ and glutamate to prevent transition to seizures.

Aims and objectives

We aim to develop a novel astrocyte gene therapy suitable for pharmacoresistent epilepsies including focal, multifocal and genetic epilepsies. If effective it can be adapted to enter clinical trials to bring transformative benefits to epileptic patients.

Method

All animal procedures were approved by the local ethical committee and performed in accordance with the United Kingdom Animals Scientific Procedures Act (1986) and associated guidelines. The mouse strain C57BL/6 was used; and anaesthetised via inhalation of 4-5% isoflurane (induction) followed by 1-2% (maintenance) during all surgeries performed.

To investigate whether Kir4.1 over-expression could reduce seizure susceptibility, we injected adeno-associated viral (AAV) vectors unilaterally into the mouse somatosensory and visual cortex, either AAV9-gfaABC1D-Kir4.1-tdTomato or AAV9-gfaABC1D-tdTomato that used the astrocyte-specific gfaABC1D promoter to drive transgene expression. 3 weeks later, electrographic recordings were performed in awake head-fixed mice and acute seizures were induced by focal injection of picrotoxin to the transduced area of cortex.

In parallel, animals were made chronically epileptic by intrahippocampal injection of kainic acid into the right hippocampus. 2 weeks after acute status epilepticus, telemetry devices were implanted to record baseline electrocorticography (ECoG). Then animals were treated with AAV-PHP.eB-gfaABC1D-EGFP-Kir4.1 or AAV-PHP.eB-gfaABC1D-EGFP vector injected into the right hippocampus. From 2 weeks post-treatment, ECoG was continued to record for another 4 weeks and analysed using a semi-automated program to quantify seizures.

Results

In the mouse model of acute seizures, Kir4.1 pre-treatment in cortical astrocytes significantly reduced the number of acute seizures (0.7500 ± 0.4787) during the 80-minute post-picrotoxin period compared to tdTomato control (6.000 ± 0.7071). Data are presented as mean ± SEM; *P* = 0.0014, unpaired t test with Welch's correction, n = 4 per group, males only.

In the mouse model of chronic epilepsy, Kir4.1 over-expression in hippocampal astrocytes significantly reduced seizure frequency normalised to baseline levels compared to GFP control. *P* = 0.033, mixed-effects analysis; Kir4.1: n = 8, 3 females and 5 males; GFP: n = 10, 3 females and 7 males.

Conclusions

We conclude that astrocytes can be a promising target for disease-modifying treatment of epilepsy; and AAV-Kir4.1 astrocyte gene therapy is effective in reducing seizure frequency in rodent models of acute and chronic epilepsy.

C35

Compromised lysosome function and pathological α-synuclein handling in human iPSC-derived ventral midbrain astrocytes

Lixi Gao Li[1], David Alliband[2], Lucy Crompton[3], Jon Lane[1]

[1]University of Bristol, United Kingdom, [2]University of Bristol & UWE Bristol, United Kingdom, [3]UWE Bristol, United Kingdom

Introduction

Astrocytes, a major glial cell type in the brain, play essential roles in maintaining neuronal function. In brain regions affected during neurodegenerative diseases such as Parkinson's, reactive forms of these abundant cells are readily detected (1). Parkinson's is characterised by the loss of dopaminergic neurons in the midbrain, a process associated with the intraneuronal formation of Lewy bodies and early inflammation. Lewy bodies are composed of misfolded α-synuclein aggregation, a protein central to synaptic neurotransmission and widely accepted as a diagnostic hallmark of Parkinson's. Under physiological conditions, astrocytes help prevent buildup of extracellular α-synuclein through phagocytosis (2); however, their potential contributions to α-synuclein pathobiology and Parkinson's progression are less well understood. Our mechanistic analysis of astrocytic α-synuclein handling suggests that during inflammation, reactive ventral midbrain astrocytes are primed to release α-synuclein into the extracellular environment. Additionally, lysosomal inhibition potentiates astrocytic α-synuclein release, suggesting that mishandling in the astrocyte endolysosomal/autolysosomal compartments might contribute to Parkinson's-associated Lewy body pathology.

Methods

Ventral-midbrain astrocytes (vmAstros) (3) were differentiated from normal patient-derived iPSCs and used to measure intra- and extracellular α-synuclein levels with or without inflammatory stimulation (IL-1α(3ng/ml); TNF-α (30ng/ml); C1q (400ng/ml)). Based on candidate genes identified in an siRNA membrane trafficking library was used to screen for pathways involved in α-synuclein release (ELISA), tandem shRNAs/rescue lentiviral vectors and lysosomal inhibitors (BafA1; pepstatin A, E-64) were used tobegin assess lysosomal involvement and links to canonical and non-canonical autophagy pathways. For these assays, immunoblotting and ELISA-based analysis were used to measure intracellular and secreted α-synuclein, respectively.

Results

Using the library of siRNAs targeting membrane trafficking genes with ELISA-based analysis of α-synuclein release form reactive vmAstros, we found evidence of involvement of the lysosomal system in α-synuclein release. This has been validated using shRNA against selected components of the lysosomal V-ATPase. Focusing on α-synuclein turnover, treatment with pepstatin A (24hrs), vmAstros showed significantly higher (3.2-fold; SE<0.08) α-synuclein levels than in quiescent vmAstros (Fig. 1A; unpaired t-test, $p<0.0001$). By contrast, E-64 treatment (24hrs) did not alter endogenous α-synuclein levels (Fig. 1B; unpaired t-test, $p=0.8188$). We are continuing to examine the involvement of the endolysosomal and autophagy systems in pathological vmAstro α-synuclein handling in Parkinson's.

Conclusion

A defective endolysosomal system is implicated in pathological α-synuclein release from reactive vmAstros. This could establish a positive feedback loop of inflammation and a-synuclein release in Parkinson's. Given the increasing well-recognised links between endolysosomal dysfunction and Parkinson's susceptibility, our work to determine how this pathway is controlled at a mechanistic level is of key value.

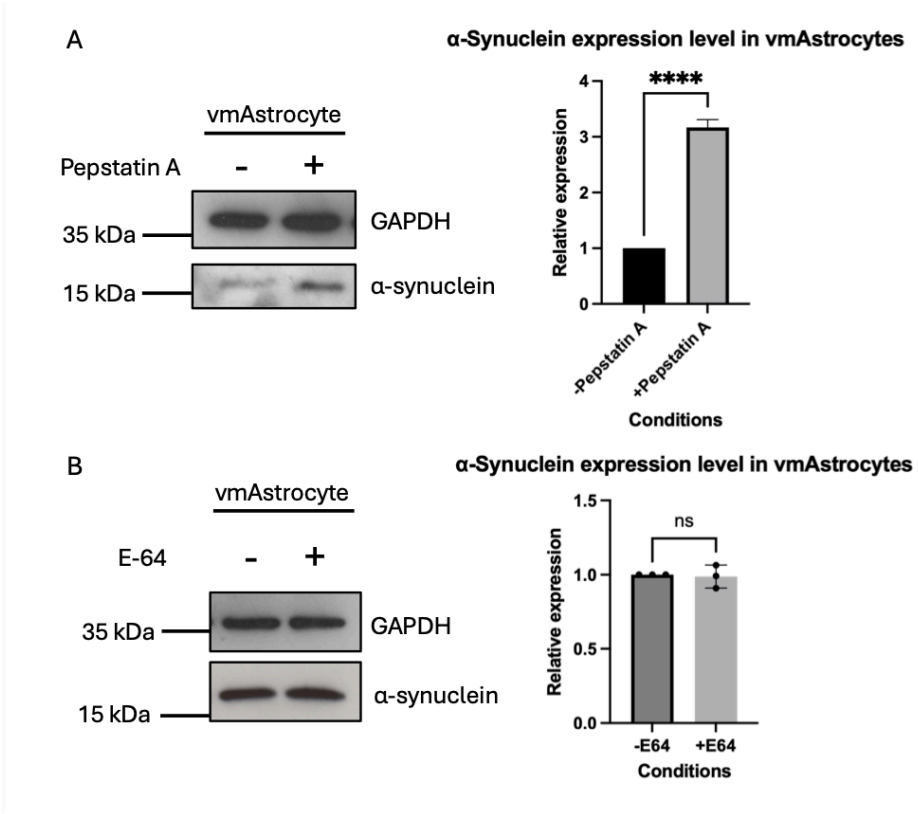


Figure1. (A) Intracellular α-synuclein level measured via Western Blotting (n=3, $p<0.0001$) in vmAstros after 24 hours of treatment with Pepstatin A (50μM). (B) Intracellular α-synuclein level measured via Western Blotting (n=3, $p=0.8188$) in vmAstros after 24 hours of treatment with E64 (20μM).

C36

Distinct mitochondrial responses to immune stimulation in MND-associated human microglia

Ilke Guntan[1], Uroosa Chughtai [1], Gaynor A. Smith[1], Owen M. Peters[1]

[1]Cardiff University/UK DRI, United Kingdom

Motor neuron disease (MND) is characterised by the progressive degeneration of upper and lower motor neurons in the central and peripheral nervous system. Approximately 10% of all MND cases are familial and driven by underlying genetic causes. Mutations in Optineurin (OPTN) and TANK-binding kinase 1 (TBK1) are rarer contributors, accounting for ~1% of familial MND cases individually. Both OPTN and TBK1 are multifunctional proteins involved in autophagy, including mitophagy, and immune homeostasis[1]. However, their role in regulating neuroinflammatory responses and mitochondrial function in resident immune cells of the brain, microglia, remains poorly understood.

The primary objective of this study was to characterise how loss of OPTN or TBK1 affects mitochondrial function in human iPSC-derived microglia under inflammatory stimulation. To address this, we utilised iPSC-derived microglia[2,3] carrying OPTN knockout (OPTN-KO) or TBK1 knockout (TBK1-KO) genotypes (JAX/iNDI lines). Cells were treated with lipopolysaccharide (LPS; 100 ng/ml; 24 hours) and the STING pathway agonist cyclic GMP-AMP (cGAMP; 1 µg/ml; 2 hours). Mitochondrial respiration was assessed using the Seahorse XF Mitochondrial Stress Test, while mitochondrial membrane potential and morphology were evaluated using Opera Phenix® high-content imaging.

Seahorse analysis revealed that OPTN-KO microglia exhibited significantly increased maximal respiratory capacity, spare respiratory capacity, and non-mitochondrial respiration following 24-hour stimulation with LPS and interferon gamma (IFNγ), compared to non-treated control microglia ($p < 0.05$[OP1] , n = 3–4, two-way ANOVA with Tukey's multiple comparisons). In contrast, TBK1-KO microglia did not show significant alterations in mitochondrial respiration under any tested condition (n = 3, two-way ANOVA with Tukey's multiple comparisons). Preliminary high-content imaging analyses suggest genotype- and stimulus-dependent alterations in mitochondrial membrane potential and mitochondrial network organisation.

Together, these findings indicate that loss of OPTN, but not TBK1, sensitises human microglia to inflammatory stimuli by altering mitochondrial bioenergetic responses. This work highlights distinct roles for OPTN and TBK1 in regulating microglial mitochondrial function during neuroinflammation. Improved understanding of microglial metabolic dysregulation in MND may provide insight into non–cell-autonomous disease mechanisms and identify novel therapeutic targets aimed at modulating neuroinflammatory responses.

C37

Central immune response in a rat model of preterm birth

Libby Harris[1], James Raine[2], Maria Dias[3], Karen O'Connor[3], Fiona McDonald[3], Valentina Mosienko[1]

[1]University of Bristol, United Kingdom, [2]University of Bath, United Kingdom, [3]University College Cork, Ireland

Preterm birth affects 1 in 10 infants worldwide, with an estimated 13.4 million cases annually (Ohuma et al., 2023). It is the leading risk factor for neonatal mortality as well as long-term physical and neurodevelopmental impairments, including autism spectrum disorder and global developmental delay. Premature infants are particularly vulnerable to breathing disorders due to immature central respiratory control and underdeveloped innate immunity, which increases susceptibility to infection. Gram-positive bacterial infections are the most common in preterm infants, often acquired in neonatal intensive care units. To develop improved treatment strategies, it is critical to understand how hypoxia and infection interact to shape central immune responses.

In this study, central immune responses were examined using a novel rat model of preterm birth that combines chronic intermittent hypoxia–hyperoxia (CIHH) with Gram-positive bacterial infection. Rat pups at postnatal day 3 were exposed to 3-hour CIHH cycles for 10 days, followed by injection of peptidoglycan and lipoteichoic acid; control pups were raised in normoxia and received saline. At postnatal day 13, brain tissue was collected, and microglial morphology in key respiratory centres, the nucleus tractus solitarius (NTS) and intermediate reticular formation (IRt), was quantified using Iba-1 immunostaining, confocal imaging, and Fiji morphology analysis plugins (Clarke et al., 2021; Kim et al., 2024). Altogether, 4 experimental groups were analysed in both male and female pups: normoxia/saline, CIHH/saline, normoxia/infection, CIHH/infection.

CIHH altered microglial morphology in male pups, with perimeter and average branch length reduced by 26% and 20%, respectively, and branch number increased by 28% compared to normoxic controls. Such changes were accompanied by a shift towards hyper-ramified phenotype, with 36% of microglia displaying this morphology versus 20% amoeboid and 18% ramified. Similar effects were observed in both the NTS and IRt. In contrast, female pups showed different responses: in the NTS, cell perimeter and branch number increased by 45% and 49%, respectively, while no morphological changes were observed in the IRt.

Pups exposed to both CIHH and Gram-positive bacteria displayed microglial morphology resembling controls, suggesting opposing effects of CIHH and bacterial infection. Such interactions may impair microglial adaptation to brain milieu changes, contributing to maladaptive neural circuitry and disease phenotypes observed in preterm infants.

C38

Deficiency of Ventral Hippocampal Astrocytic Insulin Signaling Induces Exhibition of Depression-Like Behaviors in Mice

Pei-Ling Hsu[1], Sheng-Feng Tsai[1], Yu-Min Kuo[1]

[1]Department of Cell Biology and Anatomy, College of Medicine, National Cheng Kung University, Tainan, 701401, Taiwan, Taiwan

Introduction: Depression frequently co-occurs with metabolic disorders (MetD), particularly insulin resistance, yet the mechanisms linking these conditions remain insufficiently understood. In our previous work, we established a mouse model of MetD-related depression by feeding 8-week-old C57BL/6N mice with a high-fat diet (HFD) for 12 weeks, which reliably induced both metabolic abnormalities and depressive phenotypes. We further discovered that HFD markedly reduced the expression of the glial glutamate transporters, GLAST and GLT-1, in the ventral hippocampus (vHPC), impairing local glutamate clearance and elevating extracellular glutamate levels. This glutamatergic dysregulation resulted in hyperactivation of vHPC glutamatergic projections to the nucleus accumbens (NAc), contributing to depression-like phenotypes. These findings identify reduced vHPC GLAST and GLT-1 as key determinants of MetD-related depression and highlight the need to elucidate how HFD suppresses these transporters in order to guide therapeutic development. Since insulin upregulates GLT-1 expression in primary astrocytes, our observations raise the possibility that HFD-induced central insulin resistance may drive the loss of vHPC GLAST and GLT-1 and ultimately contribute to depressive outcomes. **Objectives**: Accordingly, herein, we aimed to test the hypothesis that loss of vHPC astrocytic insulin signaling reduces local GLAST and GLT-1 expression, leading to hyperactivation of vHPC glutamatergic afferents to the NAc and ultimately the manifestation of depressive-like behaviors. **Methods**: We employed both *in vivo* and *in vitro* insulin-resistance models. To specifically interrogate vHPC astrocytic insulin signaling, we selectively deleted the insulin receptor (IR) in vHPC astrocytes using a Cre-loxP strategy. An adeno-associated virus (AAV) expressing Cre recombinase under the GFAP promoter was bilaterally infused into the vHPC of IR-floxed mice. In addition, AAV-mediated astrocytic overexpression of GLAST and GLT-1, as well as chemogenetic inhibition, were used to validate the contributions of vHPC astrocytic GLAST/GLT-1 and the vHPC-NAc glutamatergic pathway to depressive-like behaviors. (local IACUC approval: 112083) **Results**: Exogenous insulin failed to activate insulin signaling in the vHPC of HFD-fed mice ($p = 0.0079$; N = 8), which also displayed reduced GLAST and GLT-1 expression compared to controls. *In vitro*, saturated fatty acid-induced insulin resistance similarly decreased GLAST ($p = 0.0012$) and GLT-1 ($p = 0.0043$) in primary astrocytes (N = 9), indicating that loss of insulin signaling contributes to downregulations of GLAST and GLT-1. Selective deletion of vHPC astrocytic IR reduced local GLAST ($p = 0.0410$) and GLT-1 ($p = 0.0007$), increased vHPC-NAc glutamatergic activity ($p = 0.0002$), and induced depressive-like behaviors (SPT: $p = 0.0207$, FST: $p = 0.0116$; N = 21-23). Chemogenetic inhibition of the hyperactive vHPC-NAc pathway reversed depressive phenotypes (FST: $p = 0.0211$; N = 21-23). Overexpression of GLAST and GLT-1 in the vHPC corrected glutamatergic transmission dysregulations ($p = 0.0043$) and ameliorated depression-like behaviors (SPT: $p = 0.0222$, FST: $p = 0.0093$; N = 21-23). **Conclusions**: These results demonstrate that impaired astrocytic insulin signaling in the vHPC downregulates GLAST and GLT-1, leading to hyperactivity of the vHPC-NAc glutamatergic pathway and the manifestation of depressive-like behaviors. This positions astrocytic insulin signaling as a critical mechanistic driver of depression and a promising therapeutic target for MetD-related depression.

C39

Development of a genetic toolkit to modulate vertebrate myelination in a neuron subtype-specific manner

Inés Jiménez Pulido[1], Katy Marshall-Phelps[1], Phoebe Lyster-Binns[1], Rafael Almeida[1], Lida Zoupi[1]

[1]The University of Edinburgh, United Kingdom

In the central nervous system, oligodendrocytes wrap axons of certain neuron subtypes with a specialised cell membrane, myelin. The distribution of myelin along axons depends on the specific neuron they belong to, e.g., excitatory axons from deep cortical layers are more myelinated than those found in superficial layers (1), whilst the myelination of inhibitory neurons depends on the specific subtype (2). This diversity suggests that myelination might affect neuronal integrity and function in a subtype-specific manner.

To date, however, myelin manipulations are not neuron subtype-specific, so we have little insight into its specific roles for the individual subtypes. To study this, we are developing a genetic toolkit to modulate the myelination of specific axons of interest in an otherwise intact central nervous system. We have overexpressed the candidate inhibitor JAM2 using cortical organotypic slices and *in vivo* mice, and its ortholog Jam2a in the spinal cord of zebrafish. JAM2 has previously been shown to prevent the myelination of the soma of specific inhibitory neurons in mice (3).

In cortical organotypic slices, overexpressing JAM2 in the inhibitory parvalbumin interneurons suggests a reduced myelination of 38% and 43% in deep and superficial layers, respectively (N = 3 litters). This modulation is not significant (LMM with treatment and layer as fixed effects and litter/brain/axon nested as random effects; n = 114 axons, 17 inserts, and 3 litters; Type III ANOVA: $p = 0.09818$, $F(1, 6.386) = 3.7453$ for treatment), but it has led us to follow it up *in vivo*. Preliminary results show a significant reduction in myelination (LMM as previously; n = 77 axons, 13 brains, and 4 litters; Type III ANOVA: $p = 0.007507$, $F(1, 73) = 7.5628$ for treatment).

However, overexpressing JAM2 in the excitatory pyramidal neurons in slices does not affect their myelination (LMM as previously, nested as litter/insert/brain; n = 19 brains, 8 inserts, and 2 litters; Type III ANOVA: $p = 0.5797$, $F(1, 4.7017) = 0.3534$ for treatment), suggesting different regulatory mechanisms than for parvalbumin interneurons.

In addition, overexpressing Jam2a in zebrafish has not affected the myelination of reticulospinal axons at 4dpf (LMM with treatment and diameter as fixed effects and fish as random effects, n = 30 axons and 26 fish, Type III ANOVA: $p = 0.877439$, $F(1, 23.7113) = 0.0243$ for treatment), nor the myelination of Mauthner axons at 3dpf (LM with treatment and diameter as fixed effects, n = 20 axons and fish, ANOVA: $p = 0.89352$, $F(1,17) = 0.0185$ for treatment). Both neuron subtypes are excitatory; this is consistent with the mammalian results. We plan to test Jam2a in inhibitory neurons of zebrafish and other candidates in excitatory neurons in both models.

Overall, the development of genetic tools to modulate myelination in individual neuronal subtypes will allow us to characterise the role of myelin in those specific cells, as the rest of the neurons will maintain their normal myelin and function. Future experiments include the assessment of the morphology and function of those neurons in which myelination has been successfully reduced.

C40

Using Novel iPSC Models to study the relationship between PICALM and APOE in Late-Onset Alzheimer's Disease

Matilda Kakengi[1], Karolina Nowak[2], Rachel O'Donoghue[1], Natalie Connor-Robson[1]

[1]Cardiff University, UK Dementia Research Institute , United Kingdom, [2]University Collage London, United Kingdom

Late onset Alzheimer's Disease (LOAD) is one of the most common forms of dementia.

The endocytic pathway plays an important role in the mechanisms underlying AD pathology. The early endosome is a major site of APP generation, and dysfunction in this pathway is associated with AD both through genetics and pathology studies [1]. GWAS have revealed numerous risk genes in LOAD that are involved in endocytosis, including *PICALM*. *APOE* is strongly associated with developing LOAD, specifically *APOE4*, a known risk gene, has been known to induce Aβ toxicity whereas *PICALM* encodes is involved in clathrin-mediated endocytosis, which facilitates the clearance of Aβ [2,3].

Studies show the upregulation of *PICALM* can rescue phenotypes caused by the *APOE 4/4 genotype*, including Aβ clearance [4]. *PICALM* expression has also been seen to be reduced in AD microglia [5]. It is hypothesized that the *APOE4-PICALM* interaction could drive endocytic disease mechanisms, and that the upregulation of *PICALM* could rescue the *APOE4* induced phenotype in microglia. The aims of my research

are to investigate whether the combination of *APOE* and *PICALM* will drive endocytic and lipid handling disease mechanisms that impact microglial function.

To investigate the *APOE4-PICALM* interaction, we have generated hiPSC cell lines using CRISPR/Cas9 gene editing to produce *APOE3*+/+, *PICALM*+/- and *APOE4*+/+, *PICALM*+/- to study the combinational effect of *APOE* and *PICALM* .The first microglial differentiation is currently on-going, and we are planning to investigate endocytic pathways, phagocytic activity, lipid accumulation and wider microglial phenotypes.

C41

Serotonin availability affects anxiety-like behaviour and astrocyte morphology in stressed mice

Yasin Khan[1], Kratika Mujmer[1], Marc Ten Blanco[1], Natalia Alenina[2], Valentina Mosienko[1]

[1]University of Bristol, Faculty of Health and Life Sciences, School of Psychology and Neuroscience, Bristol, United Kingdom, [2]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

Stress is a highly prevalent risk factor for several neuropsychiatric disorders including depression and anxiety. Reports show that 37% of adults experience high levels of stress globally, which has a major economic impact, costing around $1 trillion globally each year (Gallup, 2025). Acute stress exposure leads to increased anxiety symptoms and is commonly modelled in rodents to study anxiety-like behaviours relevant to trauma-related disorders (Verbitsky et al., 2020).

Selective serotonin reuptake inhibitors (SSRIs) are the primary treatment for depression and anxiety. SSRIs are suggested to work by blocking the reuptake of serotonin at the presynaptic neuron, increasing the synaptic levels of serotonin (Angoa-Pérez et al. 2014). Although SSRIs rapidly increase extracellular serotonin, the therapeutic effect is delayed, suggesting the involvement of long-term neuronal and synaptic remodelling (Harmer et al., 2009).

Astrocytes, a type of brain glia cells, respond to stress and antidepressant treatment. Astrocytes express several 5-HT receptors, including 5-HT1A, 5-HT2A, 5-HT2B, 5-HT4R, hence could potentially respond to changing levels of serotonin following stress and antidepressant treatment (Muller et al., 2020). However, this has not been shown yet.

We investigated how serotonin deficiency and stress exposure differentially affect behaviour and astrocyte morphology in the medial lateral prefrontal cortex, amygdala and hippocampus of mice. To investigate this, we used tryptophan hydroxylase 2 knockout ($Tph2^{-/-}$) mice. Tph2 is the rate limiting enzyme for the conversion of tryptophan to serotonin centrally. Knockout of Tph2 is used to produce mice with a complete absence of serotonin centrally. We also used a 2-hour acute restraint stress procedure to generate the stressed groups.

Here we employed 4 groups of mice: $Tph2^{-/-}$ stressed, $Tph2^{-/-}$ control, wildtype (WT) stressed and WT control (n=8 per group). To analyse the anxiety-like behaviours, the mice were subjected to the elevated plus maze (EPM) test for 5 mins, 24 hrs following the last restraint session. Astrocytes were visualised using GFAP immunohistochemistry then confocal microscopy was used to image fluorescently labelled sections. Quantitative analysis was performed using an image analysis pipeline to evaluate astrocyte number and morphology, including process length, branch complexity and somal volumes. All animal procedures were conducted in accordance with UK legislation and approved by the University of Bristol Animal Welfare and Ethical Review Body.

WT stressed mice showed increased anxiety-like behaviour at 1- and 14-days post stress, shown by a 15.39% reduction in open arm entries ($p=0.0053$) and a 39.87 second reduction of time in the distal open arms ($p<0.001$). Anxiety index values were also greater for the stressed animals, by 0.15 and 0.16, at 1- and 14-days post stress respectively ($p<0.001$). We hypothesise that stress induces morphological changes to astrocytes, and that in the absence of serotonin these changes are altered, indicating an interaction between genotype and stress.

These findings will aid our understanding of astrocyte response to serotonergic dysfunction and stress exposure, which may reveal glial mechanisms contributing to human stress susceptibility.

C42

Targeting Aquaporin-4 to Treat Central Nervous System Oedema

Ophélie Langlois[1], Mina Kassim[1], Lucas Unger[1], Caolan Browne[1], Swathi Kallishetty Bhaskar[1], Xuntong Zhang[2], Mootaz Salman[3], Kevin Frankowski[2], Philip Kitchen[1], Zaid Alobaidi[1], Roslyn Bill[1]

[1]Aston University, United Kingdom, [2]University of North Carolina, United States of America, [3]University of Oxford, United Kingdom

Central nervous system (CNS) oedema is a life-threatening condition that affects millions of people each year and for which there is no cure. It results from abnormal water accumulation in the brain, often caused by trauma, stroke, or infection. Current treatments focus solely on managing symptoms, highlighting the urgent need for effective pharmacological therapies to improve clinical outcomes. Aquaporin-4 (AQP4), a water channel highly expressed in astrocytic endfeet, plays a key role in dysregulated fluid homeostasis after CNS injury, making it a promising therapeutic target. The rapid cellular swelling observed in CNS oedema is partly mediated by the upregulation and membrane localization of AQP4 in astrocytes, which involves direct interaction of calmodulin with AQP4. In a rat spinal cord injury model, our research group showed that inhibiting AQP4 relocalization to the plasma membrane by targeting this interaction effectively reduced spinal cord oedema and improved recovery (Kitchen et al., 2020). Therefore, our research proposes an innovative shift by developing small molecules that specifically inhibit AQP4 trafficking to the membrane or directly block its water channel function. By preventing or reversing oedema, we aim to reduce secondary injury cascades to promote recovery after CNS injury.

We employ an integrated discovery pipeline to identify small-molecule modulators of AQP4 function. Novel compounds are designed using computer-aided drug design and subsequently synthesized, purified, and fully characterized in-house. To target AQP4 trafficking, candidate inhibitors are screened *in vitro* for their ability to disrupt the interaction between the AQP4 and calmodulin, using flow-induced dispersion analysis. Compounds are evaluated in cell-based oedema models for their capacity to reduce AQP4 abundance at the plasma membrane under conditions that promote AQP4 relocalization, namely hypotonicity- and hypoxia-induced cell swelling. In parallel, we pursue AQP4 direct blockers. These compounds are screened *in vitro* for binding to AQP4 using a tryptophan quenching assay, with functional validation in cell-based systems. Their ability to inhibit water transport is tested by measuring water efflux in primary human astrocytes and AQP4-overexpressing MDCK cells under hypertonic conditions using a calcein quenching assay. Together with toxicity, off-target, and stability assessments, these functional assays inform medicinal chemistry optimisation toward the development of lead compounds.

Using this multi-layered approach, we have recently identified several promising hit molecules. Lead compounds will ultimately be advanced to *in vivo* testing in a CNS injury model and will build a framework for pharmacological intervention for the millions of people affected by CNS oedema worldwide every year.

C43

Profiling of tissue and stem cell models reveals disrupted transcriptional programmes linked to glial dysfunction in Alzheimer's disease

Michael Lattke[1], Sabino Mendez Pastor[1], Nurun Fancy[1], Johanna Jackson[1], Paul Matthews[2]

[1]UK Dementia Research Institute at Imperial and Department of Brain Sciences, Imperial College London, Sir Michael Uren Research Hub, 86 Wood Ln, London W12 0BZ, UK. , UK, [2]UK Dementia Research Institute at Imperial and Department of Brain Sciences, Imperial College London, Sir Michael Uren Research Hub, 86 Wood Ln, London W12 0BZ, UK. The Rosalind Franklin Institute, Harwell Science and Innovation Campus, Fermi Way, Didcot, OX11 0QS, UK., UK

In Alzheimer's disease, toxic protein aggregates lead to neuroinflammation, and progressive loss of vulnerable synaptic connections and neurons, ultimately resulting in cognitive decline. Glial cells, particularly microglia and astrocytes, clear toxic protein aggregates and lipids, control inflammatory responses, and provide factors promoting neuronal survival and synapse function. Disruption of glial functions is emerging as critical driver of AD pathogenesis, but the underlying mechanisms remain poorly understood.

Here, we present preliminary transcriptomic analyses of human tissue and stem cell models, identifying alterations of transcriptional programmes that may contribute to AD pathogenesis. We show that in stem-cell-derived microglia, genetic variants of TREM2 that are known to increase AD risk disrupt programmes predicted to control lipid metabolism and lysosomal protein degradation, while increasing expression of inflammatory mediators that may disrupt astrocyte functions. We further show that in astrocytes from human AD brain tissue (from Gabitto et al., 2024), programmes predicted to control protein synthesis and mitochondrial function are disrupted, which may impair metabolic support for neurons.

In conclusion, our work reveals transcriptional programmes predicted to control protective glial functions whose disruption may contribute to different steps of AD pathogenesis and suggests that TREM2 variants may increase AD risk by interfering with these programmes.

C44

How does astrocyte metabolism affect amyloid processing pathways in Alzheimer's Disease?

Ryan James Lewis[1], Martha Oyaghire[1], Simon Bell[1]

[1]Sheffield Institute for Translational Neuroscience, United Kingdom

In sporadic Alzheimer's Disease (sAD), metabolic dysfunctions such as glucose hypometabolism, hyperinsulinemia, and insulin resistance in the brain are present from an early stage. Astrocyte metabolism supports various neuronal functions in the brain, including memory encoding. Furthermore, astrocytes can produce amyloid-degrading enzymes like Insulin-degrading enzyme (IDE)—an energy-intensive process. Therefore, astrocyte metabolic dysfunction may significantly contribute to sAD pathogenesis. A recent study by Bell et al. (2025) demonstrated that hexokinase-1 (HK1), the rate-limiting enzyme of glycolysis, was deficient in astrocytes induced from sAD-patient fibroblasts, with HK1 transduction rescuing astrocytic metabolic phenotypes. Given that research has shown that IDE function and expression are decreased in sAD, this study aimed to see whether HK1-transduction affected IDE expression in sAD-astrocytes. Before the study, control and sAD fibroblasts were reprogrammed into astrocytes, with a subset undergoing HK1 adenoviral transduction using predefined protocols (Bell et al. 2018; Gatto et al. 2020). IDE expression and localisation were assessed by western blot (WB), quantitative polymerase chain reaction (qPCR) and immunocytochemistry. Contrary to existing literature, WB analysis showed that IDE immunoreactive bands were more intense in sAD samples compared to controls in most of our WBs (n=4/7, technical repeats). Fold-change analysis of these bands indicated that sAD astrocytes had higher IDE expression than controls (154% increase, SEM ± 34.2%, $p = 0.0342$*, Welch's t-test; n=3, biological replicates; n=4, technical replicates). However, after HK1 transduction, this trend of IDE upregulation was eliminated: before transduction, sAD samples showed a 224% increase (SEM ± 13.3%, n=2 biological replicates) in IDE compared to controls; after transduction, though, sAD IDE levels were 18.1% lower (SEM ± 8.5%, n=2 biological replicates) than pre-transduced controls. qPCR did not corroborate WB findings, likely due to poor primer quality, while immunocytochemistry detected predominantly nuclear IDE localisation, potentially reflecting suboptimal antibody conditions or altered IDE trafficking to the cytosol in sAD. Although we were unable to proceed further, analysis of the literature enabled us to hypothesise that the low-energy state of glycolytically deficient astrocytes, due to HK1-dependent glucose hypometabolism, could result in increased AMP: ATP ratios. This may drive allosteric AMP-activated protein kinase (AMPK) signalling, a known positive regulator of IDE (Lu et al. 2020), potentially explaining our findings. Future research involving AMPK chemical inhibition could inform us on whether this is the case. Due to a small sample size and failure to orthogonally validate our findings, any implications derived from our findings would be premature. However, high IDE levels may exacerbate pre-existing insulin resistance and impede insulin-dependent memory processes. Also, hyperinsulinemic states may competitively inhibit IDE-dependent beta-amyloid degradation (given IDEs' higher affinity for insulin), meaning increased IDE levels may not equate to increased IDE-dependent beta-amyloid proteolysis. Future degradation assays could validate this. Despite limitations, these findings provide a hypothesis-generating framework which could link metabolic impairment to IDE dysregulation in sAD in a way which is currently absent from the literature. As such, these findings should be utilised as a pilot study to inform future work on the effects of astrocytic glycolytic capacity on amyloid proteolysis in sAD.

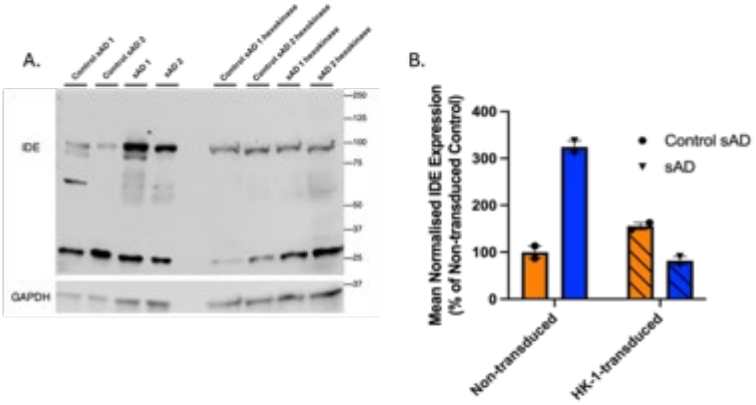


Figure 1. Transduction with a hexokinase-1 (HK1) adenoviral vector reduces IDE expression in sAD samples. A. Western blot showing IDE expression in sAD and control samples before and after HK1 transduction. **B.** Quantification of IDE expression relative to non-transduced controls. Pre-transduction, sAD samples show increased IDE expression relative to controls; post-transduction, sAD IDE expression is reduced relative to controls. Data are presented as mean± SEM. Dots represent individual samples. *n = 2, biological replicates; n = 1, technical replicate.*

C45

LRRC37A2 protects against Parkinson's disease by regulating astrocytic function and neuronal activity

DIANNE LOPEZ[1], Owen Dando[2], Shiva Kompella[3], Dana Rakotondrasolo[4], Dayne Beccano-Kelly[5], Kathryn Bowles[1]

[1]Dementia Research Institute UK – Edinburgh University, Centre for Discovery of Brain Sciences, Edinburgh, Scotland, [2]Dementia Research Institute - University of Edinburgh, Institute for Neuroscience and Cardiovascular Research, Scotland, [3]Dementia Research Institute UK – Cardiff University, School of Medicine, Cardiff, Wales, [4]Université de Poitiers - Faculté des Sciences Fondamentales et Appliquées, , France, [5]Dementia Research Institute UK – Cardiff University, School of Medicine, Cardiff, Wales

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by motor and non-motor symptoms. We have previously implicated the *LRRC37A2* gene as a potential modifier of PD risk[1]; however, its cellular role remains poorly understood, partly due to its exclusion from genetic analyses. Located at the 17q21.31 '*MAPT'* locus, a region prone to genomic instability, *LRRC37A2* exhibits copy number variations (CNVs) that differ across major haplotypes, sub-haplotypes and genetic ancestry[1,2,3]. Elevated *LRRC37A2* expression and CNVs are associated with reduced PD susceptibility[1].

Objectives

This study aims to explore the role of *LRRC37A2* in astrocyte function and its potential impact on neuronal health, further elucidating how *LRRC37A2* confers protection from developing PD.

Methodology

Transcriptomic and functional analyses were used to assess cell- autonomous effects of modulating *LRRC37A2* expression in induced pluripotent stem cell (iPSC)-derived astrocytes across European (n=4), East Asian (n=3), and African populations (n=3). To assess the non-cell autonomous effects of *LRRC37A2*, electrophysiological recordings were obtained from astrocytes mono-cultures and co-cultures of both astrocytes and dopaminergic neurons. Where appropriate, statistical difference between groups over time was determined by ANOVA and post-hoc Tukey tests.

Results

Transcriptomic profiling of astrocytes overexpressing *LRRC37A2* across different genetic ancestries revealed upregulation of genes linked to cell migration, inflammation, mitochondrial dynamics, and autophagy lysosomal pathway (ALP) with no ancestry-specific differences in expression patterns. Functionally, we found that increased *LRRC37A2* expression may exert a protective effect by modulating inflammatory signalling and enhancing astrocyte migration. Ongoing electrophysiological studies suggest that elevated *LRRC37A2* expression in astrocytes also alters potassium channel conductance, leading to enhanced neuronal activity in co-cultured iPSC-dopaminergic neurons.

Conclusion

Collectively, these findings suggest that *LRRC37A2* may play a protective role in PD by modulating astrocyte migration, inflammation, ion channel conductance, and neuronal activity. Further studies will

examine the effects of astrocytic *LRRC37A2* on mitochondrial dynamics and the ALP, as well as its non-cell autonomous effects on dopaminergic neurons carrying PD-related mutant genes. Elucidating the neuroprotective role of astrocytic *LRRC37A2* and its therapeutic potential in treating PD may lead to the development of novel therapeutic strategies for this devastating disease.

Ethical Considerations

The iPSC lines used in this study were derived from donors who provided informed consent for the use of their cells in research. The iPSC lines were anonymized to protect donor privacy. The use of iPSCs in this study complies with the UK's Human Tissue Act and the EU's GDPR.

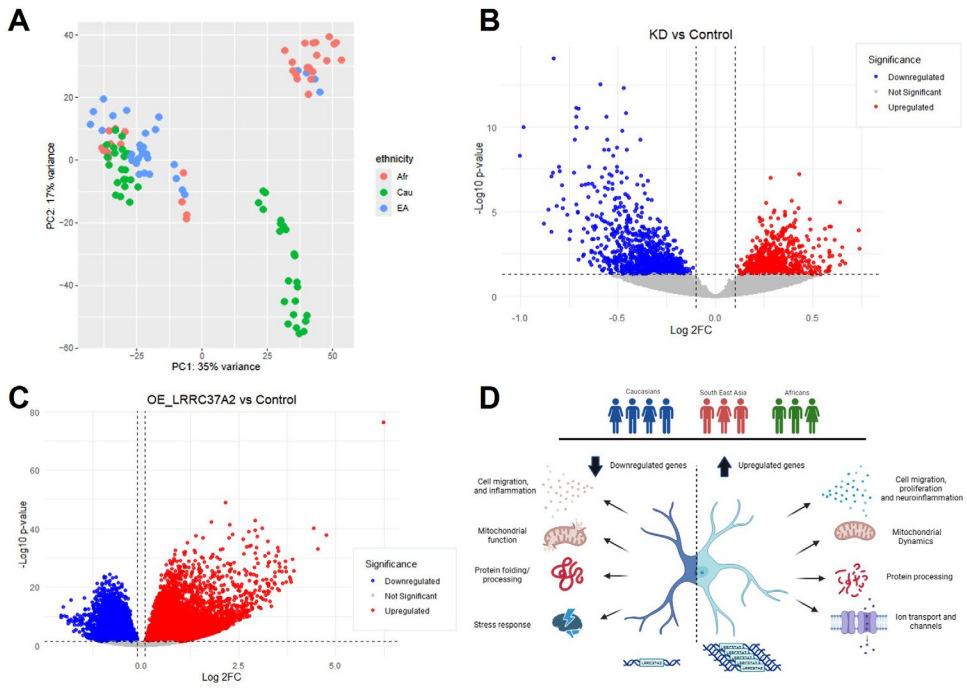


Figure 1. Transcriptomic effects of modulating *LRRC37A2* expression in iPSC-astrocytes across different genetic ancestries. A) Principal component analysis bar plots showing the effects of *LRRC37A2* in different genetic ancestries. European (N = 3, n=4 different cell lines), East Asian (N = 3, n=3 cell lines) and African ancestries (N = 3, n=3 cell lines). B-C) Volcano plots of differentially expressed genes in astrocytes expressing the knockdown *LRRC37A2 (*KD) vs scrambled control and overexpressed *LRRC37A2 (*OE_ *LRRC37A2)* vs empty vector control. D) Schematic diagram of downregulated and upregulated pathways in KD vs control and OE vs control, respectively, across all 10 cell lines. N = represent total number of individual differentiated batch, n = represents number of cell lines used.

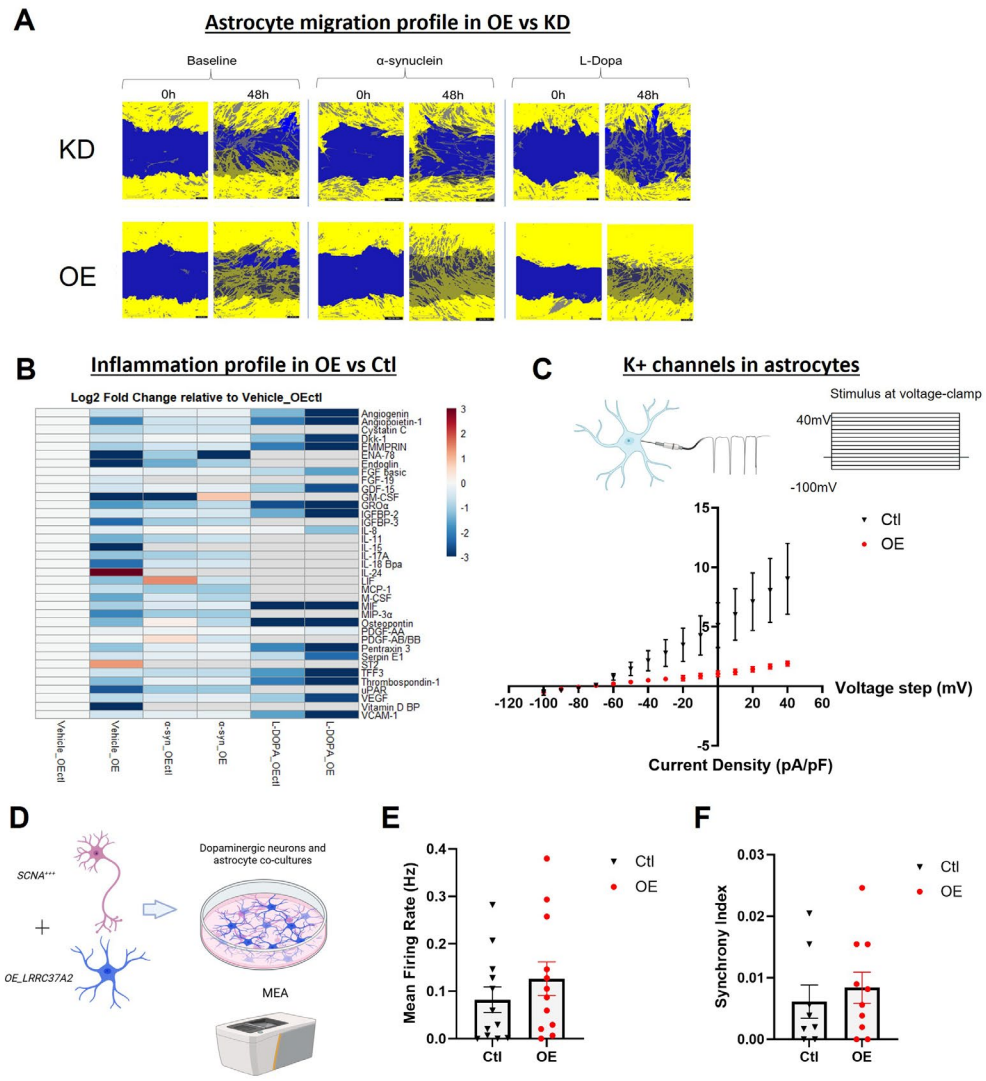


Figure 2. *LRRC37A2* expression modulates astrocyte migration, inflammation, potassium channel conductance, and neuronal activity. (A) Representative images of the scratch-wound assay performed on astrocytes under different conditions: scrambled control (KD_ctl), shRNA-mediated knockdown of *LRRC37A2* (KD), overexpression of *LRRC37A2* (OE), and empty vector control (OE_ctl). Cells were incubated with either vehicle (PBS), 200 ng/mL α-synuclein, or 500 µM L-DOPA for 3 days and imaged every 3hrs using an Incucyte machine. Yellow represents cell confluence, blue represents scratch wound. Scale bar = 600 µm. Statistical difference between groups over time was determined by ANOVA and post-hoc Tukey tests. *p<0.05 (KD vs OE). N = 3, n = 3 (n = technical replicates). B) Cytokines and chemokines released by OE and control astrocytes after treatment with vehicle, α-synuclein and L-DOPA for 2 days. (N = 1, n = 1). C) Whole-cell patch clamp recordings showing current-voltage (I/V) relationship indicating K^+ channel conductance in response to step depolarizations from −100 mV to +40 mV (10-mV increments). D) Schematic diagram of dopaminergic-astrocyte co-cultures for Multi-Array Recordings (MEA). E) The Mean Firing Rate (Hz) and (F) synchronicity index were measured in neuron-astrocyte co-cultures at baseline. N=1, n=8-12 (no. of wells). Graphs shows ±SEM.

C46

CD33 Short Isoform Promotes Efficient Amyloid-β Clearance by Coupling Phagocytic Uptake to Intracellular Degradation in Human Macrophage Models

Runze Luan[1]

[1]Manchester Metropolitan University, United Kingdom

Macrophages share core phagocytic and lysosomal clearance programs with microglia and are widely used as human cellular models to study mechanisms relevant to amyloid-β (Aβ) handling in Alzheimer's disease (AD). Genetic variation at the *CD33* locus is a well-established risk factor for late-onset AD, encoding multiple isoforms with distinct functional properties. While the CD33 short isoform (CD33-S) has been associated with enhanced phagocytic uptake, whether this translates into effective intracellular degradation and true Aβ clearance remains unclear.

Here, we employed human macrophage models engineered to express either the long (CD33-L) or short (CD33-S) CD33 isoform to dissect isoform-specific regulation of Aβ processing. Using quantitative confocal imaging with time-resolved uptake and degradation analyses, together with transcriptomic profiling (RNA-seq), label-free mass spectrometry, and targeted protein validation, we examined how CD33 isoforms coordinate phagocytosis with downstream intracellular handling of fibrillar Aβ.

Consistent with previous observations, macrophages expressing CD33-S displayed significantly increased Aβ uptake compared with CD33-L–expressing cells. Importantly, this enhanced uptake was accompanied by more efficient intracellular degradation of internalised Aβ, rather than simple accumulation. Multi-omics analyses revealed coordinated up-regulation of phagocytosis-associated components, including cytoskeletal and adaptor proteins, alongside activation of autophagy–lysosome machinery, such as ATG5- and LAMP2-associated pathways. These molecular programmes linked early internalisation to effective lysosomal processing, resulting in a marked increase in net Aβ clearance capacity in CD33-S macrophages.

Together, these findings demonstrate that the functional impact of the CD33 short isoform extends beyond enhanced uptake to promote intracellular degradation pathways critical for efficient Aβ clearance. Our work highlights CD33 isoform balance as a key regulator of macrophage clearance efficiency and provides mechanistic insight relevant to microglia-mediated amyloid handling in the context of AD.

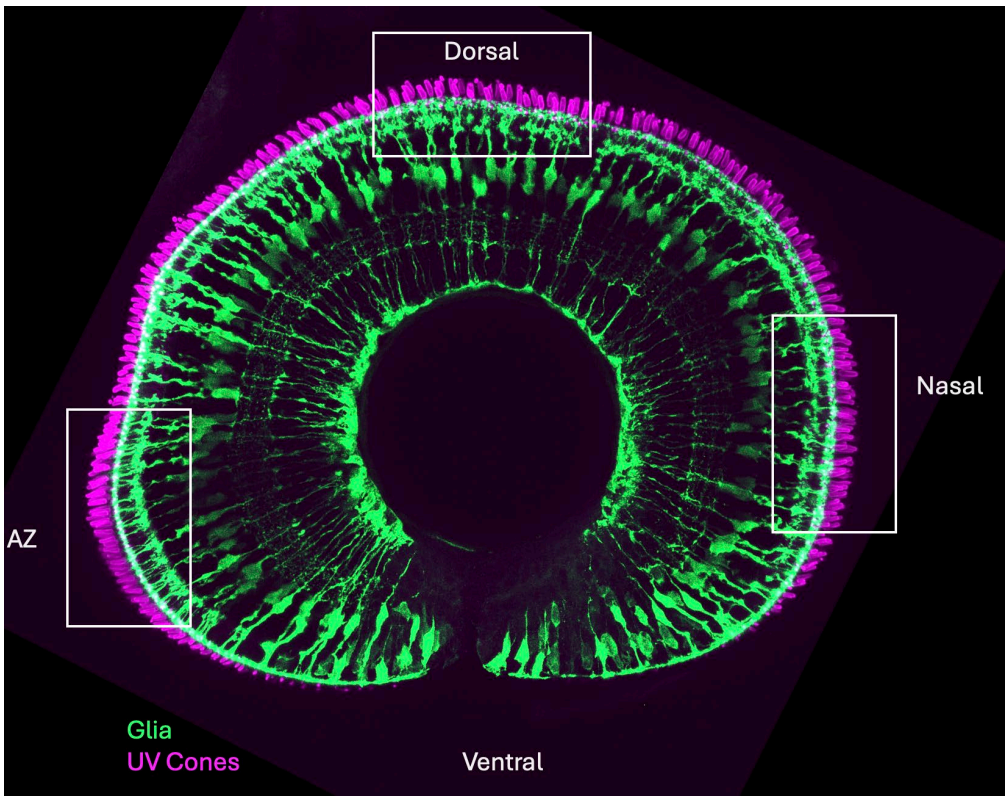
C47

Glia regulate local retinoic acid levels for neuronal specialisation in the retina

Manuela Lahne[1], Roxana Lungu[1], Takeshi Yoshimatsu[2], Ryan MacDonald[1]

[1]University College London, UK, [2]Washington University in St. Louis, USA

The functions of same type neurons can be differentially tuned to serve distinct roles. However, how such differential functional tuning arises among same type neurons during development remains poorly understood. We found that in the zebrafish retina, heterogeneity within a single glial population, called Müller glia (MG) provides local gradients of signalling molecules that specifies photoreceptor neuron functions. The acute zone (AZ), a ventro-temporal region of the retina, exhibits specializations suited for prey capture in the upper frontal visual field. Specifically, UV cones in the AZ show higher light sensitivity by elongating outer segment (OS), a light sensing cellular compartment. We found that *cyp26* genes, which encodes retinoic acid (RA) degrading enzymes, are expressed in MG in a regionally specific manner and that this regional expression pattern matches the spatial variation in cone OS length. Blocking Cyp26 activity prevented OS elongation in the AZ. Stimulation of RA signalling using receptor agonists also reduced OS length in this region. The loss of OS elongation resulted in a loss of photoreceptor sensitivity to UV stimulation. We further found that RA activated signalling pathways predominantly in MG rather than cones themselves, suggesting a cell non-autonomous mechanism for photoreceptor specialisation via MG. Removing MG completely from the developing retina abolished cone specialisation in all regions of the developing retina. These findings identify MG as a key regulator of cone functional specialisations through shaping regional RA signalling activity. Future studies are now focusing on whether glial regulation of morphogen signals in the developing nervous system is a general principle for neuronal specialisation and function.


Dorsal
Nasal
AZ
Glia
UV Cones
Ventral

C48

TGF-β inhibition ameliorates reactive changes in VCP-ALS astrocytes at single-cell resolution

Stanislaw Majewski[1], Michae Lattke[2], Tania Auchynnikava[3], Hannah Franklin[4], Joseph Tuersley[3], Yiran Wang[5], Lois Kent[3], Hubert Slawinski[3], Michael Howell[3], Adrian M. Isaacs[1], Mark Skehel[3], Benjamin E. Clarke[6], Rickie Patani[7]

[1]Department of Neurodegenerative Disease, Queen Square Institute of Neurology, University College London, UK, [2]4Department of Brain Sciences, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London, UK, [3]The Francis Crick Institute, 1 Midland Road, London, UK, [4]Department of Neuromuscular Disease, UCL Institute of Neurology, Queen Square, London, UK, [5]Department of Biochemistry, University of Oxford, Oxford, UK, [6]Life Sciences Institute, Centre for Life Sciences, National University of Singapore , Singapore, [7]Life Sciences Institute, Centre for Life Sciences, National University of Singapore, Singapore

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by motor neuron death, resulting in muscle atrophy, weakness and paralysis. In ALS, astrocytes undergo reactive transformation, ultimately contributing to neuronal death, however the causal pathways by which this occurs are poorly understood. One candidate is the transforming growth factor (TGF)-β signalling pathway, which has been found to be upregulated in ALS models and patients.

Aims

Whether TGF-β drives cell-autonomous astrocyte reactive transformation, potentially by altering astrocyte reactive sub-states, and how it may contribute to neuron death, remains unknown.

Methods

To address this, we inhibited TGF-β in hiPSC derived ALS astrocytes carrying *VCP* mutations, known to cause familial ALS, and performed single-cell RNA sequencing, single-cell proteomics and functional studies informed by our analysis.

Results

We identified changes in the proportion of transcriptomic reactive sub-states characterised by mitochondrial dysfunction, cytoskeletal remodelling and markers of reactive transformation, which were ameliorated following TGF-β inhibition. Single-cell mass spectrometry validated these findings, revealing a *VCP* mutant subpopulation featuring increased translation and proinflammatory protein expression, which was similarly abrogated by TGF-β inhibition. Finally, increased motor neuron apoptosis following treatment with astrocyte conditioned media was significantly rescued when TGF-β was inhibited in *VCP* mutant astrocytes.

Conclusions

Together, our analysis reveals that human *VCP* mutant astrocytes feature dysregulated cell-autonomous reactive sub-states, driven by TGF-β activation, which partially recapitulate aberrant astrocyte gene expression changes identified in patient postmortem single-cell studies. Additionally, inhibiting TGF-β rescues dysregulated *VCP* mutant sub-states and astrocyte mediated neurotoxicity. These results collectively indicate that selective TGF-β pathway inhibition in astrocytes may be a potential therapeutic approach for ALS.

C49

New insights into the mechanisms of axonal neurotransmitter release

Katy Marshall-Phelps[1], Phoebe Lyster-Binns[1], Maira Pyrgioti[1], Rafael Almeida[1]

[1]Institute for Neuroscience and Cardiovascular Research, University of Edinburgh, United Kingdom

Introduction

Synaptic neurotransmission is essential for neuronal communication and circuit function. However, neurons can also release neurotransmitters outside of conventional synapses. The significance of this non-synaptic release remains unclear, although growing evidence suggests an important role in neuron-glia communication. For example, myelination of axons by oligodendrocytes in the CNS is regulated by neuronal activity and neurotransmitter signalling. Yet, the mechanisms underlying axon-myelin communication via neurotransmitters *in vivo* remain poorly understood due to the technical challenge of visualising neurotransmission in intact neural circuits.

Aims/Objectives

Using larval zebrafish (*Danio rerio*) as an *in vivo* model, we employed genetically encoded fluorescent reporters to visualise neurotransmission and define the spatial and molecular mechanisms of non-synaptic release along myelinated axons.

Methods

We optimised two genetically encoded fluorescent reporters: SypHy to detect synaptic vesicle fusion[1] and iGluSnFR4s to analyse extracellular glutamate signalling[2]. To identify axonal domains associated with vesicle release, we generated a novel CRISPR knock-in line to label the heminodal/nodal marker Neurofascin a (Nfasca)[3].

Imaging was performed on 4-5 days post-fertilisation larvae (immobilised with mivacurium chloride:1.5mg/ml) using a Zeiss LSM880 Airyscan to capture discrete vesicle fusion and glutamate transients along axons. All experiments were conducted with UK Home Office approval.

Results

We observed that neurotransmitter vesicle release occurs along myelinated axons at a frequency comparable to that observed at conventional synaptic sites (axon: 45.3 ± 34.2 vs collateral: 56.4 ± 37.5 events/100µm/hr, n=20 for both; paired t-test, $P = 0.025$). Despite similar release frequencies, axonal glutamate transients exhibited distinct kinetics compared to synapses, characterised by lower peak amplitudes but larger event areas (axonal vs collateral max dF/F0: 0.67 ± 0.26 vs 2.45 ± 1.18; $n=5$; paired t-test, $P = 0.024$: Event area: 11.23 ± 3.36 vs 7.46 ± 3.74; $P = 0.004$). These findings establish that axonal

release is frequent and kinetically distinct from synaptic transmission but do not address whether events are spatially organised or stochastic.

We previously demonstrated that non-synaptic axonal vesicle fusion emerges with the onset of myelination and becomes enriched at axonal sites near to the growing ends of myelin sheaths[4]. To determine whether this spatial enrichment corresponds to heminodal and nodal domains, we labelled endogenous levels of Nfasca. Co-localisation analysis revealed a significant enrichment of vesicle events at Nfasca-positive domains (expected: 1.03 ± events/100µm/h vs observed: 8.06 ± 8.01 observed frequency; n=16; Wilcoxon matched-pairs rank test, P = 0.004). Despite this spatial association, genetic loss of Nfasca did not significantly alter axonal vesicle release frequency across genotypes (control: 39.1 ± 31.7 vs Het: 32.5 ± 25.1 vs Hom: 42.7 ± 22.3; One-way ANOVA, P = 0.11), indicating that while these domains mark sites of release, Nfasca itself is not required for vesicle fusion to occur.

Conclusions

This work shows that non-synaptic neurotransmitter release is a frequent and spatially organised feature of myelinated axons. Although axonal vesicle fusion occurs at rates comparable to synapses, the resulting glutamate signals are kinetically distinct, indicating mechanistic differences between synaptic and axonal release. The enrichment of release events at Nfasca-positive domains suggests that axonal neurotransmission is patterned by specialised membrane regions rather than occurring randomly.

C50

A Glial Mechanism of Antidepressant Action: Fluoxetine Potentiates ATP-Driven cAMP Signalling

Catriona Marston[1], Barbara Vaccari-Cardoso[1], Kratika Mujme[1]r, Sergey Kasparov[1], Anja G Teschemacher[1], Valentina Mosienko[1]

[1]University of Bristol, United Kingdom

Depression has a substantial socioeconomic burden as the leading cause of disability globally, affecting 5% of the adult population. Although the use of antidepressants to treat depression and other mental health conditions has doubled in the last 20 years, a third of patients do not respond to antidepressant therapy. Consequently, there is an unmet clinical need for improved treatment options for those living with depression.

There has been little development in this field, driven by our lack of understanding of the molecular and cellular mechanisms underpinning depression and other mental health conditions. While selective serotonin reuptake inhibitors (SSRIs), including fluoxetine (FLX), are known to elevate serotonin levels by inhibiting its uptake into the presynaptic cell, evidence suggests that the therapeutic effects of antidepressants are independent of changes in monoamine levels. Therefore, the therapeutic effects of SSRIs cannot be entirely attributed to changes in serotonin levels. Thus, other signalling pathways are likely to be targeted by FLX.

Astrocytes, once thought to be solely supporting cells, are now widely recognised to play an essential role in emotional regulation. Both cAMP and Ca^{2+} signalling are essential for astrocyte function; however, their roles in emotional regulation or antidepressant mechanisms remain largely unknown.

Here, we aimed to determine the mechanism by which antidepressant FLX alters intracellular signalling in astrocytes.

Live cell imaging of primary rat astrocytes revealed that FLX application (10μM, 5 minutes) increases intracellular cAMP levels by 27%, but not Ca^{2+} levels (*cAMP (Epac sensor): $p<0.0001$; Ca^{2+}(Twtich2B sensor): $p=0.1511$*). The FLX-driven increase in cAMP was dependent on adenylate cyclase (AC) signalling: application of AC inhibitor, NKY80, in combination with FLX attenuated FLX's effect on cAMP (*NKY80+FLX vs FLX: $p=0.0044$*). In the presence of selective serotonin 2B (5-HT2BR) and adenosine 2B receptor (A2B) antagonists (PSB603 and LY266097), FLX was unable to increase cAMP (PSB+FLX $p<0.0001$ and LY+FLX $p=0.0029$ vs FLX). The FLX-driven increase in cAMP levels was 4x less than that of the direct action of adenosine on the A2BR. We proposed that A2BR activation is secondary to 5-HT2BR activation. It has been previously demonstrated that ATP can be converted to adenosine by microglia. Although steps are taken to minimise microglial contamination, approximately 3% of microglia remain in our primary astrocyte cultures. Therefore, we used a real-time luminescence-based ATP release assay to demonstrate that FLX increases ATP release from astrocytes by 100% (*$p<0.0001$*), a process that is also dependent on 5-HT2BR (*LY+FLX vs FLX: $p<0.0001$*). Finally, in microglia-depleted cultures (PLX5622, 10μM, 7 days), FLX was unable to increase cAMP (*$p=0.0118$*). All experiments included a minimum of 2 biological replicates, with 3 coverslips/wells and at least 4 cells/region of interests per coverslip. When two data sets were compared, an unpaired T-test was used; when more than two groups were compared, a one-way ANOVA with post-hoq Holm-Šídák's multiple comparisons test was used.

To summarise, we have identified a novel antidepressant mechanism in which FLX potentiates ATP release via 5-HT2BR. ATP is converted to ADO by microglia, which, in turn, activates the A2BR, thereby increasing intracellular cAMP levels.

C51

Assessment of hypothalamic blood-brain-barrier integrity in neonatal rats exposed chronic intermittent hypoxia/hyperoxia and acute S. Aureus derived lipoteichoic acid and peptidoglycan

Anna O'Connell[1], Fiona B McDonald[1]

[1]University College Cork, Ireland

The risk of significant cardiorespiratory challenges increases with decreasing gestational age at the time of birth. Many infants less than 32 weeks of gestational age will require respiratory support in the form of supplemental oxygen to offset cardiorespiratory instability that commonly leads to hypoxia. The vulnerability of the population due to their limited metabolic reserves and their immaturity means preterm infants often need on-going access to specialist clinical care requiring prolonged hospital stays. Exposure to pathogens within the hospital increases the risk of nosocomial infection. Hypoxia and inflammation have been reported to disrupt the blood-brain-barrier, which may lead to microglia activation.

The aim of our study was to assess if the BBB within the paraventricular nucleus was compromised in neonatal rats exposed chronic intermittent hypoxia/hyperoxia and acute S. Aureus derived lipoteichoic acid and peptidoglycan (LTA&PGN). In this model we have previously demonstrated an increase in circulating cytokines, cortisol and progesterone in response to bacterial derived products.

Methods: Female dams and their litters (male and female) were either left at normoxia or placed in an environmental chamber and FiO2 was manipulated to generate a pattern of intermittent hypoxia (8%)/hyperoxia(30%)/normoxia(21%) (CIHH) between postnatal day (PND) 3 and 12. On PND13 the pups from both groups were divided between saline-treatment and LTA&PGN (i.p) treatment. Ultrasonic vocalisation was measured 3 hours after treatment on PND13 and animals were later euthanised with pentobarbital and perfused with FITC-dextran. Ultrasonic vocalisations were recorded using ultravox and analysed using Deepsqueak (n=5-10). The brains were post-fixed in 10% formalin, cryoprotected with 20% sucrose and cryosectioned using Lecia cryostat. Images were visualised using confocal microscopy at 50um. Analysis was performed by sampling extra/intra ratio of fluorescence of capillaries (n=5-6).

Results: The number and frequency of calls were not different between experimental groups. When male and female animals were exposed to LTA&PGN the extra/intra ratio of floresence of capillaries increased in the magnocellular and the intermediocellular region of the PVN (three-way ANOVA, Gas x LTA&PGN p=0.02 and p=0.01 respectively. **Conclusion:** There is no evidence of a change in vocalisation when pups are temporarily separated from their dam despite exposure to early life stress. There is some evidence of increased leakiness in pups exposed to S. Aureus derived LTA&PGN, whereas when rat pups are exposed to CIHH and LTA&PGN the extra/intra ratio of fluorescence is no longer different.

C52

Soft Tuneable Hydrogel Micropillar Systems for Mechanistic and Translational Studies of CNS Myelination

Emad Moeendarbary[1], Claire Vinel[1], Soufian Lasli[1], William Richardson[1], Beatriz Garcia Diaz[2], Graham Sheridan [3]

[1]University College London, United Kingdom, [2] IBIMA, Hospital Regional Universitario de Málaga, Spain, [3]University of Nottingham, Nottingham

Introduction:
Oligodendrocytes (OLs) generate myelin essential for CNS function, yet the physical and biochemical cues regulating myelination remain incompletely defined. Existing in vitro models often fail to reproduce the softness, geometry, and extracellular matrix (ECM) environment of native axons, limiting mechanistic insight and contributing to poor translational predictability of pro-myelinating compounds. To address this, we developed a fully hydrogel-based axon-mimetic micropillar system with independently tuneable stiffness, diameter, and spacing that supports formation of multilayered compact myelin by rodent and human OLs.

Aims/Objectives:
We aimed to (1) determine how biophysical and biochemical features (axon-like stiffness, geometry, and ECM coatings) influence OL differentiation and myelination, (2) validate the platform using ultrastructural and transcriptomic analyses, and (3) assess whether substrate stiffness modulates pharmacological responses to known myelination-promoting drugs.

Methods:
Micropillar arrays (diameters 3–10 µm, spacing 5–15 µm) were fabricated from polyacrylamide hydrogels of defined stiffness (0.5, 5, 20, 50 kPa). Primary rat $O4^+$ OLs or human foetal / hPSC-derived OLs were cultured for 7–14 days. Myelination was quantified by MBP immunostaining using a standardised wrapping score (0–3). Myelin ultrastructure was assessed by TEM. Transcriptomic profiling of OLs on flat vs micropillar gels was performed via RNA-seq (n = 3 biological replicates/condition). For compound testing, OLs were treated for 5 days with benztropine, clemastine, GSK239512, simvastatin, or wiskostatin (n = 3 replicates/condition). Statistical analyses used one-way ANOVA with Tukey post-hoc testing.

Results:
Rodent OLs robustly differentiated and formed compact multilayered myelin around micropillars, with 51.9 ± 15.1% of pillars showing multilayer wraps and an average sheath thickness of 45.9 ± 20.7 nm (n = 3). Myelin thickness correlated strongly with lamellae number (R^2 = 0.94). Geometry critically regulated OL behaviour: 10 µm pillars exhibited significantly higher full-wrapping (score 3) than 3 or 5 µm pillars ($p < 0.001$), recapitulating diameter-dependent myelination. Stiffness modulated myelination in a diameter-dependent manner: for 10 µm pillars, wrapping increased progressively from 0.5 kPa to 50 kPa ($p < 0.01$). ECM cues also influenced outcomes, with laminin increasing the proportion of fully wrapped pillars compared to PDL ($p < 0.05$). RNA-seq revealed >800 differentially expressed genes on micropillars versus flat controls, including upregulation of key myelin-related genes (Mog, Mag, Mbp), ECM remodelling pathways, and Vegfa. Drug testing demonstrated stiffness-dependent effects: benztropine and clemastine increased full wrapping ($p < 0.01$), but effects were attenuated on softer 5 kPa pillars. Human foetal and hPSC-derived OLs also myelinated micropillars, with human cells producing multilayered myelin by day 14.

Conclusion:
This tuneable hydrogel micropillar platform captures key biomechanical determinants of CNS myelination and improves the physiological relevance of in vitro assays. Its ability to reveal stiffness-dependent drug responses suggests it could reduce false-positive compound identification and advance translational discovery for demyelinating diseases.

Ethical Standards:
All animal procedures complied with UK Animals (Scientific Procedures) Act (1986) and institutional ethical guidelines. Human stem cell–derived and foetal cells were used in accordance with approved ethical protocols.

C53

Systemic infection significantly impacts the astrocyte transcriptome in Alzheimer's disease

Zoe Moore[1], Zofia Dudziec[1], Bridget Ashford[1], Lydia Castelli[1], Rachel Waller[1], Stephen Wharton[1], Julie Simpson[1]

[1]The University of Sheffield, United Kingdom

Background: Systemic infections can exacerbate cognitive decline in Alzheimer's disease (AD), yet the underlying molecular mechanisms remain poorly understood. Given the central role of astrocytes in the pathophysiology of AD, we tested the hypothesis that systemic infection promotes a shift in astrocyte function toward a proinflammatory neurotoxic phenotype, thereby accelerating pathology and cognitive decline.

Methods: An enriched population of $GFAP^+$ astrocytes were isolated from the temporal cortex by laser capture microdissection from AD cases who died with (n=6) or without systemic infection (n=6) and matched control cases who died with (n=6) or without systemic infection (n=6). The astrocyte gene expression profile was interrogated using microarray analysis to identify novel mechanisms potentially impacted by systemic infection.

Results: The presence of AD pathology resulted in the significant differential expression of 3,978 transcripts by astrocytes, the majority of which were downregulated (3,273) (FC≥1.5, p value <0.05), and primarily associated with impaired synaptic signalling ($p=3.3x10^{-7}$). In response to systemic infection 1,156 genes (742 upregulated, 414 downregulated) were significantly differentially expressed by astrocytes in AD. Key pathways impacted by systemic infection included oxidative phosphorylation ($p=1.3x10^{-4}$), reactive oxygen species ($p=2.8x10^{-5}$) and neurodegeneration ($p=1.3x10^{-4}$).

Conclusion: Overall, our findings suggest that in AD astrocytes negatively impact synaptic signalling, and are primed to adopt a neurotoxic phenotype in response to infection, providing a basis for future candidate studies based on specific pathways.

C54

Optimisation of viral tools to alter lactate in astrocytes in vivo

Kratika Mujmer[1], Marc Ten Blanco[1], Anja Teschemacher[1], Michael C. Ashby[1], Valentina Mosienko [1]

[1]University of Bristol, United Kingdom

Introduction: L-lactate (lactate) is an essential energy substrate and gliotransmitter released by astrocytes with roles in neurotransmission, memory consolidation, and stress response. Current *in vivo* tools for modulating brain lactate levels fail to widely target astrocytes, as these are driven by the promoter for glial fibrillary acidic protein (GFAP) expressed in only 10-20% of astrocytes with high regional heterogeneity. To better understand the function of astrocytic lactate, we need tools that specifically target a wider population of astrocytes.

A recent promising strategy to reduce lactate in astrocytes is the expression of bacterial-derived Lactate Oxidase (LacOx) enzyme, which catalyses the conversion of lactate to pyruvate. Primary astrocytes transfected with AVVs carrying GFAP-driven LacOx reduces lactate release by 50%. *In vivo* GFAP-driven expression of LacOx in hippocampal astrocytes also results in decreased anxiety-like behaviour in a novel environment. Here, we aim to leverage LacOx to optimise a viral tool for lactate reduction in a wider astrocyte population with high specificity.

Methods: Animal procedures were performed according to the Animal Scientific Procedures Act 1986, Home Office, United Kingdom and the Animal Welfare and Ethics Review Board, University of Bristol (PPL PP5495972). An AAV LacOx construct with reversible loxP sites and an IRES-GFP (AAV-LacOx-GFP) was stereotaxically injected in the dorsal hippocampus (dHip) and prelimbic cortex (PrlC) of Aldh1l1-Cre/ERT2 BAC transgenic mice, where Tamoxifen-inducible Cre expression was controlled by Aldh1l1. Aldh1l1-Cre/ERT2 BAC mouse line was chosen for pan-astrocytic targeting, and the inducible Cre system was selected to provide temporal control of expression. A control virus lacking LacOx (AAV-YFP) was also studied. Expression of the AAVs was studied at different titres (~10^12 to 10^9 vg/ml), and in both transgenic positive (with and without Tamoxifen induction) and negative animals. We also studied the expression of LacOx by a GFAP-driven lentiviral vector (LVV-LacOx-tdTomato), and a LacOx-lacking negative control construct (LVV-tdTomato) in the PrlC.

Results and Statistics: Transgenic positive mice injected with AAV-YFP virus showed astrocyte-specific expression in both brain areas [n(animals)=2, n(slices analysed)=5 for PrlC; n(animals)=2, n(slices analysed)=8 for dHip] whereas AAV-LacOx-GFP showed exclusively neuronal expression [n(animals)=2, n(slices analysed)=6 for PrlC; n(animals)=2, n(slices analysed)=7 for dHip]. In the CA1 dHip, animals injected with AAV-LacOx-GFP showed a significantly higher proportion of fluorescent neurons than animals injected with AAV-flox-YFP control virus [n(ROIs analysed)=19 and 20, respectively; $p<0.0001$, Mann-Whitney test]. Neuronal expression of LacOx-IRES-GFP was also observed in the dHip of transgenic negative animals [n(animals)=2, n(slices analysed)=3] and transgenic positive animals not treated with Tamoxifen [n(animals)=1, n(slices analysed)=3]. Reduction in the titres of AAV-LacOx-GFP did not improve astrocyte-specificity. Similarly to the AAVs, animals injected with LVV-LacOx-tdTomato showed a significantly higher proportion of fluorescent neurons than animals injected with LVV-tdTomato virus in the PrlC [n(animals)=2 each, n(ROIs analysed)= 8 and 11, respectively; $p<0.0001$, Mann-Whitney test].

Conclusion: LacOx-containing viral vectors have a propensity for off-target and Cre-independent expression. Further studies are required, including a time-course study of viral expression and the effect of LacOx enzyme on astrocyte health, to ascertain why LacOx evades astrocytic expression.

C55

Selective derailment of microglial specialisation drives a childhood dementia

David Munro[1], Michael Sewell[1], Sarah Choi[1], Vanessza Fentor[1], Colin Smith[1], Geneviève Bernard[2], Michael Daniels[1], Josef Priller[1], Barry McColl[1]

[1]University of Edinburgh, United Kingdom, [2]McGill University, Canada

Introduction: Biallelic mutations in *LRRC33* cause a currently untreatable childhood-onset neurological disease that we term LRRC33-related encephalopathy (LRE). *LRRC33* is expressed by intracranial macrophages, including highly specialised microglia of the brain parenchyma. LRE is a suspected microgliopathy—a type of brain disease driven directly by dysfunctional microglia—however fundamental questions regarding the cell-type specificity, timing, phenotypic diversity, and mechanisms of microglial dysfunction resulting from defective LRRC33 are unanswered.

Aims: This study aims to characterise brain macrophage phenotypes in human LRE brain tissue, and to determine the temporal, spatial, and mechanistic alterations of microglia in a preclinical model of the disease. Furthermore, we seek to investigate whether therapeutic strategies aimed at eliminating or replacing microglia can mitigate disease-associated pathology.

Methods: Using human post-mortem brain tissue, as well as *Lrrc33 and Tgfb1* deficient mouse models, we characterised microglial phenotypes by histology (RNAscope/IHC; n = 3-12 per group), *in vitro* assays (n = 4-7 per group), and scRNA-seq (n = 2-4 mice per group). Therapeutic potential was assessed in mice through pharmacological microglial elimination (n = 7-11 per group) and bone marrow transplantation (n = 3-8 per group), followed by behavioural assays. Where appropriate, data were analysed using linear mixed-effects models or ANOVA with post-hoc corrections for multiple testing to account for fixed and random effects. Two-sided tests were used throughout. P (or adjusted P) < 0.05 was considered significant. All animal procedures were performed in a UK Home Office–licensed facility in accordance with the Animal (Scientific Procedures) Act 1986.

Results: We show in both humans and mice that microglia, but not other intracranial macrophages, fail to specialise in the absence of functional LRRC33, instead giving rise to abnormal microglia distinct from all described profiles in normal physiology or other diseases. In *Lrrc33-/-* mice, sustained elimination of microglia, or their replacement with *Lrrc33*-expressing transplanted donor myeloid cells, rescues disease, demonstrating that dysfunctional microglia are actively harmful and drive LRE, and revealing a clinically relevant therapeutic approach. Mechanistically, microglial-restricted deletion of transforming growth factor (TGF)-β1 induces developmental defects equivalent to those caused by LRRC33 deficiency, implicating LRE as a neurodevelopmental disorder rooted in failed microglia-intrinsic TGF-β1 signalling.

Conclusions: Our findings identify abnormal microglia as central drivers of LRE, establish it as a distinctive early-onset microgliopathy, and demonstrate that an intact LRRC33–TGF-β1 pathway within microglia is essential for normal human brain development.

C56

Astrocytes protect Neurons and control the Blood-Brain-Barrier Integrity via Cytoskeleton-assisted Membrane Trafficking

Juliane Schiweck[1], Robert Hülse[2], Marta Ornaghi[2], Britta J. Eickholt[2], Kai Murk[2]

[1]German Center for Neurodegenerative Diseases (DZNE), Bonn , Germany, [2]Institute of Biochemistry, Charité Universitätsmedizin Berlin, Germany

The functionality and cerebral capacity of the central nervous system (CNS) depend on complex interactions of astrocytes with neurons and other cell types[1]. While their intercellular communication is well described with respect to certain signalling molecules, very little is known about how astrocytes use intracellular membrane trafficking pathways to orchestrate and exert multiple functions. We discovered an elaborate membrane compartment of tubular endosomes in astrocytes. These membrane tubules are associated with Rab8 GTPases, which are involved in various diseases, including Parkinson's disease. Tubular Rab8+ membrane compartments act as trafficking hubs for surface receptors and adhesion molecules, including β1-integrin[2]. Rab8+ membrane tubule formation involves the leucine-rich repeat kinase 2 (LRRK2) and requires the reorganisation of membrane-associated cytoskeletal scaffolds via the actin regulator drebrin. Perturbances in Drebrin-Rab8-dependent membrane trafficking cause profound defects in vivo: Following CNS injuries, drebrin loss leads to atherosclerosis-like membrane accumulations in astrocytes, followed by the disruption of astrocyte reactivity and the development of excessive neurodegeneration[2,3]. Moreover, drebrin loss impairs the organisation of cerebral blood vessels and blood-brain barrier integrity. In summary, our work shows that cytoskeleton-assisted membrane trafficking in astrocytes is an important mechanism in sustaining neuronal networks and the neurovasculature.

C57

Refining the microbial hypothesis of Alzheimer's disease: the neurotropic yeast Cryptococcus neoformans does not induce neurodegenerative mechanisms in vitro.

William Newton[1], Elizabeth Ballou[1], Jonathan Witton[1], Carolina Coelho[1]

[1]University of Exeter, United Kingdom

Cryptococcus neoformans is a neurotropic environmental fungus causing lethal meningitis in immunocompromised individuals, particularly acquired immunodeficiency syndrome (AIDS) patients. *C. neoformans* can use microglia as a reservoir for cerebral infection by subverting microglia's normal immune functions, although the exact phenotypic changes are still poorly described [1]. A growing body of evidence suggests chronic cerebral infections potentiate Alzheimer's disease (AD), for example, by the fungus *Candida albicans*. *C. albicans* infection increases production of amyloid-β, an AD hallmark, as a potential anti-microbial response [2] and drives microglia-mediated neuroinflammation [3]. Mirroring cerebral infections, genome-wide association studies (GWAS) also identified Aβ- and microglia-associated genes as strongly enriched risk factors for late-onset AD [4]. Given *C. neoformans* is a neurotropic yeast with chronic exposure, and can modulate microglial function, this work explores if *C. neoformans* could also drive AD-associated mechanisms.

We modelled microglia-*C. neoformans* interactions *in vitro* using the human HMC3 microglia cell line infected for 24 hours with the H99E *C. neoformans* or yeast-locked SC5314-Efg$^{\Delta/\Delta}$/Cph1$^{\Delta/\Delta}$ *C. albicans*. Fungi were opsonized for 30 minutes in 20% human serum, then inoculated into HMC3 cultures ± 100 U/mL interferon-γ (IFNγ). Microglial viability was assessed via lactate dehydrogenase (LDH) and resazurin assays, for cell lysis and metabolic activity, respectively, while fungal proliferation was determined via a colony forming unit (CFU) assay. Neuroinflammatory responses were measured by enzyme-linked immunosorbent assays (ELISA) for IFNγ, TNFα, and IL-1β. Enrichment of AD pathways in microglia was assessed via RNA sequencing.

C. neoformans did not increase HMC3 lysis or reduce metabolic activity, *figure 1A-D*, whilst *C. albicans* significantly reduced the latter by 72.2%, *figure 1E*. *C. neoformans* showed superior replication in the presence of HMC3s compared to media alone, *figure 1F*, supporting the subversion of microglia antimicrobial responses. *C. neoformans* only mildly potentiated IFNγ secretion in IFNγ-stimulated HMC3s, *figure 2A*, and did not induce detectable secretion of TNFα or IL-1β (data not shown). However, when compared to *C. albicans*, this potentiation became non-significant despite using multiple *C. neoformans* morphotypes with varying exposure of pathogen-associated molecular patterns (PAMPs), *figure 2B*. Transcriptomics confirmed HMC3s respond to H99E infection and/or IFNγ treatment, *figure 3A*, with 368 genes specifically modulated by H99E infection, *figure 3B*. However, H99E-modulated genes showed no significant enrichment of AD or broader neurodegeneration pathways, *figure 3C-E*. Additionally, when H99E-modulated genes were crossed-over against 188 AD GWAS, only *MME* and *ABCA1* were conserved, *figure 3F*, and both genes were regulated in an anti-AD fashion, *figure 3G-H*.

Overall, our results do not provide any evidence that H99E *C. neoformans* promotes AD-like mechanisms in a HMC3 microglia model and highlights the strong subversion of antimicrobial responses by *C. neoformans*. Further investigations into the *C. neoformans*' mechanisms of microglial evasion would require more translatable models. For example, we are currently optimizing the use of primary human monocyte-derived microglia-like cells and induced pluripotent stem cell-derived microglia as novel cryptococcal meningitis infection models, *figure 4*. Additionally, more work should be performed to refine hallmarks of AD-associated microbes for more efficient identification of currently unknown risk factors.

Note: n numbers in figure legends.

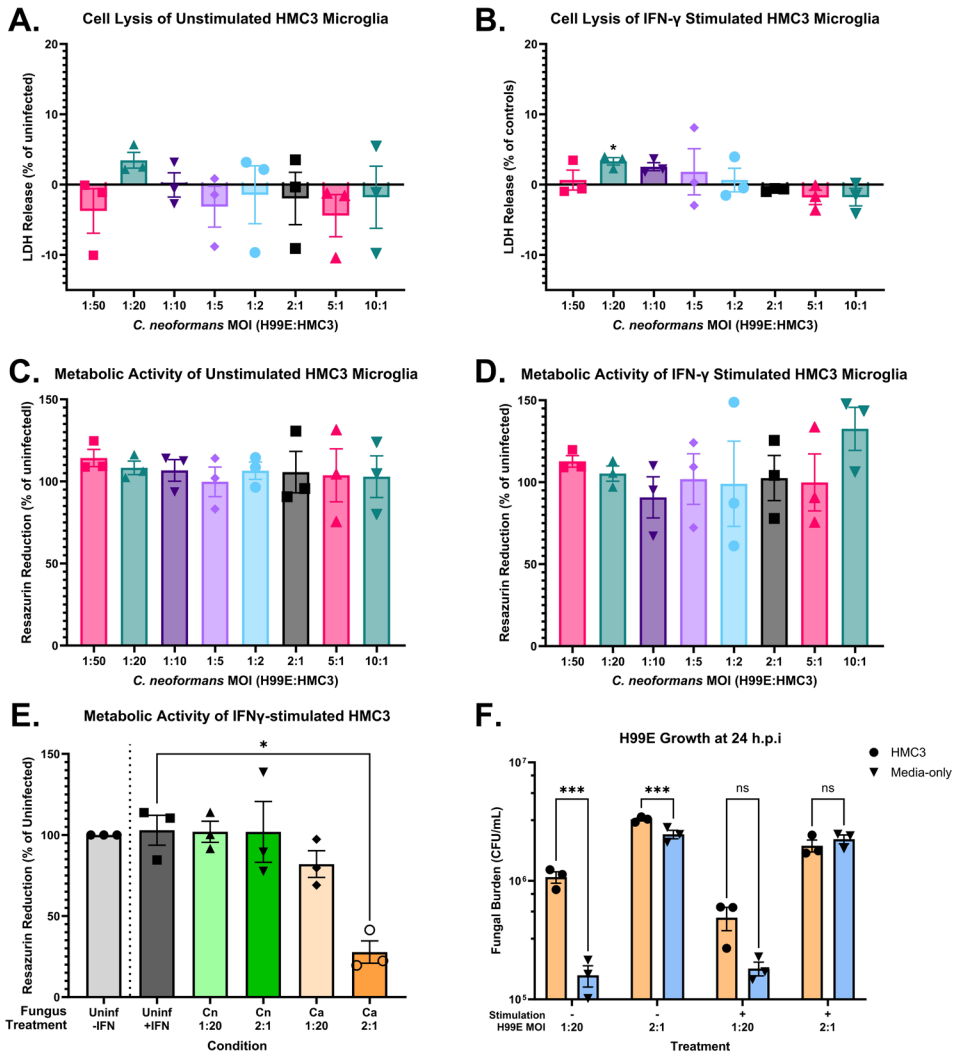


Figure 1. Infection of HMC3 microglia with H99E *C. neoformans* does not induce an anti-fungal response at 24 h.p.i.. HMC3 microglia were seeded into 96-well plates at $3x10^4$ cells/well and rested O/N. H99E *C. neoformans* (Cn) or yeast-locked SC5314-Efg1$^{\Delta/\Delta}$/Cph1$^{\Delta/\Delta}$ *C. albicans* (Ca) were inoculated into YPD and incubated O/N at 37 ^{o}C or 30 ^{o}C, respectively, with agitation. Fungi were washed and pre-opsonized in 20% human serum prior to infection for 24 hours. Subsequent, microglial viabilty was assessed via a LDH assay for cell lysis (A,B) and a resazurin assay for metabolic activity (C-E) and expressed as a % ± SEM of the uninfected control. Mean fungal proliferation ± SEM at 24 h.p.i. in the presence or absence of microglia was determined through a CFU assay (F). Three biological repeats were performed for all experiments and significance determined in GraphPad Prism using a row-matched One-way (A-D) or Two-way (E) ANOVA: non-significant = ns, $P < 0.05$ = *, $P < 0.001$ = ***.

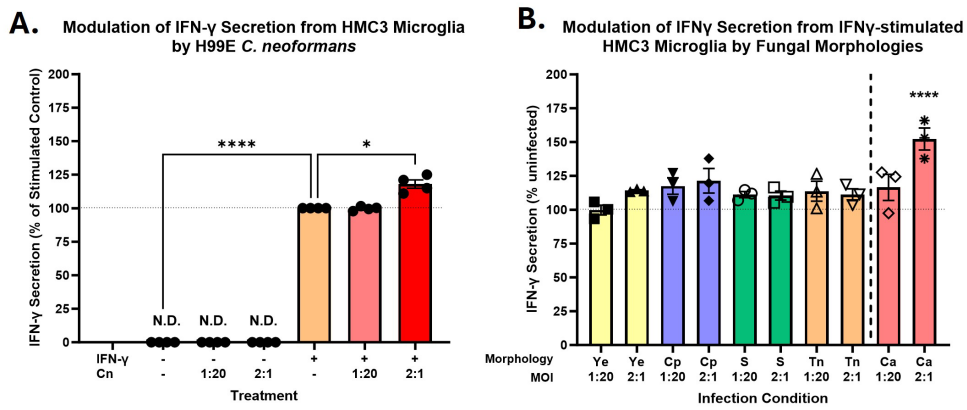


Figure 2. H99E does not induce proinflammatory responses in HMC3 microglia. An IFNγ ELISA was performed on culture supernatants from HMC3 microglia infected with H99E *C. neoformans* (Cn) or yeast-locked SC5314-Efg$^{\Delta/\Delta}$/Cph1$^{\Delta/\Delta}$ *C. albicans* (Ca) and expressed as mean % ± SEM IFNγ concentration relative to uninfected controls. IFN secretion was first compared in microglia IFNγ stimulation. Subsequently, the ability of *C. neoformans* morphotypes (rich media yeast, Ye; capsule-induced, Cp; seed cells, S; titan-induced, Tn) and Ca to potentiate IFNγ secretion in IFNγ stimulated cells was explored (B). Four (A) or three (B) biological repeats were performed and significance determined in GraphPad Prism using a One-way ANOVA: $P < 0.05$ = *, $P < 0.0001$ = ****.

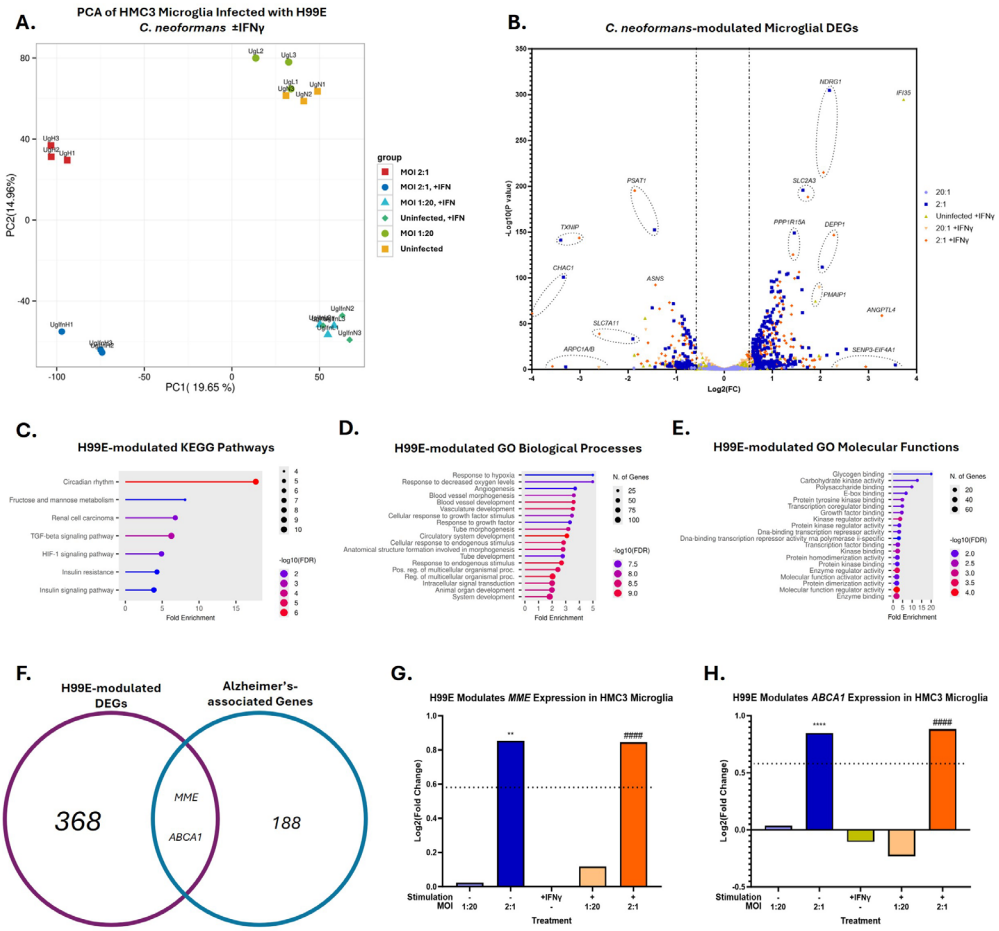


Figure 3. H99E *C. neoformans* does not promote Alzheimer's-associated transcriptional responses in HMC3 microglia. HMC3 microglia were infected for 24 hours with rich-media grown H99E *C. neoformans*, pre-opsonized in 20% human serum, for 24 hours ±100 U/mL IFNγ stimulation. Microglial RNA was purified via TriZOL and a DirectZOL mini-prep. Library construction, sequencing by Illumina Novaseq (PE150 mode), and basic bioinformatics was performed by BMKGENE. Principle component analysis (PCA) was performed on unfiltered DEGs to confirm HMC3 responsiveness (A). H99E-modulated DEGs were identified as conserved genes differentially regulated by an MOI 2:1 H99E infection irrespective of IFNγ stimulation and expressed as a volcano plot across all conditions (B). Enrichment analysis was performed on H99E-modulated DEGs using ShinyGO 0.85 identify significantly enriched KEGG pathways (C), GO biological processes (D), and GO molecular functions (E). H99E-modulated DEGs were then screened against a list of Alzheimer's associated DEGs (F) and the expression patterns plotted for cross-over genes *MME* (G) and *ABCA1* (H). Conditions were performed in triplicate from one biological repeat. Differentially expression analysis was performed by BMKGENE using edgeR to determine significance: $P < 0.01$ = ** compared to unstimulated, $P < 0.0001$ = **** compared to unstimulated, $P < 0.0001$ = #### compared to IFNγ-stimulated

C58

Targeting Microglial P2X7R Using Non-Viral siRNA Delivered Via An Injectable Hydrogel As A Treatment for Post-Traumatic Temporal Lobe Epilepsy

Anne Marie O'Callaghan[1], James Dixon[2], Adrian Dervan[1], Ian Woods[1], Tobias Engel[3], Fergal O'Brien[1]

[1]Tissue Engineering Research Group, Royal College of Surgeons in Ireland, Ireland, [2]School of Pharmacy, University of Nottingham Biodiscovery Institute, Nottingham, United Kingdom, [3]FutureNeuro, Royal College of Surgeons in Ireland, Ireland

Introduction

Temporal lobe epilepsy (TLE) is the most common acquired seizure disorder, often caused by brain injury. Anti-seizure drugs are ineffective in 33% of patients, who are classed as drug-refractory[1]. Furthermore, these systemic treatments fail to tackle the underlying disease pathophysiology. The purinergic receptor P2X7R, highly expressed on inflammatory microglia within injured brain regions, drives chronic inflammation in TLE by inducing the release of neurotoxic cytokines. Systemic P2X7R inhibition produces disease-modifying effects in murine TLE models, seen with pharmacological antagonists[1] as well as silencing RNA[2] (siRNA) which is considered a more targeted gene therapy approach. However, as P2X7R is widely expressed throughout the body, focal delivery is crucial to developing P2X7R-targeting therapies. Therefore, the key aim of this study was to develop a brain-compatible injectable hydrogel carrying siRNA targeting microglial P2X7R (siP2X7R) for focal delivery to the brain for the treatment of TLE.

Materials and methods

Fabrication of a photo-crosslinkable biomimetic hydrogel. Methacrylated hyaluronic acid (MeHA) was produced by reacting hyaluronic acid with methacrylic anhydride. Gel biophysical properties and degradation profile were characterised. *Non-viral delivery of siRNA targeting microglial P2X7R.* siP2X7R was complexed with GAG-binding Enhanced Transduction (GET) peptide[3] to produce peptide-siRNA nanoparticles. These were delivered to reactive microglia at an optimised concentration. P2X7R knockdown and cell phenotype were validated by PCR, and cytokine release quantified by ELISA. *Tuning siP2X7R release from MeHA hydrogels.* Encapsulated and naked siRNA was loaded into gels at various concentrations and release was quantified over time. Released nanoparticles were delivered to microglia and P2X7R knockdown was validated.

Results

MeHA crosslinks when exposed to blue light in the presence of a photo-initiator. *In situ* photo-polymerisation was confirmed using a soft tissue model. Hydrogels had a stiffness compatible with neural cells[4], a mesh size suitable for nanoparticle retention, and a rapid degradation profile. P2X7R knockdown of 65% was achieved following delivery of 50 nM siP2X7R nanoparticles to reactive microglia stimulated with LPS and BzATP. P2X7R knockdown ameliorated inflammatory cell phenotype and signalling, as demonstrated by reduced Iba1 mRNA expression and IL-1β cytokine release respectively. GET encapsulation improved the release profile of siRNA from MeHA hydrogels by preventing the initial burst release and prolonging release over time. Released nanoparticles successfully transfected reactive microglia and functional P2X7R knockdown was achieved.

Discussion

Injectable hydrogels can act as tuneable drug delivery vehicles for CNS applications that overcome several limitations associated with systemic drugs including difficulty crossing the blood-brain barrier[4]. Here we tuned the physicochemical properties of a hyaluronic acid-based hydrogel to mimic the native brain tissue and to optimise it for use as an siRNA delivery platform. The results of our study indicate that siRNA transfection promotes knockdown of microglial P2X7R which thus reduces the production of neurotoxic cytokines *in vitro*, and future work will involve confirming the disease-modifying effects of this system *in vivo* using a well-established murine injury model of TLE. In summary, this gene-activated hydrogel demonstrates potential to act as a novel disease-modifying treatment strategy for post-traumatic TLE patients, with future applications within the wider field of neurotrauma.

C59

Myelination deficits in the barrel cortex of Fmr1 KO mice

Laura Simoes de Oliveira[1], Lida Zoupi[1]

[1]The University of Edinburgh, United Kingdom

Fragile X Syndrome (FXS) is a neurodevelopmental disorder resulting from the lack of the protein FMRP due to the transcriptional silencing of the FMR1 gene. FXS is the leading single gene cause of autism, affecting approximately 1 in 4000 males and 1 in 8000 females. Individuals present with intellectual disability, autism, sensory hypersensitivities and epilepsy amongst other comorbidities.

FMRP is ubiquitously expressed throughout the brain and the contribution of neuronal defects to FXS has been widely studied in rodent models of the disorder, with neuronal excitability and network function in the somatosensory cortex thought to contribute to altered sensory processing in FXS. Altered myelination has been reported in FXS individuals as well as in rodent models of the condition. However, the contribution of oligodendrocyte lineage cells and myelination to sensory abnormalities in FXS is not known.

In the current project, I have assessed oligodendrocyte lineage cell numbers and myelination in the somatosensory cortex of *Fmr1* KO mice using immunohistochemistry in perfusion fixed brain sections. I have found a reduced number of mature oligodendrocytes ($p=0.006$, Two-tailed T-test) and an increase in oligodendrocyte progenitor cells ($p<0.001$, Two-tailed T-test) in *Fmr1* KO mice ($n=8$ mice) relative to WT ($n=11$ mice), possibly suggesting a developmental delay. This reduction in mature oligodendrocytes was accompanied by a 40% reduction in myelination in the upper layers of the cortex in *Fmr1* KO mice ($p<0.001$, Two-tailed T-test), suggesting altered developmental myelination may contribute to altered somatosensory cortex function in FXS. I am currently testing whether pro-myelinating drugs can rescue the myelination phenotype and recover neuronal function.

All experiments were performed in accordance with institutional (University of Edinburgh, UK) and UK Home Office guidelines (ASPA, PPL: PP5764018).

C60

Effects of Isoflavones on iPSC-Derived Oligodendrocyte Differentiation and Remyelination in Rodent Models

Sila Ozcelik[1], Nona Pop[1], Jiaju Jiang[1], Cheong-Meng Chong[2], Simon Ming-Yuen Lee[2], Dan Ma[1]

[1]Aston Medical School, Aston University, United Kingdom, [2]Institute of Chinese Medical Sciences, University of Macau, China

Introduction Myelin wraps around axons, providing rapid neuronal conduction and metabolic support. Failure of repair following myelin damage (demyelination) results in axonal degeneration and eventually neuronal loss, which is the key component in demyelinating diseases such as multiple sclerosis (MS), and this also contributes to the pathogenesis of Alzheimer's disease (AD).

In the central nervous system (CNS), myelin is formed by oligodendrocytes. Regeneration of myelin (remyelination) involves the activation and maturation of the oligodendrocyte progenitor cells (OPCs), the crucial cellular processes recapitulating the lineage progression of developmental myelination. Isoflavones, as plant-derived compounds with a structural similarity to estrogen, interact with estrogen receptors (ERs) and play a significant role in the biology and function of oligodendrocytes. It has been shown that isoflavones and estrogen have a positive influence on OPC proliferation and differentiation, promoting myelin repair[1]. This study investigated whether isoflavones promote the generation of human induced pluripotent stem cell (hiPSC)-derived OPCs and enhances remyelination in rodent models.

Methods All animal procedures accorded with the UK Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the Animal Welfare and Ethical Review Body (AWERB). Primary OPCs were isolated from cerebral cortices of neonatal Sprague-Dawley rats (P2–3) killed by pentobarbital overdose (n=3–4 independent cultures). Organotypic cerebellar slices were prepared from P4–8 rat pups (n=3). For *in vivo* studies, focal demyelination was induced in the ventral spinal cord of anaesthetized C57BL/6 mice[1,2] (9–10-month-old; n=4–6/group) via 1% lysolecithin injection; buprenorphine was administered for analgesia. Animals were treated with puerarin or calycosin (100 or 80mg/kg i.p.) or vehicle daily for 7 days post-lesion. iPSC-OPCs were generated according to Fossati protocol[3]. Mitochondrial function was assessed via Seahorse extracellular flux analysis. Data are presented as mean ± S.E.M. Statistical significance was determined using unpaired Student's t-test or one-way ANOVA.

Results We have shown that puerarin treatment significantly increases the differentiation of iPSC-derived O4+ pre-oligodendrocytes. It promotes rat OPC proliferation with an increased mitochondrial activity. In *ex vivo* cerebellar slices, puerarin (200μM) significantly improved myelination and remyelination following lysolecithin-induced damage. *In vivo*, puerarin treatment in mice (9 months) significantly increased the density of Olig2+ lineage cells and CC-1+ mature oligodendrocytes within lesions compared to controls[1]. Recently, we have found that calycosin, another traditional Chinese isoflavone, known for its antioxidant and anti-inflammatory properties, promotes OPC proliferation which is indicated by an increased percentage of Ki67+ cells in Olig2+ lineage cells, and differentiation which is indicated as increased O4+ and MBP+ cell number ($p < 0.05$). Calycosin also increases remyelination *ex vivo* ($p < 0.05$) and *in vivo* ($p < 0.05$), similarly to puerarin.

Conclusion Our findings suggest that isoflavones are beneficial to oligodendrocyte lineage progression and myelin repair, with a potential to be developed into preventive or therapeutic agents for CNS diseases associated with myelin damage.

C61

The Ageing Brain in Heart Failure: Senescent Glia in the Paraventricular Nucleus

Alessia Paranchini[1], Bianca Cabungan[1], Dipa Baral[1], Ahmed AlTuwaijri[2], Prashant Jay Ruchaya[1]

[1]University of East London, United Kingdom, [2]King Saud University, United Kingdom

Microglia and astrocytes are essential regulators of central nervous system homeostasis; however, during ageing and chronic disease these glial populations undergo maladaptive phenotypic transitions characterised by loss of homeostatic function, heightened reactivity, and acquisition of pro-inflammatory and senescent features. Such changes are increasingly implicated in sustained neuroinflammation and the process of inflammaging. The paraventricular nucleus (PVN) of the hypothalamus serves as a critical integrative centre for autonomic and neuroendocrine control, exerting powerful influence over sympathetic outflow through projections to cardiovascular regulatory nuclei. Disruption of glial homeostasis within the PVN therefore represents a plausible mechanism linking neuroinflammation to autonomic dysregulation in cardiovascular disease.

Using immunohistochemistry, we examined glial activation and cellular senescence within the PVN in an isoproterenol (ISO; 150 mg/kg)-induced mouse model of heart failure across age groups. All experimental procedures were conducted in accordance with the regulations for animal testing, directed by the Home Office and stipulated under the under the Animal (Scientific Procedures) Act 1986. ISO treatment resulted in robust microglial activation in the PVN of both young (n = 9; 12-week-old; 429.00 ± 85.10) and aged (n =6; 23-month-old; 766.83 ± 76.28) mice, accompanied by marked astrocytic reactivity in young (109.47 ± 14.20) and aged animals (815.39 ± 205.37). Notably, ageing significantly amplified the magnitude of glial activation, indicating an age-dependent vulnerability of the PVN to inflammatory remodelling. Consistent with this, expression of the senescence marker $p16^{INK4a}$ was elevated in the PVN following ISO treatment in young (3.09 ± 1.81) and aged mice (18.24 ± 5.11), suggesting that cellular senescence contributes to the pro-inflammatory glial milieu.

Importantly, post-ISO administration of the senolytic combination dasatinib (5 mg/kg) and quercetin (50 mg/kg) in aged mice significantly attenuated PVN glial reactivity, reducing microglial (502.62 ± 97.44) and astrocytic activation (533.37 ± 31.24), alongside a marked decrease in $p16^{INK4a}$ expression (15.58 ± 3.32 vs. 3.09 ± 1.81). These findings indicate that senescent cells actively contribute to PVN centred neuroinflammation and that their selective clearance can partially restore glial homeostasis.

Collectively, our data demonstrate that heart failure and biological ageing synergistically drive a reactive and pathological glial phenotype within the PVN, implicating glial senescence as a key mechanistic link between neuroinflammation and maladaptive sympathetic regulation. This study provides proof-of-concept evidence that region specific targeting of senescent and over activated glial cells may represent a novel therapeutic strategy for mitigating neurogenic contributions to cardiovascular disease.

C62

The role of LRRK2 in microglial senescence in Parkinson's disease

Ashlesha Patil[1], Mohammad Suka[1], Dr Nurun Nahar Fancy[1], Dr Paul Matthews[1], Dr Diego Gomez-Nicola[2]

[1]Imperial College London, United Kingdom, [2]University of Southampton, United Kingdom

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by motor and non-motor symptoms, including cognitive impairment.[1] Pathologically, PD is defined by dopaminergic neuron loss and accumulation of α-synuclein (α-Syn) aggregates.[1] Aging is the greatest risk factor for PD, and cellular senescence—an irreversible cell-cycle arrest—is a key hallmark of aging tissues. Senescent cells adopt a senescence-associated secretory phenotype (SASP), releasing pro-inflammatory factors that promote chronic neuroinflammation.[2] Increasing evidence suggests that pathological protein aggregation induces senescence in multiple brain cell types, including microglia.[3] As the resident immune cells of the central nervous system, microglia play a central role in maintaining brain homeostasis, but disease-associated microglial states can exacerbate inflammation.[4] Leucine-rich repeat kinase 2 (LRRK2), a kinase strongly implicated in both familial and sporadic PD, is highly expressed in microglia and regulates inflammatory signalling.[5] Previous studies demonstrate that LRRK2 can induce neuronal senescence via p53–p21 signalling and promote α-Syn aggregation[6], while inhibition of LRRK2 ameliorates neuroinflammation and cytotoxicity.[7] However, the role of LRRK2 hyperactivity in α-Syn–driven microglial senescence remains unclear.

Aims/Objectives

This project aims to determine whether α-Syn promotes microglial senescence through increased LRRK2 expression and activity in PD. The objectives are to (i) map the spatial relationship between LRRK2 expression, senescence markers and α-Syn pathology in human PD brain tissue, (ii) investigate how α-Syn drives senescence in iPSC-derived microglia carrying the common LRRK2 mutation, G2019S, and (iii) assess whether inhibiting LRRK2 activity can attenuate α-Syn–driven senescence in iPSC-derived microglia.

Methods and Research Plan

Post-mortem human brain tissue from PD cases across Braak stages (Lewy body pathology), Parkinson's disease dementia (PDD), dementia with Lewy bodies (DLB) and age and sex-matched controls (male, n = 12 and female, n = 3 for each group) will be analysed using imaging mass cytometry (IMC). The prefrontal cortex, a region affected by α-Syn pathology in cognitively impaired PD cases, will be examined. This approach can quantify senescent microglial populations and assess their spatial relationship to α-Syn pathology.

Complementary in-vitro studies will be using human iPSC-derived microglia carrying the LRRK2 G2019S mutation along with heterozygous and homozygous knockouts (KO). iPSC-derived microglia will be exposed to α-Syn pre-formed fibrils to assess LRRK2 activity and senescence induction. To directly test the contribution of LRRK2 activity, iPSC-derived microglia will be treated with LRRK2 kinase inhibitors.

Results

Meta-analysis of published single-nucleus RNA-sequencing datasets (n = 11) indicates increased expression of canonical senescence gene sets in microglia from PD, PDD and DLB compared with healthy controls (meta logFC = 0.5 and p value = 1e-04). Immunofluorescence staining on fixed-formalin

parafilm-embedded (FFPE) tissue from PDD patients show presence of senescence markers, LRRK2 and pathology markers. iPSC cells containing the LRRK2 G2019S mutation, heterozygous and homozygous knockouts have been validated and characterised for differentiation into iPSC-derived microglia.

Conclusion

This work aims to define how α-Syn pathology mediated LRRK2 activity drives microglial senescence in PD. By combining spatial analysis of human tissue with in-vitro iPSC-derived microglial models, this project offering mechanistic insight into senescence-associated pathology in microglia in PD.

C63

Investigating Transcriptomic Data from Alzheimer's Disease Patients Reveals Sex-Differences in Microglial Gene Expression

Anupa Sara Paulose[1], Marie-Victoire Guillot-Sestier[1]

[1]University College Dublin, Ireland

Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder with hallmarks of cerebral β-amyloid (Aβ) plaques, tau hyperphosphorylation, and gliosis (1) leading to cognitive dysfunction (2). Importantly, women account for two-thirds of diagnosed cases (3), present a higher risk of AD (4), and worse clinical manifestation and progression than men (5). Sex-related differences in neuroinflammation and gliosis are suggested to impact AD prevalence and progression. Furthermore, sex differences have been described in the transcriptome, metabolism, morphology, and function of microglia in human AD patients (6) and animal models of the disease (7,8). However, the mechanisms by which these sex-dependant changes impact on AD progression are still unclear.

Aim of study: Previous RNA sequencing studies have investigated sex differences in a case-versus-control manner. We are interested in studying the differences between male and female AD patients to better understand sex-biased alteration of microglial function within the disease.

Methods: We utilized publicly available human single-nucleus RNA sequencing datasets containing data from AD patients and non-AD individuals (control). Specifically, we sourced datasets from two repositories: the Gene Expression Omnibus (9) and the AD Knowledge Portal on Synapse (10), selecting those that met our predefined criteria, and carried out our analysis (see Figure 1: workflow of study).

We selected datasets from prefrontal cortex (number of AD samples (M/F) – 9 (6/3), number of control samples (M/F) – 9 (6/3)), dorsolateral prefrontal cortex (number of AD samples (M/F) – 11 (3/8), number of control samples (M/F) – 10 (4/6)), entorhinal cortex (number of AD samples (M/F) – 9 (4/5), number of control samples (M/F) – 9 (5/4)), and somatosensory cortex (number of AD samples (M/F) – 9 (4/5), number of control samples (M/F) – 9 (5/4)). Using RStudio, we performed differential gene expression analysis using the edgeR and MAST statistical methods, comparing microglial cells from male and female patients. To ensure the analysis was AD-specific, we excluded genes that exhibited the same sex-differential expression patterns in control individuals.

Results: We identified genes that present the same sex-biaised expression profile between the two statistical methods, thereby refining understanding of sex-specific differences in microglial gene dysregulation in AD. Specifically, we found that in female AD patients, genes associated with metal binding and homeostasis, cell adhesion, mitochondrial metabolism and structural stabilization were downregulated compared to male AD patients. Interestingly, no genes were commonly dysregulated in female vs male AD patients between all the different brain regions, which suggests the sex-differences are region specific.

Conclusion: Our findings highlight specific microglial functions that may drive sex-specific differences in AD and inform further studies *in vitro* and *in vivo* aiming at clarifying biomolecular mechanisms with hopes to guide the development of personalized therapies.

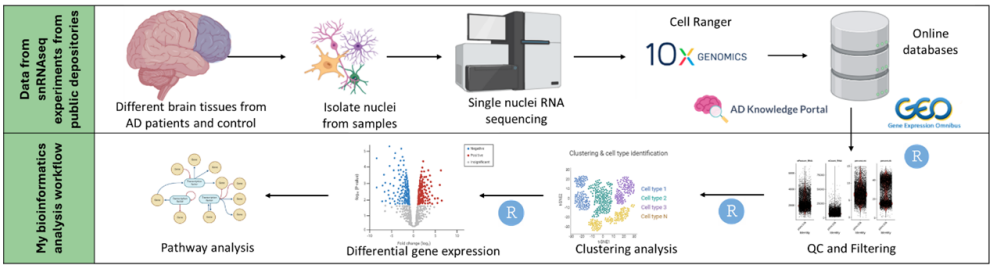


Figure 1: Analysis workflow for transcriptomic data from control and AD patient's brain tissue. Selected processed single-nuclei (sn) RNA sequencing datasets from Gene Expression Omnibus (GEO) and AD Knowledge Portal were analysed in R-studio through the Seurat package. Quality Control (QC) and filtering were conducted, followed by clustering of the cell types. Analysis was carried out in microglial cluster to identify differential gene expression between female and male samples in both Control and AD patients. Pathway analysis was performed to identify the biological pathways regulated by these genes. *Image created with Biorender.com*

C64

Outside the brain: the role of peripheral glia in lung development, homeostasis and disease

Daniel Petrossian[1], Julia Karjalainen[1], Luca Verger[1], Sofia Hain[1], Kady Jones[1], Fränze Progatzky[1]

[1]The Kennedy Institute of Rheumatology, UK

Peripheral glial cells are the non-neuronal component of the peripheral nervous system and were traditionally considered to play mainly supportive roles. However, recent studies have revealed unexpected heterogeneity and immune-modulatory functions of peripheral glia in certain tissues, particularly enteric glia in the gut. Whether peripheral glial cells play similar roles in other barrier tissues remains poorly understood.

The lung is a major mucosal barrier and a critical site of immune defence, yet the organisation and function of peripheral glial cells in the lung are largely uncharacterised. In particular, little is known about how lung-associated glial cells develop, how they are maintained during homeostasis, or whether they contribute to immune responses and disease.

Here, we present early work aimed at characterising peripheral glial cells in the lung across development and homeostasis. We describe the spatial organisation and cellular composition of the pulmonary glial network across distinct anatomical regions, including airways and parenchyma. Using single-cell RNA sequencing analyses, we begin to define the molecular features of lung-associated glial populations and explore potential functional states. We additionally perform preliminary analyses to assess glial responses in the context of infection.

Together, this work represents an initial step toward defining a previously undercharacterised cell population in the lung and lays the foundation for future studies investigating the roles of peripheral glial cells in lung development, homeostasis, and inflammatory disease.

C65

Defining monocyte/macrophage migration to and within the brain during acute viral encephalitis

Fabian Schuette[1], Alistair Gamble[1], Elise Pitmon[1], Heather Mathie[1], Jun Fu[2], Adrian Francis Stewart[2], Eve Anderson[3], Douglas Strathdee, Gerard J Graham[4], Marieke Pingen[5]

[1]Chemokine Research Group (CRG), School of Infection & Immunity, University of Glasgow. , UK, [2]Technische Universität Dresden, Germany, [3]Cancer Research UK Scotland Institute, UK, [4]Chemokine Research Group (CRG), School of Infection & Immunity, University of Glasgow, UK, [5]Chemokine Research Group (CRG), School of Infection & Immunity, University of Glasgow and Wales Applied Virology Unit (WAVU), College of Medicine, Cardiff University, UK

Introduction. Cell migration plays a central role in tissue homeostasis and mounting effective responses during infection and inflammation. This is particularly evident in the brain, where recruited immune cells can readily cause irreversible damage. Consequently, cell migration to and within the brain is carefully orchestrated and appears central to various neuropathologies, including encephalitis, stroke and multiple sclerosis.

Cell migration is predominantly controlled by a chemoattractant cytokines (chemokines) and their receptors. This process is poorly understood for migration to and within the brain, despite the therapeutic potential. Indeed, we have previously demonstrated that targeting cell migration improved the outcome in a mouse model of lethal viral encephalitis[1].

Here we have used viral encephalitis as a model for acute viral infection to investigate migration of monocytes and macrophages to and within the brain. Monocytes are circulating immune cells that contribute to host defence and tissue repair, and serve as macrophage precursors. CCR2 is the main chemokine receptor for monocyte bone marrow egress and migration in the periphery. CCR2 is expressed in a discrete locus with CCR1, CCR3 and CCR5. In humans, CCR5 deficiency is associated with increased morbidity during viral encephalitis.

Aim: To characterise chemokine-mediated cell migration of monocytes/macrophages to the brain.

Methods/ethics. Animal experiments were performed with approval by local ethics and UK home office. Our *in vitro* mixed glial/neuronal cell culture system uses embryonic day 17 mouse brains[2]. Subcut injection with Semliki Forest Virus strain A7(74) was used to induce viral encephalitis.

Results. Using FISH-imaging on myelinating rodent cultures, we previously observed that certain immune genes are expressed differentially by brain resident cells[3]. We now fully characterised the immune response to IFN-b, a key cytokine in the antiviral immune response in our mixed glial/neuronal cultures. RNAseq on cells separated using fluorescence-activated cell sorting after 24 hours of IFN-b or mock treatment demonstrated that each cell type initiated a unique immune profile. All cell types expressed multiple chemokines relevant for monocyte migration in response to IFN-b (fold change padj < 0.05, n=4 biological replicates).

We next investigated monocyte migration in our *in vivo* viral encephalitis model. Using our chemokine receptor reporter mice[4], we found a reduction in CCR2 expression compared to bone marrow and blood (average 46% vs 85% and 97%, n=6 mice). Surprisingly, we observed an increase in CCR5 expression (average 28% monocytes CCR5+ in brain vs 2% in bone marrow and 5% in blood, n=6).

To determine the importance of CCR2 for monocyte migration to the brain, we compared monocyte migration in mice lacking the full cluster CCR1/2/3/5, or deficient for CCR1/3/5 but not CCR2. Full locus deletion reduced the presence of monocytes and their macrophage progeny in the encephalitic brain by 97%, and this phenotype could be fully restored by re-establishing only CCR2 expression (WT vs full KO $p=0.0006$; WT vs CCR2 restored $p=0.2028$; $n=4-6$).

Conclusions. CCR2 expression is critical for monocyte migration to the inflamed brain. We hypothesise that CCR5 is important for movement within the brain, and further experiments to investigate this are ongoing.

C66

Regional specificity and morphological features of microglia are determined by prion disease subtype in chronic CNS neurodegeneration

Sasha Pokrovskaya[1], John W. Wills[2], Barry Bradford[1], Neil Mabbott[1]

[1]The Roslin Institute, The University of Edinburgh, UK, [2]Biomineral Research, Department of Veterinary Medicine, University of Cambridge, UK

Introduction: Prion diseases are a group of rare disorders affecting the central nervous system of animals and humans. Prion diseases occur following structural modification and accumulation of the misfolded cellular prion protein, PrP, causing spongiosis of the grey matter, reactive gliosis and neurodegeneration. Despite ongoing research into the mechanisms underlying prion disease neuropathology no cure is currently available for treatment of the disease, making it a uniformly fatal condition. Microglia are innate immune cells residing within CNS parenchyma that are highly sensitive regulators of brain homeostasis and function. Microglia undergo morphological and functional changes, adopting either pro-inflammatory and anti-inflammatory states.

Aims & Objectives: This study aims to explore and characterise microglial heterogeneity in chronic CNS neurodegeneration in prion-infected mice using a range of microglia markers: AIF1 (pan-microglia), TMEM119 (homeostatic microglia), and LGALS3 (disease-associated microglia).

Methods: Brains were analysed from mice infected with a range of distinct prion agent strains (ME7 scrapie, *n*=6; 79V scrapie, *n*=5; 22L scrapie *n*=6; 139A scrapie, *n*=6; and healthy control, *n*=6). Different combinations of prion agent strains were included in this study to allow comparison. Histopathological assessment of mouse brain tissue was performed using anti-AIF1, anti-TMEM119, and anti-LGALS3 antibodies to detect microglia, alongside anti-PrP to detect the abnormal accumulations of prion disease-specific PrP.

Statistical analysis: Kaplan-Meier statistical analyses were performed on the survival data and Two-way ANOVA was used to analyse the severity of disease pathogenesis. The morphology of microglia cells was analysed, generating matrices of single-cell features. Two-way ANOVA and appropriate post-hoc comparisons were performed to compare distribution of microglia cells. Significance level was set at $p<0.05$.

Results: My research identified the interplay between the distribution of microglial phenotypes and the infective prion strain agent. Inverse correlation was observed between TMEM119 and LGALS3 microglia subsets, highlighting differential activation states. During the early stages of prion disease, microglia predominantly displayed an anti-inflammatory, homeostatic profile (TMEM119+ microglia), as prion pathology progresses, microglia population expands and shifts towards neurotoxic, pro-inflammatory state (LGALS3+ microglia). This study found that LGALS3 expressing microglia are predominantly associated with the white matter tracts, while TMEM119 microglia are found in the grey matter areas. The regional distribution of microglia subtypes and its morphological features were determined by the infective prion strain; with grey matter-associated microglia exhibiting increased branching index compared to cells residing within myelin-rich white matter areas.

Conclusions: This study identified the regional and morphological heterogeneity of microglia in prion disease. Further work is necessary to improve our understanding of the interplay between different

microglia phenotypes and their regional distributions in neurodegeneration, as it would allow the development of novel therapeutic targets.

C67

APOE4-mediated toxic gain of function in human induced pluripotent stem cells exacerbates amyloid-β induced toxicity in oligodendrocytes

Nona Pop[1], Sila Ozcelik , Irundika Dias, Dan Ma

[1]Aston Univesity, United Kingdom

Introduction:

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive decline and memory loss, with the accumulation of amyloid-beta (Aβ) plaques as a pathological hallmark. The ε4 allele of apolipoprotein E (APOE4) is the strongest genetic risk factor for late-onset AD, known to exacerbate Aβ aggregation and clearance deficits. While neuronal-centric models dominate, growing evidence implicates broader cellular dysfunction, including compromised white matter integrity and myelin loss, in AD progression. Myelin, produced by oligodendrocytes (OLs), is maintained by oligodendrocyte precursor cells (OPCs). Both Aβ toxicity and APOE4-related pathways have been independently linked to disrupted OPC function and myelination (1-3). It has been reported that Amyloid-β inhibits OPC proliferation and differentiation both in vitro and in vivo; factors such as BDNF rescue amyloid-β-induced OPC toxicity (1). Our lab has previously shown that iPSC-derived ECs (iPSC-EC) rescue OPC from TNF-a induced cell death and promote OPC proliferation and differentiation (2). Recent evidence of APOE4 inducing a toxic gain of function in iPSC-derived ECs, suggests they may contribute to drive a toxic milieu, alongside the Aβ toxicity (3). The present research aims to examine weather ECs carrying the APOE4 genotype exacerbate Aβ-induced deficits in OPC maturation and confirm that healthy parental controls carrying APOE3 genetoype have a positive protective effect. Building from that, the study aims to identify potential underlying molecular mechanisms.

Methods:

Using an in vitro co-culture system, rat primary OPCs were exposed to oligomeric Aβ1-42 and co-cultured with isogenic human induced pluripotent stem cell-derived ECs (iPSC-ECs) of either the protective APOE3/3 or the risk-associated APOE4/4 genotype. OPC differentiation was assessed by immunocytochemistry for the mature oligodendrocyte markers Olig2 and MBP (n=3 independent experiments). mTORC1 pathway activation was evaluated by quantifying phosphorylated ribosomal protein S6 (pS6) in Olig2+ cells. Data were analysed using one-way ANOVA with Kruskal-Wallis post-hoc tests.

Results:

We report that Aβ1-42 exposure significantly impaired OPC differentiation, reducing the proportion of Olig2+MBP+ cells compared to control conditions ($p<.001$). Co-culture with APOE3/3 iPSC-ECs completely rescued this deficit ($p<.05$ vs. Aβ alone). In contrast, co-culture with APOE4/4 iPSC-ECs failed to confer protection, with OPC maturation remaining significantly impaired ($p<.01$ vs. vehicle control). Mechanistically, Aβ toxicity combined with APOE4/4 EC co-culture induced a pathological

hyperactivation of the mTORC1 pathway, evidenced by a significant increase in the proportion of pS6+Olig2+ cells compared to all other conditions ($p<0.001$). This is in line with previous reports of mTORC1 hyperactivation in ageing and offers novel context for potential disease mechanisms.

Conclusion:

These findings demonstrate that APOE4 confers a toxic gain-of-function in endothelial cells, transforming them from protective bystanders into active contributors to Aβ-induced OPC dysfunction. The loss of protection is associated with aberrant mTORC1 signalling in OPCs, revealing a critical disruption of the OPC-EC communication axis. Future work will focus on further elucidating this critical axis of OPC-EC communication that could be targeted to preserve white matter integrity in AD, particularly in high-risk APOE4 carriers.

Ethical Standards: All experiments involving primary rat cells were conducted in accordance with relevant institutional and national guidelines and UK legislation. Animals were humanely treated and where relevant humanely killed.

C68

Impact of Matrix Viscosity on Astrocyte Morphology and Reactivity in 3D Tissue Models

Norah-Jane Prendergast[1], Runze Xu[2], Mina Aleemardani[1], Scott Miners[1], Adam W. Perriman[3], James P.K. Armstrong[1]

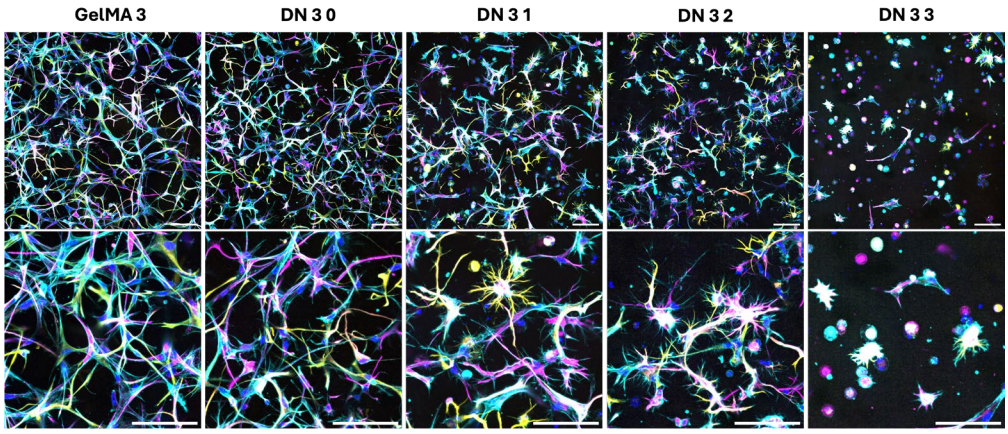
[1]University of Bristol, United Kingdom, [2]Tsinghua Univeristy , China, [3]Australian National University , Australia

The neural extracellular matrix (ECM) provides biochemical and mechanical cues that regulate cellular behaviour and function, with astrocyte-ECM interactions playing a key role in central nervous system homeostasis[1]. The composition and elasticity of the ECM are key factors guiding astrocyte phenotype and function[2,3]; however, the influence of ECM viscosity has not been investigated in this context. This study uses engineered tissue models to investigate how changes to the stress relaxation of the biomaterial can be used to guide the morphology and reactivity of human primary astrocytes.

Primary human cortical astrocytes were encapsulated within double-network hydrogels with tuneable viscoelastic properties. These hydrogels were composed of a covalent network of gelatin methacryloyl and varying quantities of a reversible covalent network of gelatin-adipic acid dihydrazide and dextran aldehyde[3]. After 7 days of culture, the astrocyte-laden hydrogels were either (a) imaged via confocal fluorescence microscopy following fixation and staining for GFAP, S100b and F-actin or (b) processed for transcriptomic analysis through RNA extraction and RT-qPCR analysis.

By modulating the viscosity of these hydrogels, we were able to access striking astrocyte morphologies that have not previously been observed *in vitro*. Specifically, we observed a morphological transition along the viscosity axis, ranging from large and elongated astrocytes (low-viscosity hydrogels) to small and highly branched astrocytes (high-viscosity hydrogels). The astrocytes cultured in more viscous hydrogels exhibited a reactive-like phenotype, characterised by an increase in average process length, process number and ramification complexity. Furthermore, increasing ECM viscosity also led to significant decreases in the area, perimeter and maximum Feret diameter of nuclei within these astrocytes, correlating with nuclear morphologies previously observed in reactive astrocytes[5]. RT-qPCR analysis revealed that astrocytes cultured in the high viscosity hydrogels maintained their levels of GFAP, S100b, ALDH1L1 and SLC1A2, with a significantly upregulated expression of reactivity markers ICAM1, C3 and IL-6. Importantly, the absence of any inflammatory stimuli in the culture media, suggested that the pro-inflammatory state was guided by the stress relaxation of the biomaterial.

Studies are ongoing to further characterise the transcriptomic, epigenomic, and functional changes in these cultures, but our findings to date highlight the importance of matrix viscosity in modulating the morphology and phenotype of human primary astrocyte cultures. Understanding the mechanistic basis underpinning how astrocytes transduce stress relaxation cues into phenotypic changes will provide novel insights into astrocyte-ECM interactions and may pave the way for improved *in vitro* models (*e.g.*, of the blood-brain barrier) and novel therapies for treating astrocyte-implicated pathologies.


GelMA 3
DN 3 0
DN 3 1
DN 3 2
DN 3 3

C69

Neuroimmune Modulation in Schizophrenia: Investigating the Presence of Altered Microglial Phenotypes and their Response to Antipsychotic Treatment

Catarina Raimundo[1], Amelia Brown[2], Andreea-Ingrid Baloc [3], Anthony Vernon [3]

[1]Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London | Wellcome Trust PhD Training Programme Neuro-Immune Interactions in Health & Disease, United Kingdom , [2]Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London | Centre for Developmental Neurobiology, Institute of Psychiatry, Psychology and Neuroscience, King's College London , United Kingdom , [3]Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, United Kingdom

Background: Microglia are the main resident immune cells of the brain, contributing to the brain's development, protection and homeostasis[1]. Given the versatility of microglia's roles, disruptions in microglia functioning have been implicated in the pathophysiology of schizophrenia[2]. While antipsychotics, the main treatment for schizophrenia, are believed to exert immunomodulatory effects[3,4], their precise impact on microglia remains unknown.

Aims: This study aimed to characterize the molecular and functional profiles of human induced pluripotent stem cell (hiPSC)–derived microglia from schizophrenia patients relative to healthy controls, and to assess how acute antipsychotic exposure modulates these phenotypes.

Methods: hiPSCs from schizophrenia patients (n=4) and healthy controls (n=4) were differentiated into microglia-like cells as previously described[5]. A pilot dose-response study was conducted to aid in the selection of the appropriate antipsychotic treatment dosage, zooming into cellular viability and cytotoxicity. Microglia were exposed to an acute treatment (24 hours) of clozapine, olanzapine and haloperidol (0, 1, 10, 100, 1000nM). Upon dosage selection, at day 14, microglia were treated with clozapine [1uM], haloperidol [50nM], olanzapine [100nM] or vehicle [DMSO] for 24 hours. To investigate differences in motility, hiPSC-derived microglia were labelled, and live imaging (2 hours) was conducted at the Opera Phenix High Content Microscope. To assess differential phagocytic capacity of microglia according to *treatment* and *diagnosis*, phagocytosis of zymosan beads was measured through the BD LSRFortessa flow cytometer. The Proteome Profiler Human Cytokine array was used to evaluate changes in cytokine secretion in pooled media samples. Lastly, to investigate differential transcriptomic signatures, bulk RNA-Sequencing was conducted.

Results: Clozapine treatment did not affect cell viability across the distinct doses (H=2.935, p=0.569) and showed no cytotoxic effects (H=0.9461, p=0.918). The same was observed for haloperidol, with no trace of cytotoxicity (H=0.3614, p=0.9855) nor reduced cellular viability (H=4.124, p=0.3895). Exposure to increasing doses of olanzapine did not exhibit significant cytotoxic effects (H=4.050, p=0.3993), however an effect of olanzapine dosage on cell viability was reported (H=16.00, p=0.0030). *Posthoc* analysis revealed a significant 25% viability decline from baseline relative to 1µM (p=0.0109). Based on the pilot results, microglia were treated for 24 hours with 50nM of haloperidol, 100nM of olanzapine, 1µM of clozapine or vehicle (DMSO) for all subsequent experiments. Antipsychotic treatment and diagnosis did not impact the percentage of phagocytic microglia (*DiagnosisxTreatment*: $F(3,16)=0.0099$, p=0.999, *Diagnosis* $F(1,16) = 0.0196$, p=0.890, *Treatment:* $F(3,16)=0.0295$, p=0.993), nor the amount of phagocytosed particles (*DiagnosisxTreatment*: $F(3,16)=0.0602$, p=0.980, *Diagnosis:* $F(1,16)=3.03\times10^{-5}$, p=0.996, *Treatment:* $F(3,16)=0.0391$, p=0.989). Motility analysis also reported no significant effects of

Treatment or *Diagnosis*. Qualitative assessment of cytokine profiles suggested a heightened SERPINE1 secretion at baseline in the patient population, further enhanced by clozapine and haloperidol treatment. Transcriptomic analysis is currently ongoing.

Conclusion: This study suggests that antipsychotic treatment does not impact functional phenotypes in microglia. The largely absent phenotypic differences between patient-derived samples and controls might indicate that microglia may require interactions with neurons to unmask disease-relevant abnormalities. To test this hypothesis, hiPSC-derived microglia–neuronal co-cultures will be established, employing a matched and mismatch patient-control design, to determine how cellular context shapes microglial behaviour.

C70

Distribution and localisation of the water channel protein AQP4 in organotypic brain slice cultures

Lauren Roberts[1], Benjamin Hewitt[2], Roslyn M. Bill [1], Philip Kitchen[1], Lucas Unger[1], Hannah Botfield[2]

[1]Aston University, United Kingdom, [2]University of Birmingham, United Kingdom

Aquaporins (AQPs) are water channel proteins found throughout the body. In the brain, AQP4 is the most abundant AQP and is highly expressed in astrocytes. Astrocytes regulate brain water homeostasis by relocalising AQP4 from intracellular vesicular pools to the cell surface, with the highest density of AQP4 found at astrocyte endfeet covering cerebral blood vessels. This subcellular relocalisation of AQP4 has a crucial role in the regulation of AQP4 function that is independent of AQP4 expression. The glymphatic system clears waste products and excess fluid from the brain, and glymphatic dysfunction has been implicated in pathological conditions such as Alzheimer's disease and cerebral oedema. Due to the enrichment of AQP4 at astrocyte endfeet and the reduction of glymphatic function in AQP4-/- mice, AQP4 is thought to have a pivotal role in glymphatic function. Recently, organotypic brain slice cultures (OBSCs) have been used to model diverse diseases *in vitro*. Although we cannot model the glymphatic system directly in OBSCs, we can study its cellular and structural elements. However, we do not fully understand the effects of the lack of blood flow and intracranial pressure in OBSCs on AQP4 localisation and function in astrocytes. To investigate this, the distribution and localisation of AQP4 were compared between acute, 3-day and 8-day OBSCs from adult rats cultured at the air-liquid interface using a serum-free media formulation. Immunohistochemistry was performed to visualise and quantify differences in the expression and localisation of AQP4 in astrocytes between acute and cultured OBSCs. We further established that the OBSC model can be used to measure changes in AQP4 localisation in response to treatments that increase the membrane water permeability of cultured primary human astrocytes. Understanding the changes in AQP4 localisation in response to the lack of blood flow in the OBSC model will aid in future research to investigate elements of the glymphatic system and contribute to the development of therapies to promote fluid clearance. Using the OBSC model to measure changes in AQP4 localisation to the astrocyte endfeet following drug treatments could be a useful method to identify potential glymphatic-enhancing drugs for further study *in vivo*.

C71

Microglial phenotypes shape vulnerability and resilience to Early-life adversity

Arthur Rocha-Gomes[1], Jefferson Manoel do Nascimento Silva[1], Valentina Mosienko[2], Hélio Zangrossi Jr[1]

[1]University of São Paulo, Brazil, [2]University of Bristol, United Kingdom

Early-life adversity (ELA) consists of exposure to stressful situations during critical developmental periods and constitutes a major risk factor for poor mental health. Despite this, only around 30% of individuals exposed to ELA go on to develop persistent vulnerability, which often emerges later in life as anxiety disorders [1]. Microglia are immune cells involved in modulating brain maturation and neurogenesis and have been associated with perturbed developmental trajectories and negative mental health outcomes following ELA [2]. Yet, it remains unclear whether vulnerability and resilience to ELA are linked to distinct microglial phenotypes.

Here, we tested the hypothesis that vulnerability trajectories are associated with striking alterations in microglial morphology following exposure to ELA, using a translational ELA mouse model, the Limited Bedding and Nesting (LBN) [3] paradigm. C57BL/6J mice were subjected to LBN between postnatal days 2 and 9 (PND2-9). In adulthood (PND90), we assessed anxiety-like behaviour in male and female offspring using the elevated plus maze, open field, and light-dark box tests, as well as responses to a panicogenic stimulus (20% CO_2) [3]. To stratify individuals as vulnerable or resilient in adulthood, we applied a Z-score approach [4], a statistical method that standardises behavioural outcomes across tests. Microglia morphology was analysed in the hippocampus, a brain region that plays a central role in the regulation of anxiety responses, using Iba-1 immunofluorescence and confocal microscopy [5]. Student's *t*-test was used for statistical analysis, with a significance level set at $p < 0.05$. This study was approved by the Animal Ethics Committee of the University of São Paulo (protocol: 1200/2023).

We observed anxiety- and panic-like responses in the open field ($p < 0.05$) and CO_2 exposure ($p < 0.05$) tests only in ELA-male offspring compared to Control, whereas no differences were detected in the elevated plus maze or light-dark box tests for both sexes. Following Z-score stratification, 29% of male and 17% of female mice were classified as vulnerable after ELA exposure. In the hippocampus, vulnerable ELA males exhibited a 24.9% reduction in microglial perimeter ($p < 0.05$) and a 54.1% increase in soma size ($p < 0.05$), while vulnerable females showed a 62.7% reduction in microglial perimeter ($p < 0.05$), compared with their respective ELA-resilient and Control groups. Notably, correlation analyses revealed a strong association ($R^2 = 0.68$; $p < 0.01$) between higher Z-score indices and reduced microglial perimeter, suggesting that this parameter may be indicative of vulnerability following ELA.

Together, these findings indicate that ELA differentially shapes microglial phenotypes in vulnerable and resilient individuals of both sexes, and that these alterations are associated with increased susceptibility to anxiety responses in adulthood.

C72

Microglia coordinate transition of Abeta pathology to Tau pathology in Alzheimer's disease and contribute to longevity

Andrew Graham[1], Naciye Magusali[1], Umran Yaman[1], Krystel Fernandes[1], Eftychia Bellou[2], Melissa Barber[1], Xinran Hao[1], Carlo Sala Frigerio[1], John Hardy[1], Maryam Shoai[1], Valentina Escott-Price[2], Dervis Salih[1]

[1]UCL, UK, [2]Cardiff University, UK

Introduction:

Human genetics have revealed the importance of microglia in Alzheimer's disease (AD). Recent work has shown that the microglial response to amyloid pathology dictates the transition to Tau pathology, or not. Human genetics for AD has currently identified 75+ risk genes. However, how these risk genes and new genes combine in pathways to coordinate microglial function and how these processes contribute to Tau pathology and resilience to AD are not well understood.

Aim/Objective:

The objective of our studies is to gain new insights into the biological processes which underlie AD and longevity (or resilience to AD) using an unbiased approach to integrate common human genetic variation of human lifespan and AD from GWAS.

Methods:

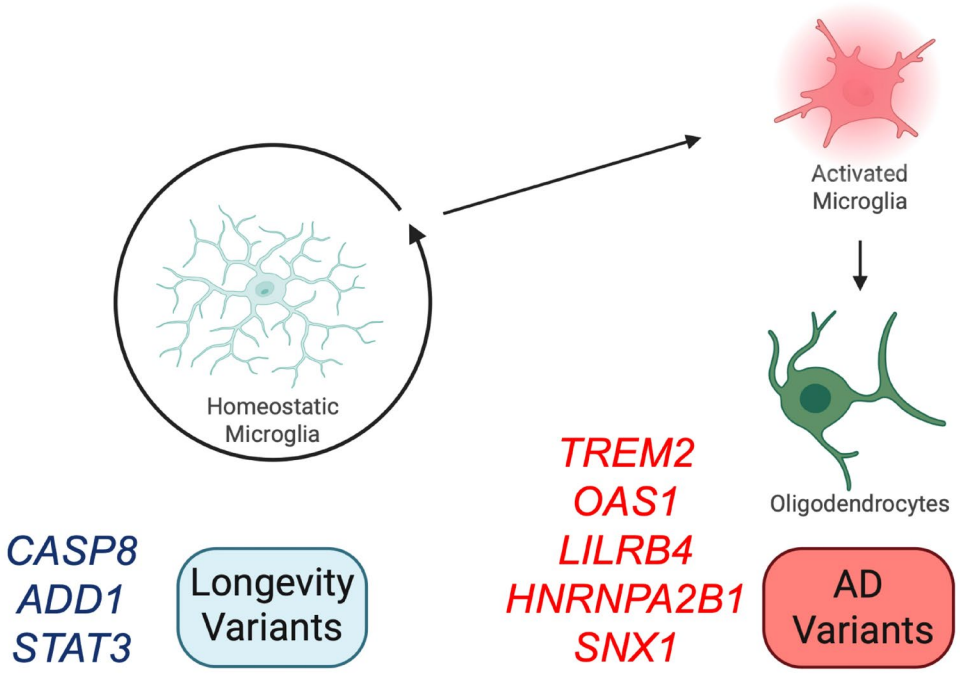
AD GWAS summary statistics data from the IGAP stage 1 GWAS meta-analysis of 21,982 AD cases and 41,944 cognitively normal controls (Kunkle et al. 2019). Three longevity GWASs covering healthspan (300,477 individuals), parental lifespan (1,012,240 parents) and longevity (defined by 11,262 individuals surviving to the 90th percentile of life and 25,483 controls whose age at death corresponds to the 60th survival percentile; Timmers et al. 2020). Bulk hippocampal RNA-sequencing (RNA-seq) datasets were generated from wild-type C57BL/6J mice, amyloid mice (*APP*K670N/M671L and *PSEN* M146V mutant lines) and tau mice (*MAPT* P301L) of ages 2-, 4-, 8- and 18-months-old (n = 2-7 mice per genotype per age group; data available from Mouseac.org; Salih et al. 2019), and non-diseased human hippocampi (from 196 individuals from the Genotype-Tissue Expression (GTEx) Project v8; gtexportal.org/home/datasets; GTEx et al. 2015). Single-cell hippocampal RNA-seq of microglia isolated from wild-type mice of ages 3-, 6-, 12- and 21-months-old (n = 2 mice per sex/age, pooled; Sala Frigerio et al. 2020). A two-step approach was used to identify biological pathways dysregulated with age and that are enriched for genes associated with AD risk or longevity. First, common human genetic variation associated with AD or ageing at the gene-based level were identified by calculating the joint summary of all SNPs in the gene. Second, assessment of statistical enrichment of associated AD and longevity genes in age- and AD-related gene co-expression networks, generated from the transcriptomic profile of the human and mouse hippocampus (Graham et al. 2025).

Results:

Our work reveals that genetic variation associated with AD was enriched in both human microglia (P = 1e-5, enrichment bootstrap-based test) and oligodendrocytes (P = 0.033, enrichment bootstrap-based test), which show the strongest increases in gene expression with ageing, and contribute to Tau pathology. Whereas, longevity-associated genetic variation was enriched in homeostatic microglia (P = 0.032, enrichment bootstrap-based test), which may drive "inflammageing." Thus, we observe that variants contributing to resilience, AD and Tau pathology balance different aspects of microglial function.

Conclusions:

Our findings have important implications for developing markers indicating the physiological age of the brain and Tau pathology, alongside better diagnosis of disease and new targets for therapeutic intervention.



C73

Aquaporin-1 (AQP1) is associated with mTOR hyperactivity in tuberous sclerosis complex (TSC)

Hanya Sandel [1], Philip Kitchen [1]

[1]Aston University , England

Aquaporin-1 (AQP1) is associated with mTOR hyperactivity in tuberous sclerosis complex (TSC)

Hanya Sandel, Roslyn Bill, Philip Kitchen

Aston University, Birmingham, United Kingdom

Tuberous sclerosis complex (TSC) is a rare multisystem genetic disorder caused by mutations in the *TSC1* and/or *TSC2* genes, which lead to hyperactivity of the mammalian target of rapamycin (mTOR) pathway. Manifestations of TSC include tumour-like lesions in the brain (called tubers) that lead to 90% of patients developing epilepsy. When uncontrolled, the epilepsy can lead to sudden unexpected death in epilepsy (SUDEP), which is the main cause of death for TSC patients. One treatment option for TSC is the use of mTOR inhibitors, such as everolimus. However, while everolimus is effective for halting tumour growth in TSC, efficacy for controlling epilepsy remains variable.

Aquaporins (AQPs) are a family of water channel proteins. 13 human AQPs have been identified (AQP0-AQP12) so far, but little is known about the expression of AQPs in TSC and upon hyperactivity of the mTOR pathway. We have found significant dysregulation of five different AQP genes from RNA-sequencing in tuber samples. Among these, we observe a > 300-fold increase in expression of AQP1. We validated that AQP1 protein expression was also increased in mouse embryonic fibroblasts (MEF) from both TSC1-/- and TSC2-/- mice relative to control. Finally, we have shown that 24-hour treatment with everolimus in the TSC-/- MEF reversed the dysregulation of AQP1 expression. Interestingly, our data also show a significant loss of AQP1 glycosylation in TSC1-/- but not TSC2-/- cells, suggesting a novel role for TSC1 in the regulation of protein glycosylation. Thus, our data indicate a link between mTOR pathway hyperactivity and the regulation of AQP1, with potential implications for AQP1 in the pathogenesis of TSC. Future research will explore dysregulation of AQP1 in TSC resected brain samples from tubers to offer a deeper insight into aquaporin dysregulation in TSC.

C74

What Happens to Oligodendroctyes That Survive Myelin Damage?

Tobias Shaw-McGrath[1], Katy L.H. Marshall-Phelps[1], Luise Seeker[2], Julia Van De Korput[1], Daniel Soong[1], Anna Williams[1], David Lyons[1]

[1]The University of Edinburgh, United Kingdom, [2]Stanford University, United States of America

Loss of central nervous system myelin is a hallmark of debilitating neurodegenerative diseases such as multiple sclerosis and Alzheimer's disease. Myelin is required for neuronal homeostasis, without which progressive neurological dysfunction occurs. Oligodendrocytes generate myelin by wrapping their processes around axons in the central nervous system. During neurodegenerative diseases, oligodendrocytes sustain damage and lose their myelin, dubbed demyelination. The loss of myelin was thought to be fatal for oligodendrocytes, and the generation of new myelin was thought to require newly differentiated oligodendrocytes. Recently, however, oligodendrocytes have been observed to survive damage and even make new myelin thereafter, albeit with reduced efficacy. However, the mechanisms behind this survival and the factors influencing their remyelination capacity remain unclear. This project aims to identify biomarkers of surviving oligodendrocytes, assess the role of surviving oligodendrocytes in remyelination, and explore therapeutic strategies to enhance their functionality.

I will utilize zebrafish models of myelin damage and human multiple sclerosis tissue to identify biomarkers and pathways that may influence the fate of oligodendrocytes surviving demyelination. Through following individual oligodendrocytes in zebrafish and using the HaloTag dyeable fluorescence system to pulse-chase oligodendrocytes, I will characterise the role of surviving oligodendrocytes in vivo. Furthermore, using CRISPR and pharmacological approaches, I will manipulate surviving oligodendrocytes, aiming to improve their remyelination capacity. By leveraging innovative imaging tools and cross-species analyses, this research has the potential to transform our understanding of oligodendrocytes in disease and contribute to novel therapeutics.

C75

Astrocytic cell plasticity promotes glioblastoma progression and immune evasion

Mathew Clement[1], Alex Gibbs[2], Ayesha Begum[1], Vasileois Eftychidis[1], Suresh Kaushik[1], Florian Siebzehnrubl[1]

[1]Cardiff University, United Kingdom, [2]Cardiff University and Wales Cancer Research Centre, United Kingdom

Glioblastoma (GBM) is the most frequent and most lethal type of brain cancer in adults, with poor survival rates of 15-20 months with therapy. The crosstalk between GBM and host cells in the tumour microenvironment (TME) promotes tumour progression, heterogeneity, and therapy resistance that together exacerbates poor survival. We have previously shown that host astrocytes can positively or negatively affect brain tumour invasion into the surrounding parenchyma, depending on the status of astroglial reactivity.

Here, we investigate cell-cell interactions between GBM and host astrocytes in patient-derived orthotopic xenografts and syngeneic in vivo models. We find that GBM-astrocyte crosstalk promotes the expression of the cell-plasticity associated transcription factor, ZEB1, both in GBM and in host astrocytes. We have previously demonstrated that ZEB1 is a key regulator of GBM cancer stem cells and mediates tumour invasion and therapy resistance. ZEB1 is expressed in neurogenic stem/progenitor cells in the developing and adult brain, where it promotes self-renewal. The functions of ZEB1 in astrocytes remain unclear.

Using a conditional-inducible transgenic mouse model for deletion of Zeb1 in murine astrocytes we find that loss of host astrocyte plasticity restricts tumour invasion and increases animal survival remarkably. Single cell transcriptional profiling of tumour-bearing control and Zeb1-deleted mice identifies key alterations of astrocyte states as well as immune cell activation which contribute to GBM tumour progression. Additionally, we identify ligand-receptor candidates that mediate crosstalk between astrocytes, GBM and immune cells and which may constitute therapeutic targets for reprogramming the host TME to reduce GBM progression.

Our findings demonstrate that host astrocytes in the TME are powerful regulators of GBM growth and that reprogramming host astrocytes can block brain tumour progression.

C76

How does paranodal adhesion mediate myelin formation and repair ?

Sophie Siems[1], Phoebe Lyster-Binns [1], Rafael Almeida [1]

[1]University of Edinburgh, INCR , United Kingdom

Accurate myelination of vertebrate neuronal circuits is crucial for their function, as the exact amount and distribution of myelin ensheathing an axon influences the timing and energy efficiency of neurotransmission. This is especially evident in multiple sclerosis (MS), where myelin regeneration after damage often produces thinner, shorter, or misdirected sheaths that fail to support normal nerve function. However, the molecular mechanisms that ensure accurate myelin growth and repair to maintain optimal conduction remain poorly understood. Recent studies suggest that adhesion proteins at the axon-myelin interface regulate myelin growth and targeting, as their genetic manipulation mistargets myelin to neuron somas and reduces sheath length, similar to MS defects. However, how individual adhesion proteins are dynamically regulated along axons to ensure accurate myelination is unknown. To address this, we developed a reporter knock-in zebrafish for Nfascb (Nfascb-EGFP), a critical myelin adhesion protein. This model allows us to visualize endogenous Nfascb in real time at single-cell resolution and revealed that Nfascb clusters specifically at the edges of mature myelin sheaths, suggesting a role in sheath growth and stabilization after formation. In parallel, we established a zebrafish line that enables proximity labeling of GFP-tagged proteins in oligodendrocytes. Combined with our Nfascb-GFP line, this approach allows us to identify molecular interactors of Nfascb that may serve as key regulators of adhesion formation, and thereby support accurate myelin growth and repair.

C77

Stargazing: How are Astrocytes Made? Role of Zeb1 in Transcriptional Regulation of Astrocyte Specification across Developement and Adulthood

Niharika Singh[1], Florian A. Siebzehnrubl[1], Isabel Martinez-Garay[2], You Zhou[1]

[1]Cardiff University, United Kingdom, [2]Universidad de Valencia, Spain

Astrocytes are integral to neural function, yet the transcription factors regulating their specification, maturation and maintenance remain under-characterised. Conditional knockout of Zinc-finger E-box binding homeobox 1 (*Zeb1)* in neural progenitor cells of the adult hippocampus impaired *de novo* astrocyte generation, driving fate commitment towards the neurogenic fate. This placed *Zeb1* as a potential candidate in the transcriptional regulation of astrogliogenesis. While *Zeb1* is expressed in all adult astrocytes, its specific targets and functional roles are unknown. Whether these roles are globally homogenous or regionally specified to suit the microcircuitry are also not known. Combining bulk RNA-Sequencing and morphometric reconstruction, we investigated *Zeb1* function in an inducible, astrocyte-specific knockout mouse model (*GLAST::CreER*^{T2}, Rosa26-tdTomato, Zeb1 $^{flox/flox}$).

Transcriptomic profiling of FACS-purified astrocytes across five brain regions revealed significant differential gene expression in the cortex, hippocampus, and striatum, suggesting Zeb1 exerts context-dependent regulatory control. Long-term morphological assessment (8 weeks to 6 months post-induction) demonstrated that Zeb1 deletion reduces astrocytic density in the cortex and thalamus. Furthermore, knockout astrocytes exhibited a progressive, "shrunken" phenotype with significantly diminished arborisation and breached tiling.

These findings identify Zeb1 as a potential critical regulator of astrocyte structural integrity and heterogeneity. Uncovering these region-specific pathways holds potential to provide a crucial foundation for understanding the selective vulnerability of neural circuits in pathology.

All animal work and procedures were approved by the United Kingdom Home Office Regulations under the Animal Scientific Procedures Act (ASPA) 1986. The permitted work was carried out on an AB & C personal license under a registered project license at Cardiff University.

C78

Non-Canonical Hedgehog Signalling Enhances Astrocytic Insulin Sensitivity and Mitochondrial Dynamics

Holly Smith [1], Natalia Riobo-Del Galdo[1], Beatrice Filippi[1]

[1]University of Leeds, United Kingdom

Introduction

Insulin resistance is a hallmark of type 2 diabetes, affecting over 4 million people in the UK[1].While insulin signalling is classically associated with peripheral glucose homeostasis, insulin receptors are highly expressed in the brain, including in astrocytes, which integrate metabolic and hormonal cues to regulate whole-body homeostasis. Notably, short-term high-fat diet exposure induces mitochondrial fragmentation in astrocytes, which is sufficient to cause central insulin resistance[2]. However, the molecular pathways regulating astrocytic insulin sensitivity remain poorly defined.

Hedgehog signalling is best known for its role in development and oncogenesis but also regulates metabolism in peripheral tissues and the central nervous system. In addition to canonical Gli-mediated transcription, hedgehog signalling is involved in rapid non-canonical pathways. [BF1] Pharmacological hedgehog inhibitors enhance glucose uptake in mice[3] and hedgehog signalling has been implicated in regulating mitochondrial dynamics in the brain[4]. Whether hedgehog signalling modulates insulin action in astrocytes remains unknown.

This study aimed to determine whether either canonical or non-canonical hedgehog signalling regulates astrocytic insulin sensitivity and mitochondrial dynamics.

Methods

DITNC1 rat astrocytes were treated chronically (30 hours) with the Smoothened inhibitor Vismodegib (100 nM) or the Gli inhibitor Glabrescione B (10 μM), or acutely with Vismodegib (100 nM, 1 hour), followed by insulin stimulation (100 nM, 30 minutes) after serum starvation. Insulin resistance was induced using palmitate (200 μM, 24 hours) prior to acute Vismodegib treatment. Insulin signalling was assessed by western blotting for phosphorylated AKT and inhibitory phosphorylation of Dynamin-related protein 1 (Drp1). Mitochondrial morphology was quantified following chronic Vismodegib treatment using Mitotracker Red staining and confocal microscopy with Airyscan, assessing aspect ratio and form factor. Statistical analyses used t-tests or one-way ANOVAs with Tukey post-hoc tests ($p<0.05$).

Results

Chronic Smoothened inhibition with Vismodegib significantly enhanced insulin signalling, producing a 5.5-fold increase in insulin-stimulated AKT phosphorylation compared to the control, insulin treated, cells ($p=0.0006$, $n=9$, 1A). In contrast, chronic inhibition of canonical hedgehog signalling using the Gli inhibitor Glabrescione B failed to increase insulin-stimulated AKT phosphorylation ($p=0.5633$, $n=3$, 1B), indicating a non-canonical mechanism.

Consistent with this, acute Vismodegib treatment (1 hour), a duration insufficient to alter Gli1 expression as assessed by RT-PCR, significantly increased insulin-stimulated AKT phosphorylation (2-fold increase, p=0.0164, n=6, 1C) when compared with control-insulin treated cells. In a palmitate-induced insulin resistance model, acute Vismodegib robustly rescued insulin signalling, resulting in an 18.8-fold increase in insulin-stimulated AKT phosphorylation compared to palmitate-treated controls (p<0.0001, n=6, 1D).

Chronic Vismodegib treatment also increased inhibitory phosphorylation of the mitochondrial fission protein Drp1 (1.6-fold increase, p=0.0237, n=9, 1E). Mitochondrial morphology analysis revealed increased elongation, reflected by an increase in aspect ratio (3% increase, p=0.0095, n=6, 1F) and a reduction in form factor (2.21% decrease, p=0.0146, n=6, 1G).

Conclusion

Non-canonical hedgehog signalling enhances astrocytic insulin sensitivity and modulates mitochondrial dynamics independently of Gli-mediated transcription. Acute Smoothened inhibition is sufficient to rescue insulin resistance, while chronic modulation promotes mitochondrial elongation. Together, these findings position hedgehog signalling as a previously unrecognised regulator of astrocytic insulin action, with potential to improve insulin sensitivity in insulin-resistant brain states, including diabetes, obesity, and neurodegeneration.

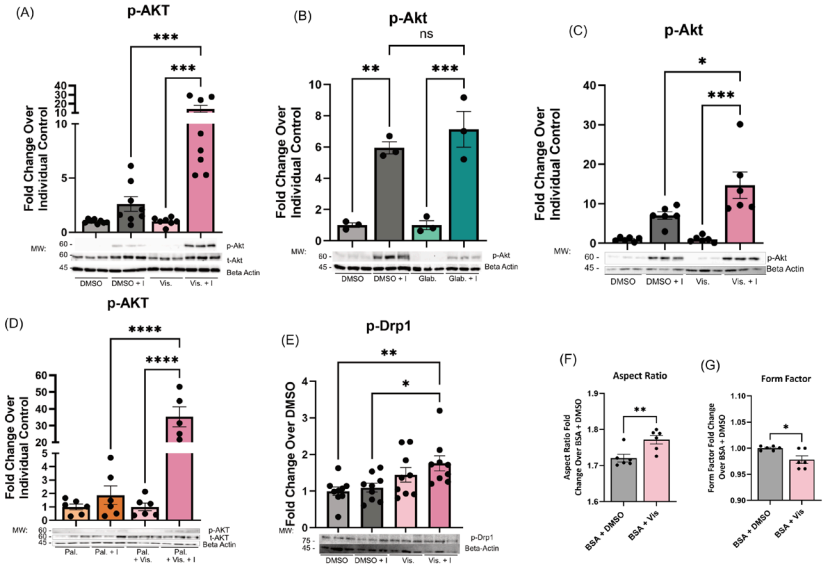


Figure 1: Non-canonical hedgehog inhibition enhances astrocytic insulin signalling and mitochondrial dynamics. (A) Chronic Smoothened inhibition with Vismodegib (Vis, 100nm, 30 h) significantly increases insulin-stimulated AKT phosphorylation in DITNC1 astrocytes following 30 minutes of insulin treatment (100nM), compared to vehicle-treated controls. (B) Chronic inhibition of canonical hedgehog signalling using the Gli inhibitor Glabrescione B (Glab,10μM, 30 h) did not enhance insulin stimulated AKT phosphorylation, indicating a Gli-independent mechanism. (C) Acute Vismodegib treatment (100nM, 1h) significantly increases insulin-stimulated AKT phosphorylation. (D) In a palmitate-induced insulin resistance model (200μM palmitate, 24h), acute Vismodegib treatment restored insulin-stimulated AKT phosphorylation. (E) Chronic Vismodegib treatment increased inhibitory phosphorylation of the mitochondrial fission protein Drp1. (F-G). Mitochondrial morphology analysis following chronic Vismodegib treatment revealed increased mitochondrial elongation, reflected by an increase in aspect ratio (F) and a decrease in form factor (G). For all western blot analysis, phosphorylated protein levels were normalised to total protein and β-actin, and expressed as fold change relative to individual vehicle-treated controls. Data are shown as mean ± SEM with individual data points representing biological replicates (n=3-9). Statistical significance was determined using t-tests or one-way ANOVA with Tukey post-hoc tests. *p<0.05, **p<0.01,***p<0.001, ****p<0.0001, ns = not significant.

C79

Peri-synaptic astrocytic remodelling drives region-specific circuit vulnerability in Alzheimer's disease mouse models via MFG-E8-dependent microglia crosstalk

Dimitra Sokolova[1], Francesca Puletti[1], Shari Addington Ghansah[1], Yongjing Zheng[1], Tatiana Georgiades[1], Sebastiaan De Schepper[1], Jemima Burden[2], Jenny Lange[1], Ling Wu[3], Bethany Geary[4], Annerieke Sierksma[5], Laís S. S. Ferreira[1], Gerard Crowley[1], Javier Rueda-Carrasco[6], Angeliki Koutsiouroumpa[1], Sara Gutierrez Pelaz[3], Philip Muckett[1], Raja Nirujogi[4], Mathieu Bourdenx[1], Barbara Caldarone[7], Joseph Kittler[8], Matthew Gentry[9], Oliver J. Freeman [10], Baljit S. Khakh[3], Soyon Hong[1]

[1]Dementia Research Institute, University College London, United Kingdom, [2]Laboratory for Molecular Cell Biology, University College London, United Kingdom, [3]David Geffen School of Medicine, University of California, United States of America, [4]Medical Research Council (MRC) Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, United Kingdom, [5]VIB Center for Brain & Disease Research, Leuven, Belgium, [6]UK Dementia Research Institute, University College London, United Kingdom, [7]Harvard NeuroDiscovery Center, Brigham and Women's Hospital and Harvard Medical Schoo, United States of America, [8]Department of Neuroscience, Physiology and Pharmacology, University College London, United Kingdom, [9]Department of Biochemistry & Molecular Biology, College of Medicine, University of Florida, United States of America, [10]Neuroscience BioPharmaceuticals R&D, AstraZeneca, United Kingdom

Introduction: Region-specific synapse loss is an early hallmark of Alzheimer's disease (AD) that precedes neuronal loss and amyloid-β (Aβ) plaque deposition. Microglia mediate synapse elimination in AD, however the mechanisms that confer synapse vulnerability in specific circuits remain unclear. Astrocytes regulate synapse homeostasis through peri-synaptic processes, but their contribution to early circuit AD pathology is poorly understood.

Objective: We sought to determine whether astrocytes shape local synapse vulnerability during early amyloidosis and to define the molecular mechanisms underlying astrocyte-microglia crosstalk (1).

Methods: Using high-resolution Airyscan imaging, correlative light-electron microscopy (CLEM), *in vitro* microglia-synaptosome engulfment assays with astrocyte conditioned media, *in vivo* astrocyte-specific viral manipulations and behavioural assays, we characterised astrocyte phenotypes and synapse dynamics in the slow-progressing Aβ hAPP NL-F knock-in (KI) model and the more aggressive Aβ hAPP NL-G-F KI model. Gain- and loss-of-function approaches included viral astrocyte-specific MFG-E8 overexpression and viral CRISPR-Cas9 deletion, as well as microglia-specific integrin-β3 deletion using the tamoxifen-inducible Cx3cr1-CreERT2 model.

Results: Using tdTomato labelling and CLEM, we re-discovered a distinct region-specific population of astrocytes with terminal glycogen- and p62-enriched bulbous peri-synaptic processes that emerges at 6 months in the NL-F KI (N=5 per genotype), prior to plaque deposition and gliosis, when synapses are lost. Bulbous astrocytes accumulated in circuits vulnerable to AD pathology like the hippocampus (N=5 mice). Glycogen- and p62-enriched astrocytic structures were detected in human AD tissue within

vulnerable regions (N=12 control and AD cases), supporting translational relevance. Functionally, bulbous astrocytes exhibited reduced peri-synaptic coverage (N=5-7 mice), impaired baseline and phenylephrine evoked GCaMP Ca^{2+} signalling (N=3 mice), and were associated with localised increases in microglial Homer1^{+} synapse engulfment (N=5 mice) and excitatory (Homer1+/Bassoon) synapse loss (N=6 mice). Bulbous processes were enriched for the phagocytic ligand milk fat globule-EGF factor 8 (MFG-E8) (N=5 mice), which interacts with integrin receptors to facilitate engulfment. Astrocyte-specific MFG-E8 overexpression in wild-type mice induced microglial Homer1 engulfment (N=6 mice) followed by excitatory synapse loss and increased bulbous astrocyte abundance (N=4-5 mice). Microglia-specific integrin β3 deletion abolished MFG-E8-driven Homer1 engulfment *in vivo* (N=4-5 mice per group), confirming integrin-dependent signalling. Conversely, CRISPR-Cas9 astrocytic *Mfge8* deletion normalised microglial Homer1 engulfment and excitatory synapse density in the NL-F KI (N=4-6 mice per group) model and reduced plaque burden, dystrophic neurites, gliosis, contextual fear deficits, and bulbous astrocyte accumulation in the NL-G-F KI (N=7-12 mice per group).

Conclusions: These findings identify a previously unrecognised astrocyte-driven mechanism that establishes local synapse vulnerability in early AD through MFG-E8-integrin-dependent astrocyte-microglia signalling, positioning astrocytes as upstream regulators of circuit-specific synapse loss (2).

Statistical analysis: Analyses were performed using animal averages, with appropriate parametric or nonparametric tests based on normality testing, correction for multiple comparisons, and significance set at $\alpha = 0.05$ (mean +/- S.E.M). Both male and female mice were used.

Ethics: All animal experiments were performed in accordance with the UK Animal Scientific Procedures Act 1986 and approved by the UK Home Office with institutional veterinary oversight at University College London. Human post-mortem tissue was obtained from the Newcastle Brain Tissue Resource with informed consent and appropriate ethical and material transfer approvals.

C80

A phenotype-stratified approach to study astrocytic metabolic pathways in a mouse model of chronic stress

Marc Ten-Blanco[1], Arthur Rocha-Gomes[1], Kratika Mujmer[1], Valentina Mosienko[1]

[1]School of Psychology and Neuroscience, University of Bristol, Bristol, BS8 1TD, United Kingdom

Chronic stress is a major risk factor in the aetiology of several psychiatric disorders, including depression, which affects around 5.7% of the adult population worldwide -around 332 million people- according to the World Health Organization. Despite the availability of pharmacological treatments, therapeutic efficacy remains limited. Nearly two thirds of patients fail to respond adequately to the first antidepressant prescribed, and among these, approximately 30% are considered treatment-resistant, showing insufficient response to two or more antidepressant trials. Given the marked heterogeneity in clinical manifestations and treatment responses, multiple components of its pathophysiology have been extensively studied in an effort to improve disease management. In this context, recent studies highlight the key role of astrocytes in stress-related disorders. Beyond providing metabolic support to neurons, astrocytes actively regulate neuronal function by maintaining neurotransmitter homeostasis, for example through the clearance of excess glutamate -known to be aberrantly elevated in depression- via specific transporters. In addition, the astrocyte–neuron lactate shuttle supplies lactate as an energy substrate to active neurons, while it can also act as a signalling molecule with reported antidepressant-like effects. Consistent with this, postmortem studies of patients with depression report reduced astrocyte density and decreased expression of astrocytic markers, suggesting that astrocyte dysfunction is implicated in disease pathology. However, the contribution of astrocytic metabolic impairment to stress-induced behavioural phenotypes remains incompletely understood.

The aim of this study is to evaluate the effects of chronic mild stress on astrocytic metabolic pathways. To this end, we employed a 10-week Unpredictable Chronic Mild Stress (UCMS) protocol consisting of seven mild stressors applied once a day for 2 hours in random order and at variable times. Control (n=8) and UCMS-exposed (n=12) C57BL/6J male mice were phenotyped longitudinally using body weight monitoring, nest building assessment, sucrose preference and elevated plus maze tests. All animal procedures were performed according to the UK Animal Scientific Procedures Act 1986, and the Animal Welfare and Ethics Review Board, University of Bristol (PPL PP5495972). Interestingly, UCMS-exposed animals showed a significant reduction in nest building behaviour (-23%) and body weight gain (-24%) compared to controls. However, rather than focusing on group-level behaviour, we integrated behavioural and physiological measures across tests by calculating a global z-score for each animal. This approach enabled the identification of extreme phenotypes, from which 4 mice of each group displaying the most representative control or UCMS-exposed profiles were selected for further molecular analyses. Blood and limbic brain regions were collected 24 hours after the end of the UCMS paradigm for corticosterone quantification and bulk RNA sequencing, with subsequent identification of astrocyte-enriched transcripts.

Our experimental approach combines behavioural phenotyping–based animal selection with transcriptomic profiling to identify stress-related transcriptional alterations in astrocytic metabolic pathways, with particular emphasis on genes previously reported to be involved in astrocyte metabolism and stress response. Together, this work seeks to refine the relationship between chronic stress, behavioural phenotype expression, and astrocyte metabolic dysfunction, thus providing insights into glial contributions to stress-related neuropsychiatric disorders.

C81

Adenosine signalling mediates metabolic communication between neurons and astrocytes

Shefeeq Theparambil [1], Olga Kopach[2]

[1]Lancaster university , United Kingdom , [2]Insititute of Neurology, UCL, United Kingdom

Increases in brain neuronal activity require the delivery of more metabolic substrates to support higher energy usage. Lactate contributes to the extracellular pool of readily available energy substrates, and its local concentration rapidly increases in response to neuronal activation. The 'astrocyte-to-neuron lactate shuttle' hypothesis proposes that neuronal activity is fueled by lactate provided by neighboring astrocytes. However, it is not entirely clear how astrocytes sense neuronal activity, and which extra- and intracellular signalling pathways are recruited to mobilize astrocytic glycogen stores, increase the glycolytic rate, and release lactate 'on demand', i.e. in a neuronal activity-dependent manner.

There is evidence that neuronal activation is associated with the release of purines: ATP and adenosine. Using genetically encoded fluorescent cAMP and PKA sensors, Epac and AKAR4, we recorded robust increases in intracellular [cAMP] and [PKA] in astrocytes in response to ATP and adenosine, leading to activation of glycogenolysis, increased glycolytic rate, and facilitated release of lactate. Robust activity-dependent [cAMP] increases in hippocampal and cortical astrocytes were recorded in brain slices. Pharmacological or genetic inhibition of this signalling pathway in astrocytes reduced the lactate release and impaired transmission in the CA3-CA1 synaptic pathway. Collectively our data suggest that metabolic signaling between neurons and astrocytes is mediated by the release and actions of ATP and adenosine. Purinergic signalling appears to play an important role in the mechanisms underlying the activity-dependent supply of chemical energy substrates to support the metabolic needs of brain neurons processing information.

C82

Epigenetic Regulation of Microglial Gene Expression in Alzheimer's Disease

Sophie Thomas[1], Sophie Shaw[1], Uroosa Chughtai[1], Joe Burrage[2], Emma Dempster[2], Owen Peters[1]

[1]Cardiff University/UKDRI, United Kingdom, [2]University of Exeter, United Kingdom

Alzheimer's disease (AD) is a neurodegenerative condition that is the most common cause of dementia in the UK. Key pathological hallmarks of AD include the extracellular accumulation of Amyloid-β (Aβ) plaques, the intracellular deposition of tau neurofibrillary tangles, and an increase in neuroinflammation which together contribute to cognitive decline. Genome wide association studies have identified numerous AD risk genes that are associated with microglial function. However, regulation of gene expression is not only influenced by the underlying DNA sequence but also by epigenetic regulation of temporal and spatial transcription. To date, our knowledge of how epigenetic regulation of microglia may contribute to AD onset and progression is limited. Previous studies have identified differentially methylated microglial genes in post-mortem human brains with advanced AD pathology[1]. However, epigenetic changes associated with the early stages of disease are yet to be fully explored.

In this study, we aimed to define the DNA methylation profile of human microglial genes associated with extracellular Aβ oligomer exposure and triage differentially methylated genes of interest.

Human iPSC derived differentiated microglia[2,3] (KOLF 2.1J; N= 4 independent differentiations) were stimulated with either LPS/IFN-γ or oligomer enriched synthetic Aβ 1-42 for 24 hours and compared to an untreated control group. Microglia were then subjected to quantitative genome wide profiling for differential methylation using the Infinium Methylation EPIC BeadChip Platform (850k CpG sites). Differentially methylated sites between the experimental conditions were identified using a previously established analysis pipeline, and candidate genes triaged for further functional investigation. Ongoing work will assess the impact of mis-expressing these genes on AD relevant microglial functions.

Profiling of the human microglial methylation landscape in response to disease relevant extracellular challenges will expand our understanding of how epigenetic regulation drives transcriptional changes in AD, particularly in the early stages of disease. Better defined early epigenetic responses to Aβ may contribute to an improved understanding of disease onset and progression and offer novel targets for therapeutic intervention.

C83

Analysis of Glial Cell Morphology and APOE Sequence Diversity in Mammalian Models of Age-Related Neurodegeneration

Meg Watt[1], Fiona Houston[1], Danielle Gunn-Moore[2], Alexandra Malbon[2], Sophie Scrimgeour[2]

[1]The Roslin Institute, The University of Edinburgh, United Kingdom, [2]The University of Edinburgh, United Kingdom

Age-related neurodegenerative changes in spontaneous mammalian models provide insights into diseases like Alzheimer's disease (AD), which is characterised by amyloid-beta plaques, neurofibrillary tangles (tau pathology), and neuroinflammation. We explored age-related morphological changes in astrocytes and microglia in cat and sheep brains, which can develop AD-like neuropathology. We also investigated comparative sequences of *APOE* (a major risk factor for human AD) homologues in these species, to identify variants associated with increased neuropathology and/or cognitive dysfunction.

Transverse sections of brain regions were cut from formalin fixed, paraffin embedded blocks from cats and sheep of various ages, including cats diagnosed with cognitive dysfunction syndrome (CDS). Age groups for sheep were young (2-4 years, *n*=8), middle-aged (6-7 years, *n*=8) and old (11-12 years, *n*=7). Cats were divided into young (2-6 years, *n*=5), aged (14-20 years, *n*=7) and an older group of cats diagnosed with CDS (16-19 years *n*=5). Sections were labelled using antibodies for glial cell markers (Iba1 for microglia; GFAP for astrocytes) using standard immunohistochemical techniques. For cats, images of grey and white matter were captured from the parietal and rostral cortex, and the dentate gyrus. In sheep, analysis was performed in the frontal cortex, parietal cortex, temporal cortex and dentate gyrus. ImageJ macros were used to determine cell size and density. Analysis of microglia process length and number was also performed. Statistical analysis was performed in R studio using the lme4 and lmerTest packages to allow for Linear Mixed-effect Models and Generalised Linear Mixed-effect Models to be fitted. A region of the *APOE* gene, including exons 4 and 5, was amplified by PCR from genomic DNA of 24 Cheviot sheep included in histopathology analyses, and sequenced by Sanger sequencing. In addition, genomic and cDNA sequences of *APOE* from 18 different sheep breeds were retrieved from annotated genomes available in Ensembl rapid, and from whole genome sequences of 46 Scottish Blackface sheep. Multiple sequence alignments were performed on Geneious Prime to analyse *APOE*.

In sheep, astrocyte density increased with age in grey matter cortical areas and the dentate gyrus. Tau tangles were associated with higher astrocyte density in specific cortical areas. In older cats with CDS (16-19 years) decreased microglial branch length (indicating activation) was observed in the parietal and rostral cortex compared to younger cats (2-6 years). Older cats with both amyloid-beta and tau pathology had higher microglial density than those with only amyloid-beta plaques or no identified pathologies in parietal cortex white matter. These glial changes may indicate cell activation and/or neuroinflammation, contributing to neuropathology similar to human AD. Analysis of *APOE* in the sheep breeds revealed multiple variants, and demonstrated all sheep encode identical amino acids at positions 112 and 158 to those that define the human *APOE* ε4 allele.

This research offers approaches for comparative investigations to understand mechanisms underlying neurodegenerative disease. Future investigations should explore these age-related and pathology-related changes to astrocytes and microglial cells using techniques that could target pathways of glial reactivity to reveal mechanisms of senescence, and provide insight into the glial cell phenotypes.

C84

Using Drosophila melanogaster to investigate glial lipid droplet biology in Alzheimer's disease

Bea Westerman-Loe[1], Leonardo Amadio[1], Natalie Connor-Robson[1], Gaynor Smith[1], Owen M. Peters[1]

[1]Cardiff University/ UK DRI, United Kingdom

Alzheimer's disease (AD) is the most common form of dementia, with late-onset AD (LOAD) accounting for approximately 95% of all cases. Evidence from genome-wide association and epidemiological studies has implicated disrupted lipid homeostasis in the progression of LOAD[1,2]. Lipid droplets are dynamic organelles which store neutral lipids to regulate lipid metabolism and energy homeostasis. Dysfunctional lipid droplet production, dynamics and composition may contribute to AD pathology, with alterations in microglial lipid droplets observed in response to amyloid accumulation[3]. Although they have been implicated in contributing to neurodegeneration, the precise mechanistic relationship between AD pathology and disrupted lipid droplet biology is not yet fully understood.

We have used the fruit fly *Drosophila* melanogaster to explore the relationship between glial lipid droplets and AD pathology. *Drosophila* are a powerful invertebrate model for studying AD, with numerous conserved LOAD-associated genes and well-defined neurodegenerative phenotypes. Importantly, their nervous system is complex, containing neuron and glial subtypes with orthologous functions to mammalian counterparts and well conserved lipid droplet-associated cellular mechanisms. We aimed to investigate how specific AD-associated genes contribute to dysfunctional glial lipid droplet biology, and conversely, how altered glial lipid droplet dynamics influence neurodegenerative pathology.

Previous *Drosophila* research has elucidated fundamental aspects of glial lipid droplet biology, including a neuroprotective mechanism whereby glial cells uptake peroxidated neuronal lipids and sequester them within lipid droplets[4]. Building on this work, through confocal imaging of a nerve in the adult *Drosophila* wing, our innovative approach allowed us to investigate changes in glial lipid droplet dynamics in response to i) genetic changes and ii) Wallerian degeneration of neurons. By using restricted expression of genetically encoded fluorescent markers, we were able to quantify changes in the size and abundance of glial lipid droplets. This rapid, medium-throughput approach allowed us to screen lipid droplet phenotypes associated with dozens of AD and lipid associated genes (n = 40, groups containing male and female flies). Using this system, we have made proof of principle measurements demonstrating that disruption of key lipid droplet machinery, including knockdown of *Drosophila* genes such as Seipin (*BSCL2*) and Lsd-2 (*PLIN2*), cause perturbed lipid droplet phenotypes. We are currently investigating how manipulating AD risk gene expression, and neuronal expression of amyloid, impacts on glial lipid droplet dynamics.

In ongoing work, we will test how the mechanisms identified in our invertebrate study contribute to human iPSC-derived glia lipid droplet associated phenotypes. We anticipate our study will provide mechanistic insights into the contribution of dysfunction glial lipid droplet biology to AD, which could inform the identification of novel therapeutic targets.

C85

Oligodendrocyte precursor cells use dscams to form tiled networks across the central nervous system

Lucy Wheatley[1], Tim Czopka[1]

[1]Institute for Neuroscience and Cardiovascular Research, United Kingdom

Oligodendrocyte precursor cells (OPCs) exist in abundance throughout the developing and adult central nervous system (CNS), where they evenly distribute and form tiled networks with their elaborate processes. It is known that OPC tiling is established and maintained by principles of self-avoidance and contact-mediated repulsion. However, it is unknown what signalling mechanisms OPCs use to form and maintain these adaptive networks, nor do we understand why OPCs form these process networks throughout the CNS.

Using RNA-sequencing analyses complemented by whole-mount RNA labelling in zebrafish we screened candidate genes with known roles in self-avoidance and contact mediated repulsion for enriched expression in OPCs. We identified the *dscam* gene family (consisting of *dscama*, *dscamb*, and *dscaml1)*, as most highly expressed by OPCs but not in mature oligodendrocytes. Moreover, we observed that all OPCs co-express all three *dscam* genes.

To test for roles of *dscams* in OPC tiling we used injection-mediated CRISPR knockdown of *dscamb and dscaml1* in transgenic zebrafish that have their entire OPC population fluorescently labelled. These 'CRISPants' exhibit a striking disruption of OPC network tiling with reduced process density and large areas that are not interspersed by OPC processes. We also observed less regular spacing of OPC somas with both clumping of somas and larger regions devoid of OPCs. In addition to these population effects, high-resolution analysis revealed that OPCs in *dscamb/l1* CRISPants exhibit thicker, more highly branched and frequently clumped processes. Timelapse analysis of OPC process dynamics showed that they also explore smaller territories than wildtype controls.

Together, our data reveal a key role for *dscam* signalling in the formation of tiled OPC networks and normal OPC process morphology. In our ongoing work we are generating cell type specific and sparse manipulations of *dscam* to test whether this phenotype represents an OPC autonomous deficit, or one that is mediated by intercellular signalling between neighbouring OPCs. Furthermore, we will present data investigating how *dscam*-mediated disruption of OPC tiling may affect their roles in patterning a myelinated CNS.

C86

Glial responses to different pathologies in Parkinson's Disease

Sasvi Wijesinghe[1], Florence Do[1], Anna Mallach[1]

[1]Imperial College London, United Kingdom

Introduction:

Parkinson's Disease (PD) is progressive neurodegenerative condition with unknown aetiology, recognised to have a debilitating effect on motor function. While PD is primarily characterised by the brain-wide accumulation of the protein α-synuclein into Lewy Bodies (LBs), there is also notable aggregation of associated amyloid-β (Aβ) pathology reported[1]. This is linked to a worse disease prognosis[2] and symptomatic dementia (PDD)[3], where up to 50% of PD patients develop dementia ten years within of their initial diagnosis.

Glial responses, particularly astrocytes, are known to be dysfunctional in PD. Although transcriptomic profiles of astrocytes have been noted to change in PD[4], the field is yet to characterise their disease-specific morphological features[5]. To better understand dysfunctional astrocytic responses in PD, we investigated the morphological changes of astrocytes in differing proximity to LBs and/or amyloid plaques using human post-mortem tissue, and whether this varying morphology could underlie patient outcomes.

Materials and Methods:

Post-mortem tissue blocks of the medial temporal gyrus of the temporal cortex were provided by the Parkinson's UK Brain Bank at Imperial College London (REC: 23/WA/0273). Five cases with a PD/PDD diagnosis from our full cohort of controls (n = 10), PD (n = 10), and PDD cases (n = 10) were used for initial analysis.

We conducted multiplex immunofluorescence staining for astrocytes (S100β), α-synuclein (α-syn) and amyloid plaques (Aβ) for each case. Imaged grey-matter ROIs from these stains went through a segmentation pipeline where we analyzed the morphological features of astrocytes near LBs (within 30μm) and/or amyloid plaques (within 40μm), or far from them. A one-way ANOVA was used to assess for significance with a Turkey HSD *post hoc* test used thereafter. Differences in astrocytic morphology between PD vs PDD were tested using an unpaired t-test.

Results:

When near either LBs or amyloid plaques, the size of astrocytes reduced, illustrating a trend of astrocytic morphology feature changes in proximity to pathology. Total number and length of astrocytic branches increased progressively as astrocytes were in close proximity to either an LB or an amyloid plaque, while the highest branch and length counts were in cells that were near both an amyloid plaque and a LB.

When we investigated whether these morphological features differed in cells of patients diagnosed with PD vs. PDD, we found that astrocytes from PDD cases were larger on average as compared to PD, and the total number and length of astrocytic branches were significantly higher in PDD cases vs PD ($p < 0.05$).

Conclusions:

Our results indicate a trend of morphological changes in astrocytes from the temporal cortex of PD patients in proximity to α-synuclein-containing LBs and amyloid plaques. When differentiated by diagnosis, astrocytes from PDD brains illustrate significantly different morphological features as compared to PD brains, alluding to varied glial responses in differing patient outcomes.

C87

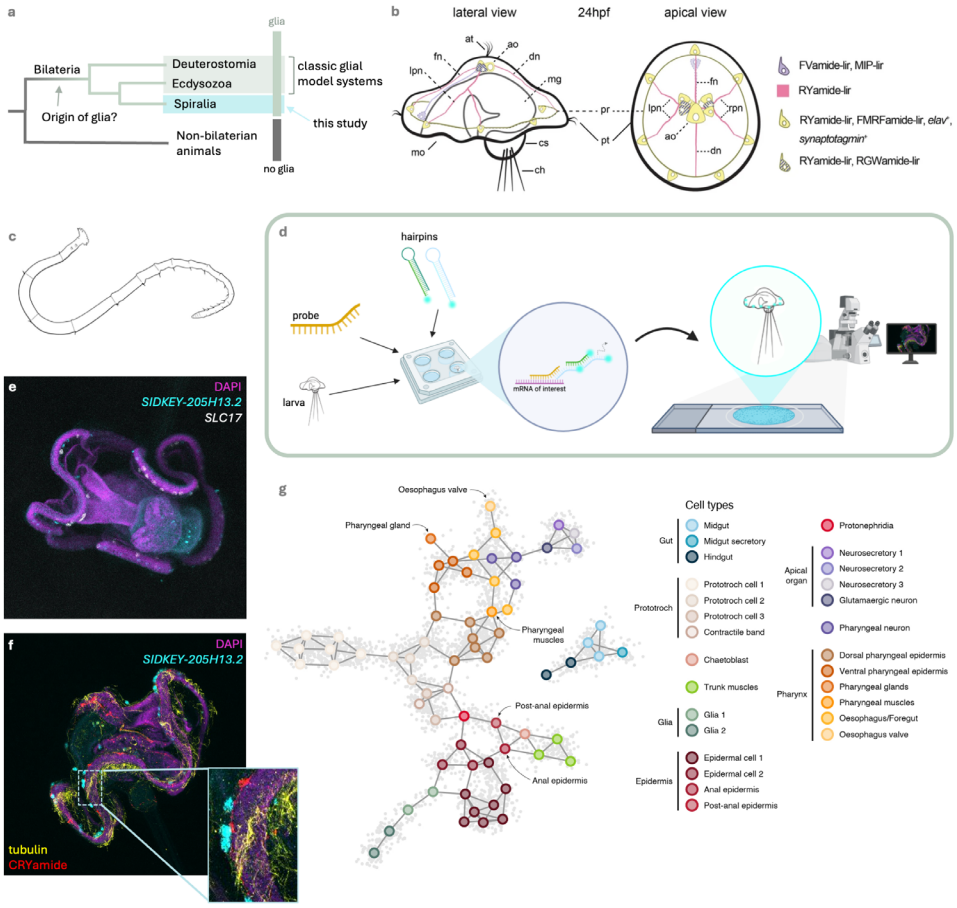
Spotlighting Spiralia: Single-cell transcriptomics reveals a novel glia population in larvae of the annelid Owenia fusiformis

Lily Winkler[1], Allan Carrillo-Baltodano[1], Angelica Miglioli[1], José M. Martín-Durán[1]

[1]Queen Mary University of London, United Kingdom

While glia studies have exploded in the last 30 years, leading to an emerging view of glia as complex and highly adaptable signalling partners to neurons, this increase in understanding has overwhelmingly focused on a very narrow range of model organisms. Across the tree of life, many animal clades and lineages remain only sketchily explored. Although glia are thought to have arisen in the bilaterian ancestor and exist across bilaterian species, the established model organisms for the study of glial physiology (including *M. musculus*, *D. melanogaster* and *C. elegans*) all fall within only two of the three major lineages of Bilateria. A paucity of studies has led to comparatively diminished understanding of glia in the third major lineage: Spiralia (Falcone, 2022; Piovani & Marlétaz, 2023). Addressing this imbalance by studying glia in spiralian invertebrates will provide a key piece of the puzzle for early glial evolution – a field of ongoing debate in which many questions about fundamental glial biology remain unanswered (Sheloukhova & Watanabe, 2024). In this study, we discovered and characterised a novel population of glia cells in the larvae of the annelid worm *Owenia fusiformis* using transcriptomics, gene expression visualisation and confocal fluorescent microscopy.

We performed single-cell RNA sequencing and subsequent computational clustering analysis using Seurat and MetaCell packages to determine the cell types present in the *O. fusiformis* larva (n = 400 larvae). Results of the transcriptomic analysis indicated the presence of a population of glial cells which expressed genes such as *glial cells missing* (*gcm*), the main glial cell fate determinant in *Drosophila*, a surprising find given the small size of the nascent larval nervous system (only 10-20 neurons at 48h post-fertilisation (when analysis was performed), Carrillo-Baltodano *et al.*, 2024). We therefore further investigated these putative glial cells: Hybridisation chain reaction (Evanko, 2004) was used to fluorescently label expression of marker genes identified via the transcriptomic analysis, allowing for the putative glial cell population to be imaged using confocal fluorescence microscopy (n ≥ 20 larvae). We found two distinct populations of glial cells nestled along (but not overlapping with) neuronal structures in the ciliated band lining the mantle of the larva. This section of the nervous system is lost in the juvenile or adult worm and is therefore likely to perform an active physiological function in the nervous system at this larval stage rather form part of a developmental pathway. We are carrying out ongoing work to further investigate the physiology of these cells – do they arise from the same developmental precursors as neurons? What functional roles might they perform in the *O. fusiformis* nervous system? What differences distinguish the two subtypes of glial cells? We are currently performing lineaging as well as gene manipulation experiments to further investigate development and function of the putative glial cells, as well as comparative experiments with other annelid species. Through this work, we hope to elucidate more about spiralian glial cells and support a more balanced understanding of glial cells across the tree of life.



a) Simplified overview of glial presence across taxa. **b)** *Owenia fusiformis* larval nervous system 24 hours post-fertilization (hpf) showing neurons with FVamide-, RYamide-, MIP- and FMRFamide-like immunoreactivity (-lir). ao: apical organ; at: apical tuft; ch: chaetae; cs: chaetal sac; dn: dorsal nerve; fn: frontal nerve; lpn: left peripheral nerve; mg: midgut; pr: prototroch ring; pt: prototroch; rpn: right peripheral nerve (reproduced with permission from Carillo-Baltodano et al., 2024) **c)** Diagram of the *O. fusiformis* adult worm. **d)** Experimental design for gene expression visualisation via hybridization chain reaction (created with BioRender.com). **e)** Representative confocal image of 2-week-old larva following HCR for *SLC17* (cyan) and *SI:DKEY-205H13.2* (grey) as well as DAPI staining for cell nuclei (magenta), showing two populations of putative glial cells (grey and cyan). **f)** Representative confocal image of 2-week-old larva following HCR for SI:DKEY-205H13.2 (cyan), DAPI staining (magenta) and immunostaining for CRYamide (neurons, red) and tubulin (cilia, yellow) with enlarged section showing relative positions of putative glia and neurons. **g)** Diagram of the cell types and clusters identified by MetaCell analysis of *O. fusiformis* single-cell transcriptomic RNA sequencing dataset.

C88

Influence of ageing and sex on astrocytes in the context of Alzheimer's Disease

Fergus Woods[1]

[1]University College Dublin, Ireland

Introduction:

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder involving chronic neuroinflammation and glial dysfunction, particularly of microglia and astrocytes. A striking feature of AD is its sex disparity, meaning women represent nearly two-thirds of cases and experience more severe cognitive decline. Emerging evidence suggests that astrocytes may contribute to this difference through sex-biased transcriptional and functional changes (1). To investigate these changes, zebrafish are an emerging in vivo model for astrocyte research, offering rapid development, optical transparency, and conserved glial biology, making them ideal for studying sex- and age-dependent glial responses.

Aims:

This study aimed to 1) identify sex-biased gene expression differences in astrocytes using publicly available single-cell RNA-seq datasets from human AD patients and 2) explore astrocytic changes between adult male and female zebrafish, in order to validate the model for future studies.

Methodology:

The publicly available RNAseq datasets: GSE157827 (2), syn21670836 (3), and GSE167494 (4) were utilised. Dataset metadata was used to stratify each dataset's samples by sex and disease status using BRAAK staging, with age- and stage-matched cohorts (minimum n = 3 per sex and condition). UMAP clustering was used for visualisation in RStudio, and astrocytes were identified based on GFAP, AQP4, and SLC1A3 expression. Differential gene expression between astrocytes from female and male patient samples was assessed using the Wilcoxon signed-rank test separately for control and AD samples. Genes similarly regulated in both conditions were excluded, and differentially expressed genes were compared across datasets.

Astrocytes abundance and morphology were analysed in male versus female zebrafish (4 months, 1 year, and 2 years; n = 5 per group) using immunofluorescence and RT-qPCR targeting GFAP. All animal experiments were conducted in accordance with EU and HPRA regulations for the use of animals for scientific purposes.

Results:

Bioinformatic analyses identified a set of genes upregulated in astrocytes from female relative to male AD patients . Analyses using Ingenuity Pathway Analysis and Gene Ontology revealed dysregulation of pathways associated with reactive astrocyte phenotypes and cytoskeletal remodelling. Notably, *HSPB8* was consistently upregulated in astrocytes from female compared to male AD patients. In zebrafish, astrocyte numbers were comparable between sexes across ages; however, a significant increase in

GFAP-positive area was observed in specific brain regions of 2-year-old female zebrafish ($p < 0.05$; two-way ANOVA with Tukey's HSD correction).

Conclusions:

My work identifies sex-specific astrocytic transcriptional and morphological changes that may contribute to the increased vulnerability of females to Alzheimer's disease. Integrated analysis of human single-cell RNA-seq datasets revealed a consistently dysregulated set of genes in female AD astrocytes. Notably, the consistent upregulation across all datasets of *HSPB8* highlights autophagic regulation as a potential mechanism underlying sex-biased disease susceptibility, as together with its chaperone partner *BAG3*, HSPB8 has recently been implicated in the regulation of autophagic flux in AD (5). Additionally, analysis in zebrafish demonstrates age- and sex-dependent differences in astrocyte morphology without changes in overall astrocyte abundance, supporting the translational relevance of this model for future investigation of the impact of HSPB8 dysregulation on astrocyte phenotype and function.

C89

TRPA1 activation modifies potassium siphoning, neuronal activity in myelinated axons, and seizure propensity

Xiaolan Wu[1], Grace Flower[1], Xiangyi Du[1], Brooke Jones[1], Pingting Cao[1], Vincenzo Giacco[1], Philip Holland[1], David Andersson[1], Robert Wykes[2], Robert Wykes[3], Nicola Hamilton[1]

[1]Kings College London, United Kingdom, [2]University Collage London, United Kingdom, [3]University of Manchester, United Kingdom

Myelin supports rapid neuronal signalling by siphoning potassium (K^+) away from axons following action potentials, a process mediated by inward-rectifier potassium channels (Kir channels). In Multiple Sclerosis (MS), demyelination disrupts this mechanism. However, the precise alterations in myelin function and their consequences for neuronal activity remain poorly understood. Patch clamp recordings indicate that elevated extracellular [K^+] can activate transient receptor potential ankyrin 1 (TRPA1) in oligodendrocytes, whose activation then leads to inhibition of Kir channels (Hamilton et al., 2016). This may impair K^+ clearance and promote neuronal hyperexcitability. Such changes could contribute to epileptogenesis, but the functional relationship between TRPA1 and Kir channels remains unclear.

Here, we investigated the role of TRPA1 in K^+ siphoning and neuronal activity using compound action potential (CAP) recordings from optic nerves of wild-type (WT) C57Bl/6J mice and oligodendrocyte-specific TRPA1 conditional knockout mice (Sox10 iCreER^{T2}:TRPA1$^{fl/fl}$; cKO). High-frequency stimulation (HFS; 100Hz) was applied to mimic seizure-like activity and induce partial conduction block via elevated periaxonal K^+, and recovery rates were quantified as a measure of K^+ siphoning capacity (Larson et al., 2018).

In WT optic nerves, application of the Kir channel antagonist BaCl2 (100μM) significantly prolonged recovery following 30 s HFS (Control: n=8, mean ± SEM: 76.6 ± 15.9s; BaCl2: n=10, 276.5 ± 25.7s; Student's *t*-test, $P<0.0001$). Similarly, activation of TRPA1 with the agonist polygodial (100 μM) impaired recovery (Control: n=10, 51.6 ± 6.0s; polygodial: n=6, 81.2 ± 13.9s; Student's *t*-test, $P=0.041$), consistent with reduced K^+ siphoning. Interestingly, polygodial application further exacerbated recovery impairment in cKO nerves following 300s HFS compared with WT nerves (WT: n=7, 270.9 ± 30.3s; cKO: n=9, 392.9 ± 19.8s; Student's *t*-test, $P=0.0043$). These results indicate that TRPA1 knockout in oligodendrocytes is not therapeutic and indeed reduces their capacity for potassium syphoning. In line with this, we find that TRPA1 knockout leads to a reduction in potassium channel function and expression in oligodendrocytes.

To assess whether oligodendrocyte TRPA1 deletion alters seizure susceptibility, epilepsy was induced in cKO mice and WT littermates using pentylenetetrazole (PTZ), and the latency to Racine scale stages was measured. In parallel, we have ongoing experiments using electrocorticography (ECoG) recordings from the motor and somatosensory cortex, to evaluate alterations in brain activity and circadian rhythms.

We found that a higher proportion of cKO mice reached Racine stage 4 (loss of posture) and stage 5 (tonic–clonic seizures) after injection of PTZ. Latency to stage 4 was reduced in cKO mice (WT: n=4, 794.3 ± 185.8s; cKO: n=9, 315.4 ± 41.1s; Student's *t*-test, $P=0.0041$), as was latency to stage 5 (WT: n=4, 891.0 ± 178.2s; cKO: n=6, 385.3 ± 81.1s; Student's *t*-test, $P=0.0195$).

Preliminary ECoG recordings revealed brief spike events in the motor cortex, but not the somatosensory cortex of cKO mice (WT: n=1/11; cKO: n=6/14; Fisher's exact test, $P=0.09$), suggesting increased vulnerability of motor cortical circuits following oligodendrocyte-specific TRPA1 deletion.

In summary, TRPA1 regulates Kir channel-mediated K^+ siphoning and neuronal excitability. Both pharmacological activation and genetic deletion of oligodendrocyte TRPA1 impair K^+ clearance, potentially disrupting neuronal stability and increasing seizure susceptibility.

C90

Astrocyte Kir4.1 expression in the retrotrapezoid nucleus varies with age, sex and in Alzheimer's disease

Yumuci Xu[1], Adam Greenstein[2], Andrew Robinson[3], John Gigg[2], Virginia Hawkins[1]

[1]Manchester Metropolitan University, United Kingdom, [2]University of Manchester, United Kingdom, [3]Manchester Brain Bank, United Kingdom

Introduction

The retrotrapezoid nucleus (RTN) is a brainstem respiratory control region where rodent astrocyte Kir4.1 channels are inhibited by increased CO2/H^+ and contribute to chemosensing and CO_2/H^+-dependent respiratory drive. All astrocytes express high levels of the inwardly rectifying potassium channel Kir4.1 and the water channel AQP4 and their disruption affects [K^+]o homeostasis, glutamate uptake, water and waste clearance, processes likely altered in Alzheimer's disease (AD). Reduced Kir4.1 expression has been reported in several neurodegenerative mouse models, but few studies have assessed brainstem alterations. Furthermore, whether astrocyte Kir4.1 is expressed in human RTN tissue, or whether its expression changes with age, differs by sex, or is altered in AD models remains poorly understood. To address this, we compared astrocytic Kir4.1 expression in the RTN of young and aged male and female rodents, including AD models, and examined Kir4.1 expression in putative human RTN tissue.

Methods

Kir4.1 expression was assessed by fluorescent immunohistochemistry in RTN and hippocampal tissue from young adult (2 months) or aged (≥9 months) TgF344-AD rats and APP23 mice, as well as in post-mortem human brainstem. Astrocytes were identified using GFAP or Aldh1L1 labelling and Kir4.1 mRNA levels in TgF344 animals were also assessed by qPCR. Kir4.1 protein expression in astrocytic somata and processes was quantified from confocal images acquired using identical settings, and U87 astrocytoma cells were assessed for Kir4.1 and AQP4 expression by immunocytochemistry. Statistical analyses used unpaired t-tests or multiple comparisons as appropriate with a minimum of 2 animals/subjects per group. Animal procedures were conducted in accordance with the Animals (Scientific Procedures) Act 1986 and human post-mortem tissue was obtained from the Manchester Brain Bank under approval from the Manchester Brain Bank Management Committee (REC Reference 24/NE/0201).

Results

Kir4.1 protein levels were higher in RTN astrocytes than in hippocampal astrocytes, with consistently greater expression in females than males across age groups. Kir4.1 expression also increased with ageing in both RTN and hippocampal astrocytes. However, Kir4.1 protein levels were reduced in aged TgF344-AD rats compared with age-matched controls, including at astrocytic endfoot domains. In 12-month-old male APP23 mice, Kir4.1 expression was detected at higher levels in GFAP-positive astrocytes in the RTN compared to hippocampus and appeared to differ less between genotypes. Kir4.1 mRNA levels were also unchanged between AD models and controls but U87 astrocyte like cells expressed both Kir4.1 and AQP4. Finally, Kir4.1 protein was detected in astrocytes within the putative RTN of aged human ponto-medullary post-mortem tissue.

Conclusions

These findings demonstrate that astrocytic Kir4.1 expression in the RTN increases with normal aging but appears altered in aged AD models, and we show for the first time that human RTN astrocytes also express Kir4.1. Disruptions in astrocyte Kir4.1 protein expression may contribute to neurodegenerative disease mechanisms and potentially to sleep-disordered breathing in cognitive decline.

C91

Optimising in vitro blood-brain barrier models using induced pluripotent stem cell-derived endothelial cells

Zhizhong YANG[1], Nikoleta Daskoulidou[1], Natalie Connor-Robson[1], Axel Montagne[2], B. Paul Morgan[1], Wioleta Zelek[1]

[1]UK Dementia Research Institute, Cardiff University, UK, [2]UK Dementia Research Institute, University of Edinburgh, UK

Background:

In vitro blood-brain barrier (BBB) models are valuable for studying neurovascular biology and testing drug permiablity. Induced pluripotent stem cells (iPSCs)-derived brain microvascular endothelial cell (BMEC) models demonstrate superior barrier properties compared to immortalised cell lines. This project compares two differentiation protocols (Bertucci and Qian *et al*.)[1,2] to identify strategies for generating robust, biologically relevant BBB models with stronger barrier integrity.

Methods:

Transwell-based BBB models were established using iPSC-derived endothelial cells differentiated through two types of protocols. Endothelial characteristics were evaluated via detecting of VE-cadherin, tube formation, and membrane transporters. Barrier integrity was assessed by sodium fluorescein permeability, transendothelial electrical resistance (TEER), and tight junction formation (claudin-5, occludin, and ZO-1). In addition, transferrin uptake assay and rhodamine accumulation assay were also performed to exam endocytic and P-glycoprotein (Pgp) efflux transport function, respectively.

Results:

iPSCs derived using Bertucci's protocol exhibited well-defined endothelial phenotype, characterized by expression of VE-cadherin and CD31, successful tube formation in 3D extracellular matrix, and intact endocytosis and efflux transport function. However, despite of tight junctions formation, those cells yielded low barrier integrity with TEER measurement around 20 $\Omega \cdot cm^2$. In contrast, iPSCs derived from Qian's protocol displayed mixed endothelial/epithelial features, including the ability to form tubes and high epithelial markers expression (E-cadherin and CD326). Despite this, it showed robust transport functions and superior barrier integrity with higher TEER measurements (~3000 $\Omega \cdot cm^2$).

Conclusions:

The two BBB models performed distinctly due to different iPSC-derived cell phenotypes and functional properties. The Bertucci's iPSC-derived BBB model is appropriate for mechanistic studies and neurovascular biology study because of its well-defined endothelial phenotype, whereas the Qian's BBB model with superior barrier integrity is better suited for drug delivery screening applications.

C92

Astrocytes' control of synaptic plasticity during brain development and circuit impairment in Alzheimer's disease.

Yuniesky Andrade-Talavera [1]

[1]Department of Medical Physiology and Biophysics. University of Seville. , Spain

Department of Medical Physiology and Biophysics. Avenida Sánchez Pizjuán 4. University of Seville. Seville. 41900. Spain.

Since the emergence of the tripartite synapse concept, mounting evidence has revealed that astrocytes can control information flow in a circuit-specific way, particularly for a form of synaptic plasticity, namely Spike Timing-Dependent Plasticity (STDP). Based on our recent findings we could say that astrocytes frame the activity coincidence for STDP as a temporal detection traffic light (e.g., favouring the induction of Long-Term Depression (t-LTD) by D-serine release at postnatal (P) days 13-21 (P13-P21) [1], closing a plasticity window due to adenosine release at P22-P30 [2] or further gating a new plasticity window for Long-Term Potentiation due to glutamate release at P34-P42 in mice hippocampal CA1 synapses [3]. These findings and the recent similarities found in the mouse primary somatosensory cortex (S1) allow us to hypothesize that in the mouse hippocampus and S1, astrocytes aid or prevent firing coincidence by providing selective gliotransmitters during coincident spiking, depending on the developmental stage. In line with this, it is known that neurotransmitter-evoked activation of astrocytes leads to astrocytic release of glutamate, D-serine, ATP, and/or adenosine, which through the activation of the corresponding pre- and postsynaptic receptors establishes a threshold for basal synaptic transmission, and enhances short- and long-term synaptic plasticity. Astrocytes gradually increase their Ca^{2+} signalling during the induction of t-LTD in a cannabinoid receptor 1 (CB1R)-dependent manner at synapses between excitatory neurons in somatosensory cortical layer 4 and layer 2/3 (L4-L2/3 synapses). Interestingly, at L4-L2/3 synapses of the primary somatosensory cortex, stimulation of the astrocyte coincident with afferent activity results in LTD. This has also been observed during coincident stimulation of Schaffer collaterals and astrocytes in area CA1 of the hippocampus, and we have observed it recently in horizontal L2/3-L2/3 synapses of S1 involving astrocytic release of D-serine as a prerequisite [4]. Preliminarily, we have observed that a form of synaptic plasticity involving brain rhythms interactions involves astrocyte signalling, and astrocytes dictate the magnitude of this plasticity and sense the timing coincidence of rhythms interaction. This type of plasticity, named theta-nested gamma oscillations-induced Long-Term Potentiation (TnG-LTP), is impaired at 2 months of age in an Alzheimer's disease model (App-tau mice), far before amyloid and tau pathology and clinical onset of cognitive impairment. This is controversial, since plasticity induced with conventional protocols appears disrupted only at later stages of the disease, probably because these protocols do not consider the oscillatory activity of the network and the interaction of theta and gamma rhythms. Thus, TnG-LTP might have an impact on functions that are acquired later during development. Additionally, astrocytes may play a role in setting a threshold for the induction of plasticity during development, and this could represent a promising mechanism to target in Alzheimer´s disease models.

C93

TDP-43 loss drives microglial phagocytosis of synapses via elevated glycolysis

Jan Zakrzewski[1], Ismail Gbadamosi[2], Ali Jawaid[3]

[1]Faculty of Medicine, Medical University of Warsaw, Poland, [2]Translational Neuropsychiatry Research Group (TREND Lab), Łukasiewicz Research Network - PORT Polish Center for Technology Development, Poland, [3]Translational Neuropsychiatry Research Group (TREND Lab), Łukasiewicz Research Network - PORT Polish Center for Technology Development, Poland

Background: TDP-43 is an RNA/DNA-binding protein implicated in the pathogenesis of several neurodegenerative disorders (NDDs), including amyotrophic lateral sclerosis, frontotemporal lobar degeneration, and Alzheimer's disease. Emerging evidence suggests that TDP-43 loss in microglia alters their functional state, contributing to exaggerated synaptic engulfment. Excessive reliance on glycolysis in microglia has been associated with heightened inflammatory outputs and pathological synaptic pruning, whereas enhanced oxidative phosphorylation (OXPHOS) moderates cytokine release and promotes engulfment of pathological cargoes. Based on these observations, we asked whether metabolic alterations represent the mechanistic link between TDP-43 loss and exaggerated synaptic engulfment.

Study aim: This study aims to demonstrate the mediating role of cellular metabolic pathways in exaggerated synaptic phagocytosis by microglia with TDP-43 loss of function.

Ethical standards and consent to participate: Not applicable.

Materials and methods: To recapitulate TDP-43 loss of function, TDP-43 was knocked down in murine BV2 and human HMC3 microglial cells using RNA interference. The effects of TDP-43 depletion on microglial metabolism were then examined via glucose uptake and lactate, ATP, and reactive oxygen species (ROS) production assays. Furthermore, live-cell metabolic flux analysis (MFA) of the two cell lines was performed after TDP-43 knockdown under glycolytic and mitochondrial stress. Finally, synaptoneurosome uptake was quantified in cells with TDP-43 knockdown after metabolic rerouting.

Statistical analysis: Data from biological triplicates across independent passages were normalised to matched non-silencing controls and pooled. Outliers were excluded and distributional assumptions assessed before hypothesis testing. Two-group comparisons used parametric tests with variance correction when appropriate, or non-parametric alternatives when assumptions were not met; time-course metabolic responses were analysed with repeated-measures models and post hoc multiple-comparisons testing, and viability across time points was assessed by within-group ANOVA with post hoc testing.

Results: TDP-43 depletion was associated with increased microglial glycolysis in both cell lines, without a concomitant decrease in mitochondrial oxidative phosphorylation as measured by metabolic flux analysis. This was corroborated by increased glucose uptake, lactate production, and intracellular ATP and ROS levels. TDP-43 knockdown also led to exaggerated engulfment of synaptoneurosomes by microglia, which was reversed by metabolic rerouting via supplementation with pyruvate-containing,

glucose-free medium.

Conclusions: Collectively, our findings identify TDP-43–dependent microglial metabolic rewiring as a potential driver of pathological synapse loss. The mechanistic links between TDP-43 depletion and a hyperglycolytic, pro-phagocytic microglial phenotype define a novel non-cell autonomous route to neurodegeneration. Importantly, pyruvate supplementation's ability to attenuate microglial metabolic and functional aberrations highlights metabolic rerouting as a promising therapeutic avenue in TDP-43-associated NDDs, warranting validation in vivo.

C94

Neuroimmune regulation of memory formation and consolidation: a systematic review

Ming Zhong[1]

[1]University of Bristol, United Kingdom

Background

Neuroinflammation is generally considered to be a pathological response. However, there is growing evidence suggesting that immune signalling within the central nervous system is a dynamic component of neural plasticity. Neuroinflammatory activity that is appropriately regulated may support memory formation and consolidation, with its effects depending on timing, intensity, and inflammatory state. This systematic review aims to synthesise current evidence on how neuroinflammation influences memory formation and consolidation under pathological and physiological contexts.

Methods

A systematic literature search was conducted in PubMed (Table.1) to identify studies examining neuroimmune and neuroinflammatory mechanisms involved in memory formation and consolidation following PRISMA guidelines (Fig. 1.). Search terms covered learning and memory, neuroimmune/neuroinflammatory processes, and the central nervous system context. Original experimental studies were included if they investigated neuroimmune or neuroinflammatory mechanisms in the central nervous system and assessed memory using explicit behavioural paradigms or synaptic plasticity endpoints (LTP/LTD) in non-human models. Reviews, human studies, and studies lacking memory-related outcomes or accessible full text were excluded. The final search was completed in January 2026, with no restriction on publication year.

Results

In total, 224 records were identified, and 57 animal studies between 2006 and 2026 were included after assessment. The included studies predominantly examined disease- or stress-related conditions, including ageing, injury, inflammation, environmental exposure, or physiological stress. All included studies employed at least one established memory paradigm or synaptic plasticity measure, supporting relevance to memory formation or consolidation. The most frequently used paradigms were the Morris water maze (35 studies) and novel object recognition (23 studies). Measures of synaptic plasticity were commonly reported, including long-term depression (22 studies) and long-term potentiation (18 studies). Additional paradigms included Y-maze, fear conditioning, Barnes maze, passive avoidance, extinction, and spatial discrimination tasks, with several studies applying multiple paradigms within the same experimental design.

Neuroimmune involvement was assessed using a broad range of biomarkers. The most frequently reported categories were cytokines (40 studies) and glial cell–associated proteins (33 studies), followed by neuropeptides/neurotrophins (18 studies) and inflammatory signalling pathways. At the molecular level, TNF-α, IL-6, IL-1β, GFAP, Iba1, and BDNF were the most commonly examined markers. Several studies additionally investigated inflammasome components, complement proteins, Toll-like receptors, microRNAs, or extracellular vesicles.

Across studies, inflammatory alterations were most often characterised by increased pro-inflammatory signalling or glial activation, which frequently coincided with impaired memory performance or disrupted synaptic plasticity. In contrast, studies reporting attenuation of inflammatory responses commonly observed improvements in memory-related outcomes.

Conclusion

Existing evidence indicates that neuroimmune and neuroinflammatory processes play a critical role in modulating memory-related behaviours and synaptic plasticity in animal models. Cytokine signalling, glial activation, and immune-related molecular pathways consistently interact with mechanisms underlying memory formation and consolidation. Future studies should more precisely distinguish immune effects across distinct memory stages to improve mechanistic resolution and translational relevance.

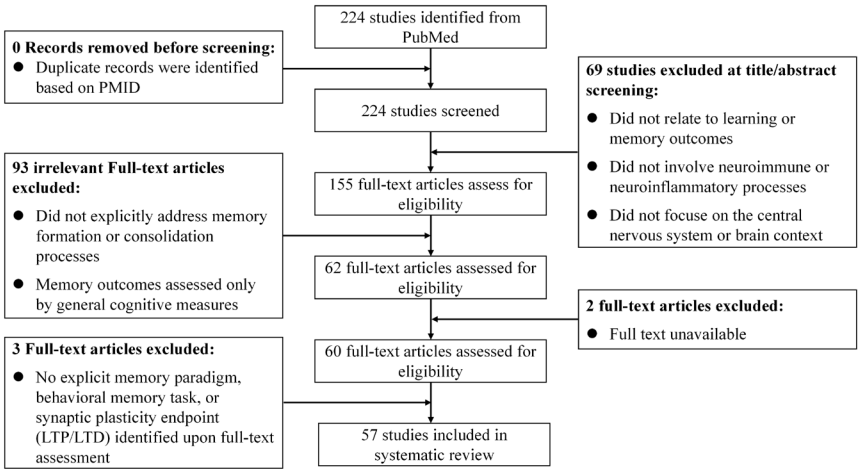


Fig. 1. PRISMA flow diagram of the paper selection process for systematic review

Table.1 Search Strategy for PubMed database

Search#	Search Strategy	Items Found
1	Neuroinflammatory diseases [MeSH	55,757
2	Memory [MeSH Terms]	115,618
3	Biomarker [MeSH Terms]	1,314,876
4	Therapy [MeSH Terms]	12,351,001
5	(#1) AND (#2) AND (#3)	1,047
6	(#5) NOT (#4)	563
	Filters	
Free full text		
English		
Animal(rodent models)		224

C95

MicroRNA-29a alleviates Spinal cord injury induced Edema by Targeting Aquaporin-4

HONGJUN ZOU[1], JINBO LIU[1]

[1]The Third Affiliated Hospital of Soochow University, China

Introduction/Objectives: Post-traumatic edema mediated by aquaporin-4 (AQP4) dysregulation is a critical determinant of secondary neuronal injury after spinal cord injury (SCI). This study aimed to investigate the therapeutic potential of miR-29a-3p in modulating AQP4 to alleviate edema and improve neurological recovery following SCI.

Methods: A mouse contusion SCI model was established (n=3/group, total n=30). Pathological validation was performed via H&E staining. AQP4 spatiotemporal expression was analyzed by immunofluorescence and Western blot (days 1, 7, 14 post-injury). Edema was assessed by spinal cord wet weight and in vivo MRI. In vitro, an IL-1α-induced astrocyte injury model was used. Bioinformatics screening and dual-luciferase assays identified miR-29a-3p as a direct AQP4 regulator. GFAP-Cre-driven Tg-29a transgenic mice (n=5/group, total n=60) enabled astrocyte-specific miR-29a-3p overexpression. Functional recovery was evaluated using the Basso Mouse Scale (BMS) and footprint analysis over 4 weeks.

Results: AQP4 expression was significantly upregulated (>6-fold vs. sham, $p<0.01$) and redistributed by day 7 post-SCI, correlating with increased tissue water content. In the injured spinal cord and in IL-1α-stimulated astrocytes, miR-29a-3p was downregulated while AQP4 was upregulated. miR-29a-3p directly targeted the 3'UTR of AQP4. Astrocyte-specific overexpression of miR-29a-3p in Tg-29a mice significantly suppressed AQP4 protein levels, reduced edema (MRI and tissue water content, $p<0.01$), attenuated glial scar formation (lowered GFAP and vimentin), and improved functional recovery (higher BMS scores, $p<0.05$) compared to wild-type SCI controls. Increased neuronal marker NF-200 expression was also observed.

Conclusions: AQP4 dysregulation is a pivotal mechanism in SCI-induced edema. miR-29a-3p serves as a key post-transcriptional regulator of AQP4. Astrocyte-targeted overexpression of miR-29a-3p effectively mitigates edema, modulates astrogliosis, and promotes functional recovery, highlighting its potential as a novel therapeutic strategy for SCI.

Ethical Statement: All animal procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.