

# **Neuroplasticity in Brain Health and Disease: Advances in Techniques, Translation and Education**

**8 – 9 April 2024 | Newcastle University, UK**

## **Abstracts**

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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

# **Neuroplasticity in Brain Health and Disease: Advances in Techniques, Translation and Education**

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**SA01**

## **Love, death, and oxytocin: the challenges of mouse maternity**

Robert Froemke<sup>1</sup>

*<sup>1</sup>New York University, NY, United States*

The neuropeptide oxytocin is important for maternal physiology and social behavior. In this talk, I will discuss new and unpublished data from our lab on when, where, and how oxytocin is released from hypothalamic neurons to enable maternal behavior in new mother mice. I will focus on maternal responses to infant distress calls, and how oxytocin enables rapid neurobehavioral changes for dams and alloparents to recognize the meaning of these calls. We have built a new system combining 24/7 continuous video monitoring with neural recordings from the auditory cortex and oxytocin neurons of the hypothalamus in vivo. With this documentary approach, we have identified behaviors of experienced and naïve adults learning to co-parent together which also activate oxytocin neurons. I will discuss circuits routing sensory information to oxytocin neurons leading to oxytocin release in target areas important for maternal motivation. Finally, I will discuss longer-term behavioral monitoring over months, examining how single mothers build nests to help ensure pup survival or how this sometimes goes awry.

**SA02**

**Impaired visual discrimination in a mouse model of SYNGAP1 -related intellectual disability and autism**

Danai Katsanevaki<sup>1</sup>

<sup>1</sup>*The University of Edinburgh, Edinburgh, United Kingdom*

Altered sensory experience is now considered a key feature of neurodevelopmental and autism spectrum disorders (ASD). This alteration results in, and often predicts, a range of cognitive, social, and behavioural affective impairments, strongly impacting the quality of life. However, the underlying neurophysiological mechanisms that disrupt sensory perception remain understudied. We investigate these mechanisms in a mouse model of *SYNGAP1* haploinsufficiency (HET), *SYNGAP1* being one of the most prevalent neurodevelopmental disorder genes. Using *in vivo* two-photon calcium imaging, we found that layer 2/3 neurons in the primary visual cortex (V1) of *Syngap* HET mice responded to visual stimuli with greater variability compared to wild-type littermate controls, resulting in a reduction in V1 coding precision and a reduced behavioural visual discriminability. This impairment was not due to altered intrinsic and synaptic properties of V1 HET neurons. Notably, when we tested V1 visual responses under anaesthesia, we found that the variability of visual responses and population coding precision in V1 neurons of *Syngap* HET mice were restored to control levels. These results indicate that anaesthesia-dependent inputs are responsible for the visual impairments found in *Syngap* HET mice and suggest a dysregulation of behavioural state modulation. Top-down, neuromodulatory inputs may therefore be promising therapeutic targets for improving sensory experience of individuals with *SYNGAP1* haploinsufficiency and more generally, other neurodevelopmental disorders.

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**SA03**

## **Mechanisms of homeostatic plasticity in the developing nervous system**

Peter Wenner<sup>1</sup>

*<sup>1</sup>Emory University, School of Medicine, Atlanta, United States*

The nervous system has an impressive capability of homeostatically maintaining function in the face of various challenges. The developmental period for the construction of neural circuits is highly dynamic and presents dramatic challenges to circuit activity as cells increase in size, synaptic connections are added and removed, and GABAergic transmission transitions from excitatory to inhibitory in the mature system. Several different homeostatic mechanisms exist to ensure that the system can maintain an appropriate level of activity. For instance when activity levels are inhibited increases in excitatory synaptic strength and intrinsic membrane excitability are observed. I will be discussing different homeostatic mechanisms in different systems to understand how this process shapes the maturation of network excitability. I will also discuss the relevance of homeostatic plasticity in neurodevelopmental disorders where altered levels of excitability are observed, such as autism.

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**SA04**

## **Educational neuroscience: Mechanisms of learning and other factors that influence educational outcomes**

Michael Thomas<sup>1</sup>

*<sup>1</sup>Centre for Educational Neuroscience, Birkbeck University of London, London, United Kingdom*

In this talk I'll give a broad overview of the field of educational neuroscience, and how insights into neural mechanisms of learning can inform classroom practices. Educational neuroscience proceeds either by informing theories of learning (that is, via psychology) or by viewing the brain as an organ whose physiological state needs to be optimised for learning in the classroom (that is, via brain health). For the former, I'll give examples of projects within the University of London Centre for Educational Neuroscience on science and maths learning in primary school, and on adult literacy programmes in low-income countries. For the latter I'll discuss recent work on the role of sleep and aerobic fitness in improving learning outcomes.

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SA05

**Better tools for better science. How open hardware is on its way to revolutionize the way we think about research and education**

Andre Maia Chagas<sup>1,2,3</sup>

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Open Hardware is a way of developing and distributing physical tools and equipment, where developers make designs and build instructions freely available online, under specific licenses that determine how the shared documentation can be used. Very similar to Open Source Software, this way of distributing know-how enables everyone to locally replicated, learn, modify and improve on existing designs. Open Hardware designs are present in many different fields from health care to computing technologies, and examples include equipment for automated COVID testing, smartwatches, farming robots, MRI machines, Geiger counters, and educational tools.

In academia, open hardware is becoming pervasive and has several positive outcomes for science and education, as researchers now have an ever growing library of designs for tools and educational resources in the form of peer-reviewed articles and other online repositories. This resource can be used to create innovative experiments, as researchers can think about what kind of research questions they would like to pursue, which kind of experiments would be needed to address those, and finally build specific tools to perform said experiments (instead of the traditional way of adapting experiments based on available equipment at a lab/institute). Moreover, leveraging open hardware we can change the way we train and educate researchers in training, as we can add practical and hands on experiments to complement and/or substitute “passive lectures” based solely on theoretical explanations. On this talk we will see the details on how this is happening, what implications it could have for research going forward and how it is being applied by different groups in different fields across the globe.

**SA06**

**Plasticity of neural population activity in the medial prefrontal cortex during maze learning**

Mark Humphries<sup>1</sup>

*<sup>1</sup>University of Nottingham, Nottingham, United Kingdom*

The prefrontal cortex (PFC) is thought to represent our knowledge about what action is worth doing in which context. But we do not know how the activity of neurons in PFC collectively changes when learning which actions are relevant. Here we show, in a trial-and-error task, that population activity in PFC is constantly plastic, independently of behavioural learning. Only during episodes of clear learning of relevant actions are the accompanying changes to population activity carried forward into sleep, suggesting a long-lasting form of neural plasticity. And only during those same episodes does there appear a consistent neural signal of working memory for successful choices. Our results suggest that representations of relevant actions in PFC are acquired by reward imposing a direction onto ongoing population plasticity.

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SA07

## Building 3D brains online, a course-based undergraduate research experience on neuroanatomy

Marina Yasvoina<sup>1</sup>

<sup>1</sup>*King's College London, London, United Kingdom*

Neuroscience can be a challenging subject for undergraduate students. The discipline often relies heavily on rote memorization and uses two-dimensional diagrams for teaching, which do not fully convey the complexity of the brain structure and function. The result is often 'surface learning', where students overwhelmingly focus on anatomical topography and not the functional complexity and connectivity of the nervous system. Yet, first-year students must establish foundational knowledge in order to successfully transition to more advanced modules and the independent research project in their final year. This leads to a stressful experience where both the teaching and the exam structure do not prepare students for the challenges ahead.

Introducing undergraduate students to authentic research experiences early in their education can bring many benefits. Students who are exposed to scientific inquiry develop critical thinking and problem-solving skills, data analysis and even skills in science communication. Recognizing this, we developed a first-year neuroscience module based on Course-Based Research Experience (CURE) and constructivist principles for assessment and learning. The module design is focused on the application of knowledge: students assemble a portfolio structured as a small-scale research project on neuroanatomy using a 3D model of a mouse brain. We have aligned each teaching session with a specific task to be completed for the project. The portfolio itself is modelled after the Results section of a scientific research paper and includes written and visual components, a summary of findings supported by literature cited and figures. Importantly, because the data can be interpreted in different ways, the project is consistent with the exploratory nature of science. The module design also aims to reduce stress for students by encouraging them to learn through practice and application, together with a structured approach to completing the portfolio.

The presentation will discuss the module design and showcase our approach to novel assessments. I will also share student feedback and module evaluation data. Finally, I will explore how CUREs can be used in a large classroom setting, and offer tips on how to adopt this approach in your own classroom.



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SA08

## Knowledge assessment through single-best answer questions

Phil Smith<sup>1</sup>

<sup>1</sup>*Cardiff University School of Medicine, Cardiff, United Kingdom*

Clinical decision-making is predominantly scenario based, weighing up uncertainties and selecting the best option (rather than a 'correct' or 'incorrect' answer) for an individual in their individual circumstances. Knowledge in clinical disciplines around the world typically assesses candidates' choices of a single best answer (SBA) to clinical scenarios, thus requiring the application of knowledge rather than simple knowledge recall.

A single best answer examination can quickly sample from the whole curriculum and have results available immediately after the test. Almost all the work of a ('best of five') SBA paper is in its preparation, with little post-test work, unlike essay marking. Their main disadvantages are their reward of recognition and their unrealism in having five pre-determined options.

Construction of an SBA question requires it:

- **To have a consistent and agreed style** (avoiding construct irrelevance).
- **To pass the 'cover test'**, i.e. be answerable without first seeing the answer options.
- **To avoid rewarding 'test wise' candidates** (especially through limiting 'convergence strategy' opportunities).

Clinical SBAs are blueprinted to topics that are common (frequently presenting) and important (life threatening or life changing). It is challenging to write SBAs on certain topics, especially those either with clearly correct or incorrect answers (e.g. clinical science) or those requiring lengthy discursive explanation (e.g. social science, ethics and professionalism).

Future directions for SBA question construction include:

- **Three answer options** instead of five (easier to write yet nearly as reliable).
- **'Open book' assessments** (particularly challenging with availability of large language model artificial intelligence).
- **Very-short-answer questions:** substituting the five options with candidate-generated limited free text that is marked by computer, giving a more realistic but challenging test.

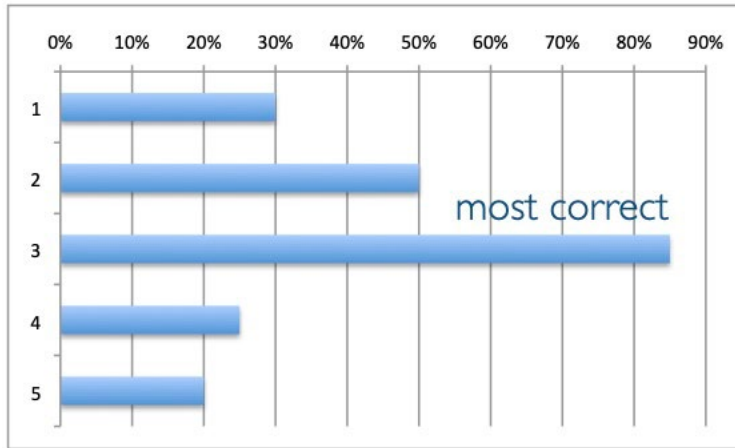
Future directions for SBA exam test delivery include:

- **From paper to computer.** UK undergraduate medical exam delivery is already being shaped by the requirements of the UK's new Medical Licensing Assessment, a 200-item SBA assessment based on clinical scenarios. Thus, increasingly medical students sit SBA knowledge tests on computers (with questions presented in random order) in invigilated venues.

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- **Computer adaptive testing**, with items presented of varying difficulty according to previous answers.



Harris B, Walsh J, Smith PE. Single-best answer question writing tips for clinicians. *Postgraduate Medical Journal* 2017; 93: 76–81. PMID: 27371033. DOI 10.1136/postgradmedj-2015-133893.

Smith PE, Mucklow JC. Writing clinical scenarios for clinical science questions. *Clinical Medicine* 2016; 16: 142–5. PMID: 27037383. DOI 10.7861/clinmedicine.16-2-142.

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SA09

## The role of the neurovascular unit in diabetic retinopathy: pathophysiology and therapeutic targets

Tim Curtis<sup>1</sup>

<sup>1</sup>*Queen's University Belfast, Belfast, United Kingdom*

Diabetic retinopathy is a neurovascular complication of diabetes that can lead to vision loss and blindness. Current treatments primarily target the advanced stages of the disease when significant retinal damage has already occurred. Consequently, there is an urgent need for innovative interventions, particularly those effective in the early stages of the condition. Recent research from our group has identified a crucial role for the acrolein-derived advanced lipoxidation end-product, FDP-lysine, in the pathogenesis of diabetic retinopathy. We have also discovered a new drug, 2-HDP, which is effective in scavenging acrolein and preventing retinal FDP-lysine accumulation during diabetes. In this talk, I will share our latest data regarding the pre-clinical effects of 2-HDP on the formation of the neurovascular lesions observed in diabetic retinopathy. Furthermore, I will introduce recent collaborative work utilising serial block face scanning electron microscopy (SBF-SEM) and computational image reconstruction, which has provided the first 3D nanoscale analysis of the neurovascular pathology associated with this condition. Our findings thus far suggest that acrolein scavenging drugs, such as 2-HDP, could offer an effective means to prevent the development of diabetic retinopathy before it reaches its advanced, sight-threatening stages. Moreover, the application of SBF-SEM presents a promising avenue for studying changes in the retinal neurovascular unit during diabetes, offering a valuable platform to inform future studies aimed at delaying or preventing the progression of this condition.

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SA10

## How to get from microglia cell shape to their function

Sandra Siegert<sup>1</sup>

<sup>1</sup>*Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria*

Brain dysfunction has a detrimental effect on our daily lives. Yet, surprisingly little is known about how microglia, traditionally classified as immune-responsive cells, maintain brain functionality and how deviation from this task will induce disease onset. As highly dynamic cells, the microglia branching tree gives a first intuition on their intrinsic state; massively reduced branching is commonly found in severe pathological conditions and indicates phagocytic activity. However, attempts to morphologically classify microglia beyond this extreme condition have been unsatisfactory. In our line of research, we noticed that strategies to simplify the branching tree are insufficient to handle the dynamic nature and often cause loss of discriminative information.

In this talk, I will introduce our developed algorithm *morphOMICs*, which is based on principles of topological data analysis, statistics, and machine-learning dimensionality-reduction techniques [1]. I will show how *morphOMICs* has been robust to quantify subtle yet functionally informative morphological adaptations. The talk will finish with a roadmap to connect microglia morphology to their intrinsic functional state taking advantage of the retina and in this way establish microglia as a sensor for the physiological state of the brain and as an early indicator for environmental changes.

[1] Colombo, G., Cubero, R. J. A., Kanari, L., Venturino, A., Schulz, R., Scolamiero, M., Agerberg, J., Mathys, H., Tsai, L-H., Chachólsk, W., Hess K. & Siegert, S. *Nature Neuroscience*, 25(10), 1379-1393 (2022).

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SA11

## Record and analyze electrical activity in organoids, cell cultures and brain slices: BioSignal Processing Unit (BioSPU)-integrated plates

Sercan Deniz<sup>1</sup>

<sup>1</sup>*3Brain AG, Pfäffikon, Switzerland*

Intelligent plates integrating BioSignal Processing Unit (BioSPU) provide a tool for recording electrogenic cell activity within the same vessel used for culturing cells and organoids.

The plates establish direct contact between microchips and biological samples, enabling electrophysiological recording and stimulation at single-cell resolution. Researchers working with neurons, cardiomyocytes, retina or beta-pancreatic cells employ this technology to: characterize disease models, monitor iPSC-derived cultures development and organoid activity, conduct neurotoxicity and neuropharmacology studies or assess the impact of inflammation on cellular electrophysiology.

BioSPU-integrated plates represent the next-gen technology for neuroscience.

### C01

#### Development of novel co-opsins to manipulate intracellular and extracellular ionic content

Amy Marshall<sup>1</sup>, Laura Alberio<sup>1,2</sup>, Andrew Trevelyan<sup>1</sup>

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Recent work has indicated that there is a very substantial redistribution of ions between intracellular and extracellular ion levels throughout the day<sup>1,2</sup>. Furthermore, ionic redistribution plays a major role in different episodic neural pathology such as seizures and cortical spreading depressions<sup>3</sup>. Particular attention has focused upon K<sup>+</sup> and Cl<sup>-</sup> levels where even small changes may affect the likelihood of pathological discharges such as seizures or cortical spreading depression<sup>4</sup>. Optogenetics allows for the control of ionic levels using light-controlled ion channels and pumps (opsins). While optogenetics is a well-established field, there is relatively untapped potential for pairing opsins in order to manipulate ionic movement. The only example of this is Cl-out, which couples an Arch outward proton pump with an inhibitory channelrhodopsin<sup>5</sup>. The outward proton pump hyperpolarises the cell, thereby creating an electrochemical gradient for extruding chloride anions when the chloride channel is opened.

We have now developed and tested five new co-opsins for the manipulation of ionic movement. We used PCR and overlapping PCR to create the constructs, inserted the vectors into HEK293 cells. The constructs contain a fluorescent tag for easy identification of expressing cells. Whole cell patch clamp was used to demonstrate light activated currents indicative of targeted membrane expression, and functionality.

1. Cl-out4KCR contains GtACR2, a general anion (chloride) channel, and KCR, a potassium channel. This couples chloride and potassium movement so that chloride will be extruded against its concentration gradient due to the outward movement of potassium. (n=10 cells)
2. K-in has KCR, a potassium channel, and ArchT3.0, an outward proton pump. ArchT3.0 moves positive charge out of the cell to manipulate the electrochemical gradient of potassium, thereby causing potassium to move into the cell through the open KCR channels. (n=5)
3. K-out combines KCR and ChR2, a general cation channel, with a reversal potential close to zero. ChR2 therefore creates the electrochemical gradient for moving potassium out of cells when KCR2 is opened. (n=11)
4. HaloKCR combines KCR and NpHR, an inward chloride pump. When NpHR is activated it will change the electrical gradient of potassium so that when KCR1 is activated, potassium will enter the cell. (n=6)
5. HaloReverseKCR changes the order of NpHR and the inflexible linker  $\beta$ HK in order to flip NpHR. With NpHR flipped, chloride will be pumped out of the cell. This will change the electrochemical gradient of potassium so that when KCR is activated potassium will leave the cell in order to create a net outward ionic redistribution. (n=5)

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We will illustrate these findings, and discuss these in relation to models of the constructs within the membrane, generated using Alphafold. We will discuss how these might be used for exploring novel pathophysiological mechanisms involving ionic redistribution.

References 1. Ding F et al. (2016). Science 352(6285):550-5. 2. Pracucci E et al. (2023). Nat Commun 14, 7108. 3. Somjen G. (2004). Ions in the Brain (book). 4. Parrish RR et al. (2023). The Journal of Neuroscience 43 (5): 685. 5. Alfonsa H et al. (2016). Nat Commun 7: 13495.

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C02

## Altered network activity patterns and transcriptomic profile in an *in vitro* human model of STXBP1 encephalopathy.

Faye McLeod<sup>1</sup>, Anna Dimtsi<sup>1</sup>, Gavin Clowry<sup>1</sup>, Andrew Trevelyan<sup>1</sup>

<sup>1</sup>Newcastle University, Newcastle upon Tyne, United Kingdom

### Introduction

Early infantile epileptic encephalopathies (EIEE) of genetic origin are devastating conditions, but the pathological mechanisms often remain obscure. A major obstacle is the difficulty of studying human cortical brain development, *in utero*. Until recently, no *in vitro* preparations could accurately reproduce the complex cellular networks found beyond the first trimester.

Using a novel approach that can maintain developing human brain tissue in the laboratory, we investigated STXBP1 haploinsufficiency, which is among the most common genetic causes of EIEE. Loss of STXBP1 impaired synaptic function and reduced glutamatergic synapse density at individual neurons (McLeod et al, Brain 2023).

### Aim

To fully understand how deficits in STXBP1 affect the early developing brain, we now aim to investigate the impact on neuronal networks.

### Methods

Human brain slice cultures were prepared from ethically sourced, 13-18 post-conception week brain tissue ([www.hdbr.org](http://www.hdbr.org)). The gross anatomical structures of the marginal zone, cortical plate and subplate are maintained for several months, while synaptic networks continue to develop. This preparation permits the study of genetic manipulations and their downstream effects on an intact developing human cortical network.

We assessed the downstream effects of short hairpin RNA (shRNA) mediated STXBP1 knockdown, using three powerful screening tools: (1) 3Brain multielectrode array recordings; (2) Ca<sup>2+</sup> imaging using genetic encoding calcium indicators; (3) gene expression profiles using NanoString nCounter technology.



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## Results

We show a striking reduction in the number of local field potentials in the subplate of slices with diminished STXBP1 levels (control:  $0.1040 \pm 0.01\text{Hz}$ , STXBP1 shRNA:  $0.03 \pm 0.007\text{Hz}$ , unpaired t-test,  $P < 0.001$ ,  $n = 2$  biological replicates). Interestingly, loss of STXBP1 does not affect the frequency of synchronised calcium transients in subplate neurons but reduces the peak amplitude (control:  $0.45 \pm 0.05 \Delta F/F_0$ , STXBP1 shRNA:  $0.16 \pm 0.04 \Delta F/F_0$ , unpaired t-test,  $P < 0.001$ ,  $n = 3$  biological replicates) and lengthens the decay kinetics (control:  $1.46 \pm 0.05\text{s}$ , STXBP1 shRNA:  $1.77 \pm 0.14\text{s}$ , unpaired t-test,  $P < 0.05$ ,  $n = 3$  biological replicates) of events. This data suggests that loss of STXBP1 alters neuronal network excitability.

Analyses of our NanoString data confirms that the manipulation achieves a reliable knockdown of STXBP1 across the tissue by  $46 \pm 2.5\%$  relative to control slices. This results in an upregulation of 81 genes associated with glutamate receptor activity, ephrin signalling, voltage gated ion channel signalling and vesicle recycling and a downregulation of 47 genes associated with apoptosis, oxidative stress and G-protein coupled receptor signalling (false discovery ratio analysis with two-sided unpaired t-test corrected for multiple comparisons,  $n = 3$  biological replicates).

## Conclusion

Reduced network excitability with loss of STXBP1 could indicate impairments in the formation of early neuronal networks, a process which correlated neuronal activity is instrumental for during prenatal human brain development. Changes in downstream gene expression may reflect potential compensatory mechanisms in response to this reduction, including an upregulation of activity-dependent synaptic signalling and downregulation of cell death and/or stress induced pathways.

McLeod et al (2023). Brain 146:850-857

**C03**

**Comparison of Cx45 and Cx36 expression in retinal organoids and mice retinas**

Gerrit Hilgen<sup>1</sup>, Mingaile Jackson<sup>1</sup>, Birthe Dorgau<sup>2</sup>, Evelyne Sernagor<sup>2</sup>, Majlinda Lako<sup>2</sup>

<sup>1</sup>*Northumbria University, Newcastle upon Tyne, United Kingdom*, <sup>2</sup>*Newcastle University, Newcastle upon Tyne, United Kingdom*

Gap junctions are widely prevalent in the nervous system and play many pivotal roles including synchronising and collecting of electrical signals. In vertebrates, gap junctions consist of two adjacent hemichannels which in turn are made up of six connexins (Cx). Connexins and their gap junctions emerge long before chemical synapses during neural development, allowing communication between developing cells, and are thought to be involved in establishing distinct neural circuits. It is unclear how much Cx45 and Cx36 are involved in retinal circuit creation in mouse retinas and retinal organoids generated from human induced pluripotent stem cells (ROs) and whether developmental parallels can be found in both systems. Here, we quantified the developmental expression patterns of Cx45 and Cx36 in postnatal day 8-16 mice and compared against ROs differentiated to days 40, 90, 150 and 200. For this, we developed a custom software that automatically analyses multiple anatomical parameters such as size, overlap and near-by location of all channels within a confocal microscopy stack. Our results show that numerous soma-somatic Cx45 and Cx36 gap junctions are existing in ROs, and both connexins were localised on and near synaptic terminals that were labelled with synaptophysin and vesicular glutamate transporter 1 in both tissues. Cx45 and Cx36 expressions in both plexiform layers of the mouse retina increased till eye opening and dropped slightly afterwards. Cx45 expression patterns in ROs showed increasing densities at later differential stages, whereas Cx36 displayed less pronounced expression than in mice. Heterotypic Cx45/Cx36 gap junctions, which can be found as part of the rod pathway between ON cone bipolar cells and All amacrine cells, were similarly expressed in mice and ROs. The percentage of Cx45/Cx36 gap junctions is higher before the critical event of eye opening in the mouse while in ROs it steadily increased. In addition, our multielectrode array recordings from ROs revealed gap-junction-coupled RGCs, consistent with similar findings from both connexins between retinal ganglion cells (RGCs) in mice. In conclusion, mice and ROs have very comparable Cx45 and Cx36 expression patterns. We show for the first time that ROs have heterotypic Cx45/Cx36 gap junctions and functional gap junctions between RGCs in ROs. These findings imply that both connexins play a key role during development in the mouse retina and ROs.

**C04**

**Spontaneous retinal waves and angiogenesis: A widefield calcium imaging perspective**

Michael Savage<sup>1</sup>, Cori Betram<sup>1</sup>, Evelyne Sernagor<sup>1</sup>

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The neonatal retina produces spontaneous waves of neural activity which propagate laterally across the ganglion cell layer, immediately underneath the developing superficial vascular plexus. Although it is speculated that early activity may guide angiogenesis, the exact relationship between these waves and the formation of the vasculature is still unclear. We have discovered an unclassified set of highly autofluorescent cell clusters (CCs) in the neonatal mouse retina. They appear under the leading edge of the vascular plexus as it expands from the optic disc to the retinal periphery as development progresses during the first postnatal week. Once the vasculature extends to the edge of the retina, these CCs completely disappear. It has been shown with large scale multielectrode array neurophysiological recordings that the origin points for retinal waves also follow a centre to periphery time course which mirrors the centrifugal development of the vasculature. To fully understand the mechanism linking these phenomena We have developed a large scale calcium imaging pipeline to study the origin of the retinal waves in relation to the distribution on CCs and the vascular plexus development. We have discovered that the majority of the wave origin points are peripheral to the vascular plexus. This leads us to believe these activity hotspots are signalling the vasculature to develop outwards from the retinal centre in order to provide oxygen to yet non-vascularised areas exhibiting metabolically demanding intense electrical activity. Our data show that one of the mechanisms by which these hotspots initiate retinal waves and signal to the angiogenic apparatus is via purinergic signalling through voltage sensitive Pannexin-1 hemichannels. Herein, we observe a reversible significant decrease in wave frequency in the presence of probenecid, a Pannexin-1 channel blocker. This further reinforces the interconnected nature of retinal development, aligning the progression of retinal waves and the expansion of the retinal vascular plexus.

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C05

## Absence of axon initial segment plasticity in human and rodent cortex on short and long time-scales.

Anna Sumera<sup>1</sup>, Laura Simões de Oliveira<sup>1</sup>, Angelika Kwiatkowska<sup>1</sup>, Lewis W Taylor<sup>1</sup>, Robert I McGeachan<sup>1</sup>, Claire S Durrant<sup>1</sup>, Paul M Brennan<sup>1</sup>, Peter C Kind<sup>1</sup>, Sam A Booker<sup>1</sup>

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### Introduction

The structural plasticity of neurons is a key mechanism that brain circuits can respond to, and correct, wholesale changes in activity. Activity-dependent structural changes have been described across all neuronal compartments, including the axon initial segment (AIS); with timescales ranging from as short as a few hours to as long as weeks [1-2]. Due to its unique cytoskeleton, the AIS accumulates voltage-gated ion-channels and is the primary location of action potential initiation [3]. Changes to AIS length have been proposed as a mechanism of homeostatic plasticity of neuronal intrinsic physiology, *in vitro* and *in vivo* [4], and has been linked to typical and neuropathological development (e.g. Fragile X Syndrome [5]). Until now, mammalian studies of AIS plasticity have largely relied on data from inbred mouse strains [1, 2, 4], which we have previously failed to replicate in acute mouse brain slices – despite the presence of robust homeostatic physiological plasticity [5]. Whether AIS structure and neuronal physiology display activity-dependent plasticity common to many species, including humans, remains unexplored.

### Methods and results:

This research examines the anatomical (i.e. AIS length) and physiological (i.e. cellular excitability) properties of neurons following modulation of neuronal activity. These experiments are performed under the same *ex vivo* conditions in mice, rats, and resected human brain tissue; and *in vivo* in mice. All statistics were performed using linear mixed-effects models to avoid potential pseudoreplication. All ethical approvals for animal and human tissue collection were present prior to experiments.

Under control conditions we find that, irrespective of species, AIS length correlates well with many action potential properties in Layer 2/3 neocortical neurons. However, such correlation displayed region and species differences, with the AIS length of human neurons generally correlating less with excitability. In contrast to previous studies [2, 4], where we manipulated circuit activity *ex vivo* over a short period of time (1-3 hours), we found no evidence for altered length of the AIS in mice (N=5), out-bred rats (N=7), or adult human brain slices (N=6) following either direct depolarisation (15 mM K<sup>+</sup>), and increased (20  $\mu$ M bicuculline) or decreased (300 nM TTX + 50  $\mu$ M AP-5) circuit activity. Surprisingly, cortical neurons from living human brain slices displayed no evidence for altered intrinsic or synaptic function even after 3 hours of depolarisation - changes that have previously been observed in mice. Finally, we modulated neuronal activity *in vivo* through

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deprivation of visual inputs by dark rearing mice for 2 weeks (N=10) or 4 weeks (N=6). In these dark-reared mice we observed no change in AIS length or neuronal excitability in layer 2/3 of the primary visual cortex, consistent with an absence of activity-dependent AIS plasticity *in vivo*.

## **Conclusion and implications:**

Taken together, our data suggest that whilst AIS length correlates with action potential function across species, we observe no evidence of rapid remodelling of neuronal excitability mediated by changes to AIS length following wholesale changes in activity. These data question the role that AIS plasticity could play in homeostatic plasticity of human brain circuits.

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**C06**

**Histamine stimulates calcium-dependent cation transmembrane current through H1R-Gq/11 pathway in human retinal glial Müller cells**

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Histamine is a ubiquitous autocooid that mediates a vast variety of physiological processes through the binding to one of its four G-protein coupled receptors (H<sub>1</sub>R, H<sub>2</sub>R, H<sub>3</sub>R and H<sub>4</sub>R). Using patch-clamp electrophysiology in conventional whole-cell mode, and calcium (Ca<sup>2+</sup>) imaging we found that histamine induced powerful conductance that was associated with augmented intracellular Ca<sup>2+</sup> in human retinal glial Müller cells MIO-M1. Inhibition of the histamine effects with H1R antagonist and phospholipase C inhibitor U73122 unveiled the signal transduction through G<sub>q/11</sub> cascade. Suppression of intracellular Ca<sup>2+</sup> signal and transmembrane current with xestospongine C confirmed further involvement of Ca<sup>2+</sup> release from endoplasmic reticulum (ER) through inositol triphosphate receptors (IP<sub>3</sub>R), and passive depletion of ER stores with reticular Ca<sup>2+</sup> adenosine triphosphatase (Ca<sup>2+</sup> ATPase) inhibitor cyclopiazonic acid (CPA) and Ca<sup>2+</sup> chelator 1,2-Bis 2-aminophenoxy ethane-N,N,N',N'-tetraacetic acid tetrakis acetoxymethyl ester (BAPTA-AM) verified store-dependence of the histamine signalling in human retinal glial Müller cells MIO-M1. Sensitivity of the histamine signals with nominal Ca<sup>2+</sup> free solution and NiCl<sub>2</sub> highlighted importance of the extracellular Ca<sup>2+</sup> influx, that was confirmed with 2APB the inhibitor of transient receptor potential (TRP) channels, presumably melastatin (TRPM) subfamily. The expression of H<sub>1</sub>R, TRPM7 and glial markers was confirmed using immunocytochemistry. Taken together, this study establishes a novel H<sub>1</sub>R/TRPM7 mechanism that in human retinal glial Müller cells might play important role in retinopathies such as fluid accumulation in macular oedema and neovascularisation in diabetic retinopathy.

**C07**

**Mitochondrial dysfunction in parvalbumin cells triggers a juvenile-onset severe neurological disorder in vivo**

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**Introduction:** Mitochondrial diseases comprise the largest group of inherited metabolic disorders. Neurological symptoms include epilepsy, ataxia, and cognitive impairment. Previous *post-mortem* neuropathological studies implicated severe oxidative phosphorylation (OXPHOS) deficiencies in GABAergic inhibitory neurons accompanied by neurodegeneration in mitochondrial disease, including Purkinje neurons of the cerebellum. This study aims to test the hypothesis that underlying hyperexcitability may arise due to neuronal network disinhibition and that metabolically demanding fast-spiking parvalbumin-expressing (PV+) neurons are highly susceptible to mitochondrial dysfunction.

**Methods:** A novel mouse model of mitochondrial DNA (mtDNA) depletion selectively within the PV+ cells was generated by a conditional knockout of mitochondrial transcription factor A (*Tfam*) gene via *cre-loxP* system. Mice were characterised at behavioural, electrophysiological, neuropathological, and molecular levels. A battery of behavioural tests was used to phenotype the mice, including open field, rotarod, novel object recognition, elevated plus-maze, and visual cliff tests. *In vitro* electrophysiology was performed in acute hippocampal slices CA3 by inducing gamma oscillations via carbachol (cholinergic agonist) and kainate (glutamatergic kainate receptor agonist).

**Approach for statistical analysis:** Shapiro-Wilk normality test was carried out on all data. Unpaired Student's *t*-test was selected for parametric data and Mann-Whitney *U* test for non-parametric data. For repeated measures measurements, repeated measures ANOVA test was chosen. A minimum of  $n = 3$  biological replicates was used per group.

**Results and conclusions:** Mutant mice exhibited a progressive phenotype, initiating at 8 weeks of age with tremor, cognitive impairment (in the novel object recognition test) and anxiety-like behaviour (in the elevated plus-maze test). Hyper-locomotion and stargazing (absence-like seizures) were detected at 10 weeks, with severe ataxia observed by 12 weeks. Hippocampal electrophysiology demonstrated a deficit in gamma oscillations in the knockout group upon cholinergic agonism, and aberrantly high area power of gamma oscillations upon stimulation with glutamatergic agonist kainate. Concomitantly with these data, a loss of calbindin-expressing inhibitory interneurons and a relative preservation of PV+ interneurons was detected in hippocampal CA3 region. Taken together, hyperexcitability is implicated as a feature of this mouse model. OXPHOS complexes I and IV within the PV+ cells of the knockout mice had differential deficiency levels which were brain region dependent. Purkinje neurons showed a reduction in mtDNA copy number, most severe combined complex I and IV deficiencies and modest cell loss. Metabolic rewiring was discovered in Purkinje neurons via an upregulation of

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aldolase C glycolytic enzyme, Krebs cycle enzyme aconitase and anaplerosis enzyme pyruvate carboxylase. The cerebellum and brainstem exhibited secondary reactive microgliosis and astrogliosis. Thalamic reticular nucleus was the second most affected brain region, which is implicated in stargazing behaviour in mice and exhibited combined OXPHOS defects, loss of GAD65-67 and protective perineuronal nets. Knockout mice had reduced weight and severely shortened lifespan. The novel mouse model recapitulates key features of neurological phenotype associated with mitochondrial dysfunction and could be used as a powerful translational preclinical model.



**C08**

**Investigation of the retinal neurovascular unit in diabetic retinopathy using 3D electron microscopy.**

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Diabetic Retinopathy (DR), a leading cause of adult blindness, is a progressive development of microvascular and neurovascular damage in the retina which leads to vision-threatening complications. The disruption of the neurovascular unit (NVU) is a primary factor in the pathogenesis of DR. The NVU is an interdependent unit made up of many cell types including pericytes, endothelium, glial cells and neurons, which work synergistically to maintain the function of the retina and allows adaptation to change in the physiological environment; for example, by regulating blood flow and by maintaining the blood retinal barrier (BRB). In order to assist with improving our understanding of retinal biology, and developing strategies to correct NVU dysfunction, detailed knowledge of the heterocellular interactions of the NVU is required. Serial block face scanning electron microscopy (SBF-SEM), and computational image reconstruction enables the first three-dimensional ultrastructural analysis of the NVU in retinal capillaries. Examination of the data in the x-, y- and z-planes is performed with the use of semi-automated computational image analysis tools including segmentation, 3D image reconstruction and quantitation of cell proximities to provide visualisation and analysis of the data to help explore these issues with respect spatial changes across and within the retina, between species and with disease. Heterocellular relationships within the retina have been assessed in mouse and human tissues in health and diabetes. Initial findings have revealed, with the onset on DR, features of the NVU showed areas of detachment from the Basement Membrane (BM), leaving intermittent gaps between the cell plasma membranes and the BM. Pericyte-endothelial interactions via peg and socket formations in non-diabetic capillaries show both cell membranes in direct contact, however, there appears to be space surrounding the peg in the socket area of diabetic capillaries. Similar electron lucent gaps were present in the endothelium of diabetic capillaries. An increase in the number of leukocytes were present in the luminal space of diabetic capillaries, which were found to make direct communication with endothelial projections. This work provides new information on the ultrastructural changes to the murine retinal NVU during the onset and progression DR, which in turn can serve as a platform to inform future studies on how to delay or prevent the progression of the diseased retina.

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C10

## Understanding the role of microglia during early retinal spontaneous activity and angiogenesis: insights from comprehensive microglial anatomical parameter tracking

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Neurones, blood vessels and glial cells interact at the neurovascular unit (NVU) interface. The development of the NVU and neuronal networks coincide during short but critical developmental periods, suggesting that their development and maturation may be intimately linked from a functional perspective. In the neonatal mouse retina, spontaneous waves of electrical activity consisting of intense bursting activity propagate across the retinal ganglion cell layer, coinciding with the expansion of the superficial vascular plexus during the first postnatal week. At the same time, autofluorescent clusters cell complexes (CCCs) appear under the leading edge of the plexus, disappearing once the plexus reaches the retinal periphery, around postnatal day 8. We hypothesise that these newly discovered transient CCCs are linked to the onset of retinal waves. We propose that they contain cells responsible for the generation of local activity hotspots, which leads to local hypoxia and results in the need for local metabolic energy, supplied by oxygen provided by the growing blood vessels. Once blood vessels reach the activity hotspots, cells responsible for triggering the waves are eliminated via microglia- the resident immune cells of the CNS. The origin of the autofluorescence in the CCCs may reflect metabolic stress as the cells become phagocytosed by microglia. By exploiting the autofluorescent nature of the CCCs, we performed fluorescence-activated cell sorting (FACS) followed by single-cell RNA sequencing (scRNA-Seq) to elucidate the genomic identity of the CCCs. Cluster analysis showed that the CCCs highly express multiple microglial markers, including, *P2RY12*, *SALL1* and *TGFBFR1*, as well as various neuronal markers. This strongly suggests that the CCCs reflect microglia in the process of cellular phagocytosis. Tracking the dynamic microglial behaviour in different compartments of the living retina during the first postnatal week, we can start to understand the function of microglia during this critical period of retinal development.

Using fluorescent time-lapse imaging of microglia in live retinas derived from CX3CR-GFP transgenic mice, we investigate how microglia interact with their neighbours under different pharmacological conditions. EGFPFluro-4(470 nm)16 filter is used to visualise the microglia, CY5 (635 nm)16 is used to visualise the blood vessels stained with isolectin B4 (IB4 594) and finally, DAPI (400 nm)16 filter is used to indicate the position of the cluster cells. Custom-coded algorithms in ImageJ enhance image quality, segment and mask microglial structures, and quantify changes in cell spatiotemporal features. Custom-written Matlab scripts extract crucial information for tracking complex microglial behaviours over time, such as cell area, number of branch points and velocity. Statistical comparisons of these behaviours are made between cells at varying distances from the CCCs.

By looking at various morphological microglial characteristics, we can establish their role during the period of retinal waves and angiogenesis by studying their phagocytic activity and motility at

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different retinal eccentricities, within and outside the vascular plexus, and within or outside CCCs areas.

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## Modelling PRRT2-related epilepsy in human brain slice cultures

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### Background

Disease causing variants in *Proline-Rich Transmembrane Protein 2* (*PRRT2*) are associated with infantile seizures and/or movement disorders and are amongst the most common epilepsy risk genes (Landolfi et al. 2021, Knowles et al. 2022). *PRRT2* is a synaptic protein with a putative role in negatively regulating exocytosis in neurons postnatally and is highly expressed in the human foetal brain (Mo et al. 2019, Li et al. 2016). However, neither its function in early human brain development, nor the underlying pathophysiological mechanisms associated with *PRRT2* loss-of-function variants have been elucidated. Our lab has established a unique model system which allows, for the first time, a study of the developmental consequences of altered prenatal gene function in an intact, developing, human cortical network (McLeod et al. 2023). This model represents a powerful platform for investigating the effects of *PRRT2*.

### Aim

Assess the impact of *PRRT2* loss-of-function on early human development.

### Methodology

Human brain slice cultures were ethically sourced from the Human Developmental Biology Resource ([www.hdbr.org](http://www.hdbr.org)) in Newcastle, which provides samples of 13–18 post-conception weeks (pcw) human foetal cerebral cortex tissue. These cultures can be sustained for several weeks *in vitro* with gross anatomical structures maintained and new synaptic networks forming.

To assess how *PRRT2* changes over time, slice cultures were fixed in 4% paraformaldehyde at different days *in vitro* (DIV) and labelled with antibodies against *PRRT2*. Different short hairpin RNA (shRNA) clones were designed to knock-down (KD) *PRRT2*, and their efficacy was tested in HEK293 cells using both immunocytochemistry and western blot techniques. The most efficient shRNA clone was subsequently packaged into an adeno-associated viral vector and applied to the human slice cultures. These were fixed and the impact of *PRRT2* loss-of-function on the morphology and synaptic connectivity of developing neurons was evaluated.

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## Results

PRRT2 immunoreactive puncta more than doubled, between 0 and 21 DIV, in the subplate region of human cortical slice cultures (n=3 biological cultures,  $p<0.05$ , Kruskal-Wallis test, Dunn's multiple comparison test). We established a clone that diminishes PRRT2 levels by about 50% without affecting cell integrity in HEK293 cells (n=3 passages,  $p<0.05$ , one-way ANOVA, Tukey's post-hoc multiple comparison test), thereby mimicking the haploinsufficient state found in patients. This was further validated with application of the viral vector to the human slice cultures. The gross morphology of subplate neurons, assessed as the number and length of primary processes, was not affected by the reduction in PRRT2 levels (n=1 biological culture, 5-6 slices, unpaired t-test). However, the levels of the postsynaptic glutamatergic marker Homer1 increased following *PRRT2* KD, while the presynaptic glutamatergic marker vGlut1 remained unchanged (n=1 culture, 5-6 slices,  $p<0.05$ , unpaired t-test).

## Conclusion

This model enables us to investigate the role of *PRRT2* in early brain development and assess the consequences of decreased protein levels on evolving early neuronal networks, thereby elucidating potential disease-causing mechanisms. Increased Homer1 levels after *PRRT2* KD could be a result of increased neurotransmitter exocytosis. This will be further investigated using electrophysiological techniques and by increasing the number of replicates.

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**C14**

**Folate binding protein-1 localisation in the plexiform layers of the mouse retina**

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Folate (vitamin B9) is the naturally occurring form of folic acid. Folate cannot be synthesised by the human body so must be acquired through diet/supplementation. Folate carries one-carbon groups for methylation reactions and nucleotide base synthesis making it fundamental for DNA replication, repair, and RNA synthesis. Folate deficiency has been implicated in retinal diseases, including nutritional amblyopia, diabetic retinopathy, exfoliation glaucoma and age-related macular degeneration. In most cases, the exact mechanism of how this contributes to disease is still unknown. There are 3 cellular mechanisms for folate transport: folate receptors, reduced folate carrier and the proton-coupled folate transporter. Folate receptors are attached to the surface of the cell plasma membrane via glycosylphosphatidylinositol. In mice, there are 3 isoforms referred to as the folate binding protein which are analogous to the -a, -b, and -d human forms. RT-PCR, immunohistochemistry and confocal imaging have been used to identify folate binding protein-1 expression in mouse RPE cells, retinal Müller cells and in several layers of retinal tissues, with particularly prominent staining in the outer plexiform layer (Bozard et al. 2010; Smith et al. 1999). To the best of our knowledge, no study has tried to localise any of these folate receptors at the subcellular level in the retina. Such cellular knowledge of folate receptor expression in the retina is critical for understanding their involvement in retinal physiology in health, disease onset and ageing, as well as cell-type specific drug development. Our research will reveal not only whether and which folate receptors are expressed in the retina, but also where they are expressed. Immunohistochemistry analysis (methods previously stated by (Hilgen 2023)) of adult mouse retinal tissue was used to establish localisation of folate binding protein-1 on key retinal cell types, particularly those with dendritic structures extending into the inner plexiform layer (bipolar, amacrine and ganglion cells). Preliminary data shows colocalization of folate binding protein-1 with SMI-32 (alpha retinal ganglion cells) on the dendritic structures in the inner plexiform layer. Experimental procedures were granted ethical approval by Northumbria University and all procedures accorded with current UK legislation.

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## Characterising retinal neurovascular dysfunction in a mouse model of Alzheimer's disease combined with Type 2 diabetes

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### Introduction:

Type-2 diabetes (T2D) is associated with an increased risk of cognitive impairment and Alzheimer's disease (AD) and it is likely that there are common pathways involved in progressive dysfunction of the brain and retina neurovascular unit (NVU). Glial pathology, neurodegeneration and vascular closure are hallmarks of both diabetic retinopathy and AD and, therefore, retinal pathology may be useful to understand brain changes occurring in both T2D and AD. This study assessed retinal pathology in mice models of AD and T2D, and a crossed model of AD and T2D to understand the pathogenesis of this important co-morbidity, especially as it relates to the NVU.

### Methods:

The NVU was assessed in retinal tissue from WT, APP/PS1, db/db and APP/PS1 x db/db mice at 14 and 26 weeks of age (n=4-6 mice per group). Immunohistochemistry was carried out to assess gliosis (GFAP), acellular capillaries (Isolectin-B4/Collagen 4), and Müller cell potassium and water homeostasis (Kir4.1, AQP4). In addition, changes to neuronal populations were assessed by staining for PKC- $\alpha$  (Rod bipolar cells), Calbindin (Horizontal cells), Brn3a (Retinal Ganglion cells) and cone-arrestin (Cone-photoreceptors).

### Results:

We observed evidence of NVU dysfunction in the retina of APP/PS1 x db/db mice including significant Müller cell gliosis at 14 weeks ( $p < 0.05$ ). In addition, Müller cells showed alteration of Kir4.1 and AQP4 localisation in APP/PS1, db/db and APP/PS1 x db/db mice at 26 weeks. APP/PS1 x db/db mice also had significantly more acellular capillaries than WT mice at 14 weeks ( $p < 0.01$ ). NVU dysfunction in the APP/PS1 x db/db retina appears to affect photoreceptors, as a significant decrease in the number of Cone arrestin<sup>+</sup> cells was observed at both 14 ( $p < 0.001$ ) and 26 weeks ( $p < 0.001$ ) when compared to age-matched WT mice. Additionally, a significant decrease in retinal ganglion cells was observed at 26 weeks in db/db ( $p < 0.05$ ) and APP/PS1 x db/db mice ( $p < 0.05$ ) when compared to WT retina.

### Conclusions:

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Overall, we observed evidence of NVU dysfunction in the retina of APP/PS1 x db/db mice. This occurs alongside severe cognitive impairment in this model. APP/PS1 x db/db had Increased gliosis and dysregulation of water and ion homeostasis together with retinal neurodegeneration features such as decreased photoreceptors, horizontal and retinal ganglion cells. Further studies are required to characterise the changes of the NVU in the retina and brain during diabetes related neurodegeneration.



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## Deciphering the mechanisms underpinning retinal vascular cell pathophysiology in a murine model of cerebral malaria

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### Purpose

Infection with *Plasmodium falciparum*, leading to cerebral malaria (CM), remains a major health threat in sub-Saharan African children. Malaria retinopathy (MR) often occurs concomitantly with CM. The brain and retina show similar neurovascular pathology including breakdown of the blood barriers and localised inflammation. This commonality offers a feasible avenue for early detection and prediction of risk and severe disease progression in the cerebral cortex. Animal models of CM have been widely used although the pathological events occurring in the retina have not been well characterised. Moreover, assessing key molecular events occurring in the retina and cerebral cortex during malaria could provide important insights into precisely how the parasite drives localised inflammation and neurovascular pathology. This project has been examining the common pathology between CM and MR in brain and retina in a murine model. In parallel *in vitro* experiments, we have also been assessing the impact of *P. falciparum* infected red blood cell (iRBC) lysates on retinal and brain endothelial barrier integrity.

### Methods

C57BL6 mice were infected with *Plasmodium berghei* ANKA and monitored for up to 10 days post infection. Levels of parasitaemia and CM-related criteria such as abnormal gait, poor reflexes and slow movement were measured daily. In mice with CM and non-affected controls, fundus imaging, optical coherence tomography (OCT) and fluorescein angiography (FA) were performed. Following sacrifice, retinal flatmounts were stained with TER119 (n=4-6), a RBC marker, to assess haemocoagulation in the retinal vasculature. Also in both mouse groups, microglia were labelled using IBA1 and assessed for ramified (resting) or amoeboid (activated) morphology. To measure barrier integrity *in vitro*, brain and retinal endothelial cells, transendothelial electrical resistance was measured using the xCelligence system following addition of *Plasmodium falciparum* lysates.

### Results

When compared to controls, the retinal fundus of mice that had CM (n=8) showed a distinct whitening in the vasculature which appeared to be linked to sequestration of red blood cells in localised regions. No gross changes were observed with OCT in either group. Post-mortem assessments of retinal flat mounts revealed areas of haemocoagulation and vessel occlusion,

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especially in the superficial plexi in the retinas of mice infected with CM. In many cases, this resulted in clear breakdown of the blood retinal barrier, including appearance of RBCs in the neuropile. Parallel *in vitro* studies investigating the effect of *Plasmodium falciparum* lysates on brain and retinal endothelial cells demonstrated a loss of barrier integrity as assessed by transendothelial electrical resistance ( $p < 0.0001$ ).

## Conclusion

*Plasmodium berghei* infected mice are a useful model of MR. In the current study we have characterised some aspects of pathophysiology in the retina of mice with CM. We can draw distinct parallels between the neurovascular changes occurring in the brain during CM and MR in the murine model. We are currently harnessing the power of spatial transcriptomics to further analyse key molecular mechanisms that drive neurovascular pathology in the retinal and brain. .

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C22

## **Altered voltage-gated ion channel expression around drusen accumulations in induced pluripotent stem cell retinal pigment epithelium model of age-related macular degeneration.**

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Altered voltage-gated ion channel expression around drusen accumulations in induced pluripotent stem cell retinal pigment epithelium model of age-related macular degeneration.

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Age-related macular degeneration (AMD) is one of the leading causes of irreversible blindness globally and is associated with increasing prevalence and socioeconomic burden. As a result, additional treatment avenues are under constant investigation. AMD can be characterised into a wet or dry form; wet AMD involves the formation of a pathological neovascular membrane that results in the accumulation of subretinal fluid and/or haemorrhaging and dry AMD is characterised by the presence of protein and lipid deposits, known as drusen, that form beneath the retinal pigment epithelium (RPE). The exact cause of AMD is unknown, although it is thought to originate within the RPE and is considered to be multi-factorial with both genetic and environmental factors playing a role. An overlooked potential contributing mechanism to AMD is the dysfunction of ion channels, or channelopathies, with their involvement in AMD pathophysiology being poorly understood.

Immunohistochemical analysis was carried out on induced pluripotent stem cell (iPSC) derived RPE cells of low and high-risk AMD (Hallam et al., 2017), using pan markers for voltage-gated sodium and calcium channels, alongside antibodies against complement C5b-9 and C3b, markers for drusen depositions in RPE. Initial data showed altered protein expression of the voltage-gated channels around the drusen accumulations, with aggregated staining relative to drusen accumulations compared to more homogeneous staining in the control low-risk RPE cells. Funds for this study were received from the Academy of Medical Sciences (GH) and the ECR support Northumbria University. Acknowledgement goes to the Northumbria Microscopy Lab for their exceptional service and support during the project.

## **Neuroplasticity in Brain Health and Disease: Advances in Techniques, Translation and Education**

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**C26**

**Ageing increases UT-B urea transporter protein abundance throughout the mouse brain**

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Facilitative UT-B urea transporters in the brain play an important role in regulating levels of urea in various cell types, including astrocytes. Numerous studies have reported increased UT-B RNA expression with ageing and in a variety of neurological disorders, such as Alzheimer's Disease, particularly in the hippocampal regions. However, much less is known about the effects of these conditions on UT-B transporter protein abundance and function. This current study therefore compared the levels of both UT-B RNA and UT-B protein in young (3-5 months old) and aged (18-24 months old) male C57BL/6 mice. Animals were euthanized to provide tissue for undergraduate teaching activities. Postmortem brain tissue was subsequently isolated for research purposes in accordance with a tissue-sharing initiative, following approval of the UCD Animals Ethics Committee. All data shown as mean  $\pm$  S.E.M. As expected, endpoint RT-PCR experiments initially showed that the UT-B/GAPDH RNA expression ratio increased in aged mouse hippocampus ( $0.27 \pm 0.12$  vs  $0.95 \pm 0.09$ ,  $P=0.0131$ ,  $N=3$ , unpaired T-Test). Importantly, this change was confirmed at the protein abundance level, as western blotting experiments revealed that the 30-35 kDa UT-B1 signal/protein ratio was also significantly increased in aged mouse hippocampus ( $0.76 \pm 0.21$  vs  $1.83 \pm 0.43$ ,  $P=0.0426$ ,  $N=5-6$ , unpaired T-Test). Further investigations showed similar increases in UT-B1 protein abundance in the cerebellum, frontal cortex and occipital cortex regions (all  $P<0.05$ ,  $N=3$ , unpaired T-Test). In contrast, no such changes occurred in the abundance of MCT1 short chain fatty acid transporters (all NS,  $N=3$ , unpaired T-Test). These data therefore confirmed that specific increases in UT-B1 protein abundance occur throughout the regions of the aged mouse brain. Further studies are now needed to investigate both the precise cellular location and functional consequence of these increased UT-B1 protein levels.