SHERRINGTON TALKS 2020 ONLINE

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

JOIN MICROSOFT TEAMS MEETING

FRIDAY 12TH JUNE

1PM

Presented by DPAG Graduate Students in their 3rd year of DPhil research study

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

UNIVERSITY OF OXFORD

SHERRINGTON TALKS 2020

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1300	**Welcome address by Professor Helen Christian MD, DPAG Director of Graduate Studies** *A Q&A will follow each speaker*		
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1320	**Harvey Davis** Stellate ganglia neurons are intrinsically hyperactive in hypertension, and cardiomyocytes help to compensate	*Professor David Paterson, A/Professor Neil Herring MD*	4
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Judges

Graduate Studies Committee Academics

Prize Giving on Monday 22 June

Andrew Tyler

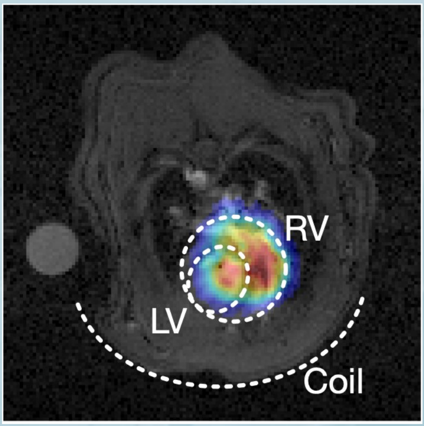
Novel Spiral Trajectories for Hyperpolarized ^{13}C Cardiac MRI

Supervisors: Professor Damian Tyler, Dr Jack Miller, Dr Justin Lau, Dr Ladislav Valkovic

Aim: Both temporal and spatial resolution are critical to interpreting hyperpolarised ^{13}C cardiac metabolic images. High spatial resolution is essential to resolve metabolic heterogeneity in tissue, for example, post myocardial infarction, where the infarct will have markedly different metabolism. Temporal resolution is also critical, there is a growing body of work advocating the use of kinetic modelling or area-under-the-curve calculations, both of which require a high quality time-course, for the determination of metabolic parameters.

Current Hyperpolarized ^{13}C readout strategies require a trade-off to be made between spatial and temporal resolution at acquisition time – This work aims to develop a novel readout which shifts this decision to reconstruction time.

Methods and Results: The proposed pulse sequence was implemented on a Varian 7T pre clinical MRI scanner. Rats were injected with hyperpolarised [1-^{13}C]Pyruvate and imaged using the proposed sequence. The results showed the hyperpolarised signal localised to anatomically plausible regions, with good delineation of the signal. Reconstructions with both high spatial and temporal resolution were made, demonstrating the value of the pulse sequence



Pyruvate signal from the rat heart

Conclusion: The novel hybrid-shot spiral readout was implemented successfully and demonstrated i*n vivo* in a rat heart. Both high spatial, low temporal resolution images and low spatial high temporal resolution images could be reconstructed from the same dataset, providing greater flexibility than was available previously, where a temporal and spatial resolution trade-off had to be made at acquisition time.

Harvey Davis

Stellate ganglia neurons are intrinsically hyperactive in hypertension, and cardiomyocytes help to compensate

Supervisors: Professor David Paterson, A/Professor Neil Herring MD

The activity of cardiac sympathetic nerves from the stellate ganglia is increased in many cardiovascular diseases contributing to the pathophysiology, however the mechanisms underlying this are unknown. Moreover, clinical studies show their surgical removal is an effective treatment, despite the biophysical properties of these neurons being largely unstudied. I have demonstrated that stellate ganglia neurons from prehypertensive spontaneously hypertensive rats are electrically hyperactive, and that M-current downregulation underpins this phenotype. I describe multiple pharmacological mechanisms to ablate this hyperactivity, using single cell RNA-sequencing as a guide. Further to this, I demonstrate that control cardiomyocytes grown in co-culture with stellate ganglia neurons can ablate this activity through a releasable factor. These data provide a new understanding of the sympathetic nervous system in hypertension and may shed light upon how the body can compensate for heightened sympathetic hyperactivity.

Eboni Bucknor

Investigating the function of *Oxr1* in the adult mouse brain

Supervisors: A/Professor Peter Oliver, A/Professor Simon Butt, Dr Silvia Corrochano

Background: Oxidation resistance 1 (Oxr1) is critical for oxidative stress sensitivity in neurons and contains the highly conserved TLDc domain of unknown function. Oxidative stress markers have been associated with many common neurodegenerative diseases and we have demonstrated that over-expression of Oxr1 is protective in cellular and mouse models of amyotrophic lateral sclerosis (ALS). By understanding such oxidative stress response pathways, we hope to identify novel therapeutic targets applicable to numerous neurological disorders. Recently, patients with loss-of-function mutations in *OXR1* have been identified that develop childhood ataxia, epilepsy and cerebellar degeneration. Interestingly, *Oxr1* knockout mice present with selective degeneration of cerebellar granule cells, ataxia and early postnatal death; yet the rapid onset of symptoms means that the significance of Oxr1 function outside of the cerebellum is yet to be described.

Aims: To determine the role of Oxr1 in the adult mouse brain and elucidate the molecular mechanisms of this protein.

Methods: We have generated an Oxr1 inducible knockout mouse model, disrupting all isoforms of the gene, to study potentially later-onset phenotypes in both neuronal and non-neuronal cells in the adult mouse.

Results and Conclusions: We have shown that robust global knockdown of *Oxr1* in the adult mouse leads to a surprisingly severe motor and neurological phenotype, with histopathological analysis revealing significant neuroinflammation across the brain as well as localised cell death in the cerebellum and olfactory bulb. Our data demonstrate for the first time the critical role for Oxr1 in the adult mouse brain.

[1,2] Eboni Bucknor,[1,3] Silvia Corrochano, [1,2] Peter L. Oliver

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Bryan Ng

Investigating Aβ-induced toxicity in tau-deficient human neurons

Supervisors: Professor Richard Wade-Martins, Dr Tara McCaffrey, Dr Natalie Connor-Robson

Aim: We generated the first *MAPT*$^{-/-}$ (tau) human induced pluripotent stem cell (iPSC) lines and studied the effects of tau deficiency in human biological context. The aim is to understand whether the absence of human tau expression results in neuroprotection from Aβ-induced toxicity that is relevant in Alzheimer's disease (AD).

Methods and Results: The isogenic *MAPT*$^{-/-}$ iPSC lines were generated with CRISPR-Cas9 technologies via the delivery of Cas9-gRNA ribonucleoproteins into iPSCs in collaboration with Dr Sally Cowley's lab from the Dunn School of Pathology. We then established a versatile and scalable cortical neuron differentiation protocol which successfully produced a heterogeneous population of functional neurons manifesting cortical identity in co-culture with rat astrocytes. Furthermore, I extracted brain homogenate from an AD patient to serve as the source of extrinsic Aβ in addition to synthetic $A\beta_{1-42}$ oligomers. Various imaging, biochemical and electrophysiological experiments were conducted in these iPSC-derived cortical neurons in order to elucidate AD-relevant phenotypes in vitro.

iPSC-derived *MAPT*$^{-/-}$ cortical neurons exhibited lower firing amplitude and frequency compared to *MAPT*$^{+/+}$ neurons at baseline, while expressing similar number of synapses. On the other hand, *MAPT*$^{-/-}$ neurons demonstrated impaired axonal outgrowth over 5 days of live imaging. Upon extrinsic AD brain homogenate and/or $A\beta_{1-42}$ oligomer insults, *MAPT*$^{-/-}$ neurons showed protection from axonal degeneration, cytotoxicity and hyperactivation as compared to *MAPT*$^{+/+}$ neurons. However, the *MAPT*$^{-/-}$ background was unable to prevent Aβ-induced loss of synapses.

Conclusion: Taken together, the absence of tau expression in human neurons resulted in phenotypic changes at baseline and appeared to result in neuroprotection in *MAPT*$^{-/-}$ neurons from Aβ-induced toxicity.

Britt Hanson

The Application of CRISPR/Cas9 for Molecular Correction Therapy of Neuromuscular Disorders

Supervisors: Professor Matthew Wood, Dr Tom Roberts

Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA) are highly debilitating, fatal and currently incurable hereditary neuromuscular disorders. DMD is caused by out-of-frame mutations in the dystrophin gene, encoding an essential protein for skeletal muscle functioning. Dystrophin is comprised of multiple redundant internal repeat domains and removal of diseased exons could result in production of a truncated, yet adequately functional, protein. Conversely, SMA manifests from knockout mutations within the neuronal survival motor neuron 1 (*SMN1*) gene. This is partially compensated by SMN2 but a C to T polymorphism within *SMN2* exon 7 induces aberrant splicing and only 10-15% functional protein production. DMD and SMA disease genotypes are amenable to splice correction. Current therapies using antisense oligonucleotide-mediated skipping of disease-causing exons, whilst highly effective, require lifelong repeat administration. To circumvent this therapeutic hurdle, this study applies CRISPR/Cas9 gene editing for single-intervention permanent correction of DMD and SMA. The *Staphylococcus aureus*-derived CRISPR/Cas9 system, delivered using clinically safe and effective AAVs, is being employed in a dual-cut strategy for excision of the murine disease causing *Dmd* exon 23 in a severe double knock out mouse model lacking both dystrophin as well as the developmental orthologue, utrophin. Preliminary results show editing in the heart and diaphragm (critical for lifespan extension), and also peripheral skeletal muscles. An ongoing study with increased vector dosing and altered Cas9:guide RNA ratios is being carried out to determine whether the scope of editing and effect on pathology can be further improved. Concurrently, the application of homology independent target integration (HITI)- and microhomology -mediated end joining (MMEJ)-directed knock in of the wild-type *SMN1* cDNA sequence is being investigated for SMA therapeutic development. This is a novel approach to *in situ* genetic correction of SMA, independent of patient genotype. Prime editing of a negative *SMN2* splice regulator is being investigated alongside as an alternative SMA therapeutic strategy with expected higher fidelity. The overarching goal of this study is to contribute towards developing and gaining a deeper understanding of novel CRISPR/Cas9 therapeutic applications for these, and potentially a plethora of other, devastating and currently incurable human genetic diseases.

Britt Hanson[1], Sofia Stenler[2], Anna M Coenen-Stass[3], Nina Ahlskog[1], Matthew J Wood[1], Thomas C Roberts[1]

James Rowland

Generalisation of stimulus representation across somatosensory cortex areas in a cellular-resolution photostimulus detection task

Supervisor: Dr Adam Packer, A/Professor Simon Butt, Dr Michael Kohl

Mice use whiskers to explore their environment. Whisker stimulation elicits a neural response in primary (S1) and secondary (S2) somatosensory cortex, two highly interconnected and hierarchically organised brain regions. Their interaction has been related to stimulus detection, although its precise functional role remains unclear. Here, we interrogate this circuit during a stimulus detection task, by assessing how S1-S2 interactions facilitate stimulus perception.

We have conditioned mice to detect 2-photon optogenetic stimulation of random ensembles of S1 cells. This allows us to control the number of stimulated cells on a trial by trial basis, and to separate the initial stimulus representation from the ensuing network response. Simultaneously, we record the calcium activity of both stimulated and unstimulated cells in S1 and S2, rendering an all-optical approach to study neural dynamics. In short, we are able to directly stimulate S1 neurons, hence defining the initial stimulus in S1, while recording the subsequent S1 and S2 neural response.

We observe elevated, sustained neural population activity in both S1 and S2 after perceived photostimulation of S1. This hints that the somatosensory system may encode information by propagation of long-lasting activity between cortical regions. Hence, we uncover a putative mechanism of how interregional communication can transform stimulus information to facilitate stimulus detection.

Tony Zhou

Improved Multinuclear MRI with Novel Radiofrequency Coil Design

Supervisor: Professor Damian Tyler, Mr Chris Randell, Dr Jack Miller, Dr Justin Lau

Aim: X-nuclei magnetic resonance imaging (MRI) provides additional metabolic information on top of conventional ^{1}H MRI with MR-active isotopes such as ^{13}C and ^{31}P. However, low concentrations of X-nuclei result in inherently low signals. We propose a novel radiofrequency (RF) coil design with 30% increased signal reception to offset the limitations of multinuclear MRI.

Methods and Results: Advancements in computational power has allowed for electromagnetic (EM) simulations to determine the performance of complex RF coil conductor designs. A novel 'multilayer' coil design was investigated consisting of overlapping conductors to see an increased magnetic flux over a central region of interest. Experimentally obtained magnetic field profiles of the multilayer coils were compared with conventional coils in a clinical MRI scanner.

Conclusion: Surface coil designs have largely remained stagnant, comprised of simple circular and square loop conductors. Multinuclear MRI demands a greater coil sensitivity due to lower signal from the X-nucleus. A novel multilayer RF coil tailored for high sensitivity was designed from the ground up using EM simulations. Both EM simulations and on-scanner experiments show the multilayer coil boasts a 30% increase in signal over conventional surface RF coils.

Snapper Magor-Elliott

A new technique for diagnosing pulmonary embolism

Supervisor: Professor Peter Robbins

Our overarching hypothesis is that detailed measurement of the profiles by which respiratory and/or tracer gases emerge from the lung contains information on the health and functioning of the pulmonary blood vessels. This information can then be recovered using a novel computational model of the cardiopulmonary system to estimate volumes of the lung that are ventilated but not perfused as in pulmonary embolism. Our group had previously modelled the lung using this data successfully. However, a weakness in this model was the estimate for the mixed venous input to the lungs and the use of an open loop circulation. To remedy this, I created a detailed and scalable physiological model of the circulation and body gas stores (CBGS model) to account for this key component of the physiology in our detection of pulmonary embolism. Initial validation of the CBGS model was achieved both through simulations of experiments in the literature and those undertaken in our laboratory. The basis for our novel pulmonary embolism detection technique involves using a combined lung-CBGS model with data collected from our prototype molecular flow sensor during a washout protocol.

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