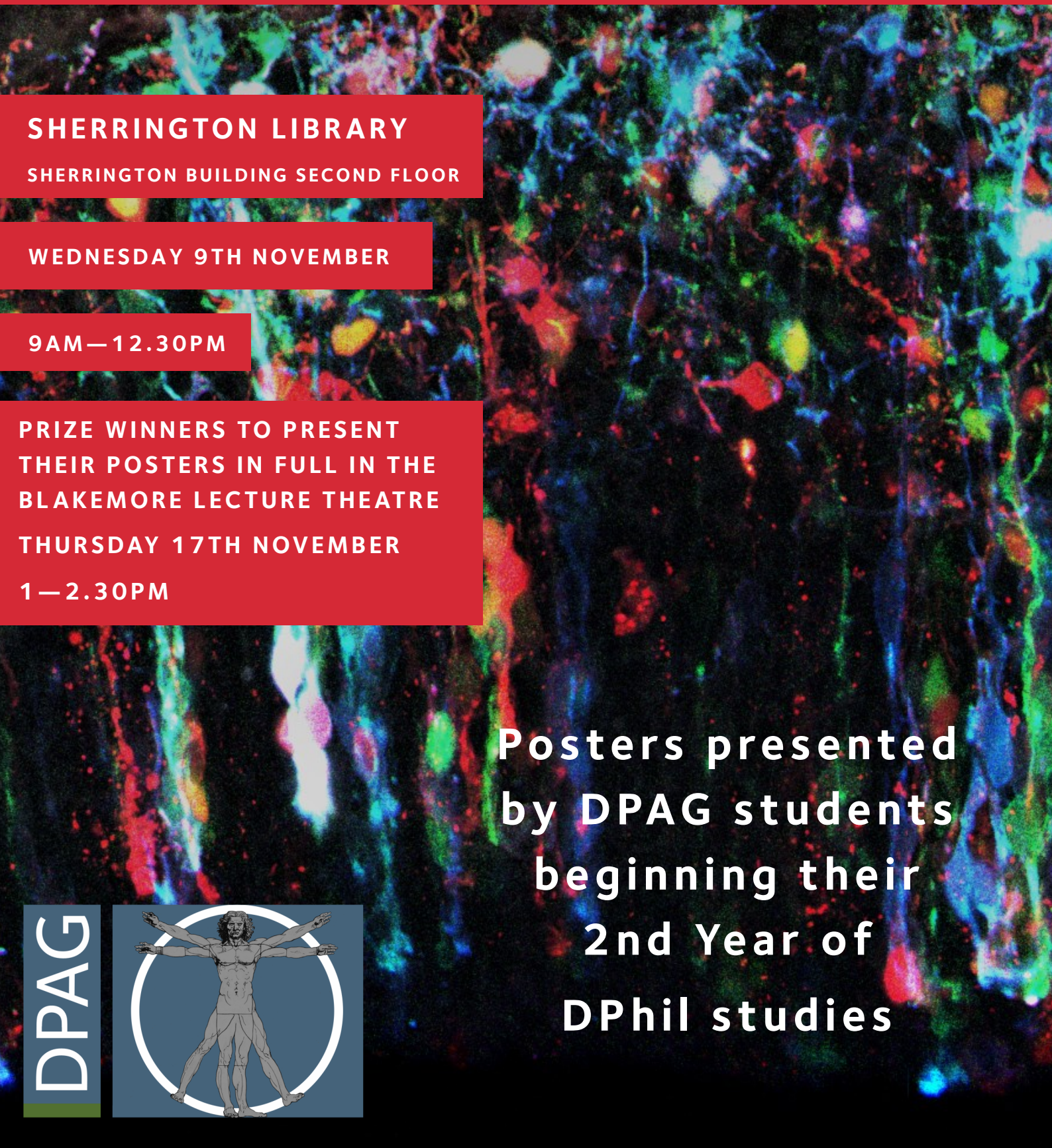
# SECOND YEAR STUDENTS POSTER DAY 2022

# 'A YEAR OF PROGRESS'

## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS



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**WEDNESDAY 9TH NOVEMBER**

**9AM—12.30PM**

**PRIZE WINNERS TO PRESENT THEIR POSTERS IN FULL IN THE BLAKEMORE LECTURE THEATRE**

**THURSDAY 17TH NOVEMBER**

**1—2.30PM**

Posters presented by DPAG students beginning their 2nd Year of DPhil studies

DPAG

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Lea Ballenberger**

## Dissecting the molecular mechanism of the sleep homeostat in Drosophila melanogaster

Supervisors: Professor Gero Miesenböck & Professor Stephen Goodwin

Sleep is crucial and sleep deprivation has detrimental effects on the organism. Although sleep is present in all animals studied so far, the precise mechanism by which it is induce has not been fully understood. To study the molecular mechanism, we use the fruit fly Drosophila melanogaster as a model organism which allows us to perform genetic manipulations and electrophysiological recordings in vivo.

Neurons projecting to the dorsal fan-shaped body (dFB neurons) have been identified to be the output of the sleep homeostat. Their electrical excitability changes depending on the sleep pressure of the animal and is influenced by potassium currents via the leak channel Sandman among others. Sandman channels are hypothesised to translocate from intracellular storage vesicles to the plasma membrane, likely under the control of the RhoGAP crossveinless-c (cv-c).

### Research Aim

This project aimed to understand the interaction between the two key players Sandman and cv-c and their influence in sleep and electrical excitability.

### Methods and Results

I performed behavioural experiments in the fly to study changes in sleep as a consequence of the lack of Sandman and/or cv-c. The sleep loss of cv-c mutants can be rescued by a simultaneous knock-down of Sandman, suggesting that Sandman is locked in the membrane in the absence of cv-c. The same conclusion can be draw when studying potassium currents and spiking activity from dFB neurons.

### Conclusion

Together, these results support the interaction of cv-c and Sandman in regulating excitability of dFB neurons as well as sleep behaviour of the fly.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Dr Mona Abdelfatah Mokhtar Mohamed Barkat**

### 3D-Printed Cortical Columns to Repair Traumatic Brain Injury

Supervisors: Professor Zoltán Molnár & Associate Professor Francis Szele

Traumatic brain injury (TBI) is considered a catastrophic clinical issue because of its devastating morbidity and mortality. Disappointing outcomes of the pharmacological clinical trials motivate the identification of new potential therapeutic approaches.

Nowadays, cell-based therapy for TBI is considered a highly promising strategy. However, the laminar organization of the cerebral cortex makes pre-assembling the transplanted neurons in the form of a 3D layered-neuronal implant a point being considered before implantation.

I hypothesize that transplantation of a 3D layered-neuronal implant would improve the outcome of traumatic brain injury. The study comes with three objectives, generation of layer-specific cortical neurons, fabrication of 3D layered-neuronal implants, and transplantation of the implants into a stab wound TBI.

The generation of layer-specific cortical neurons is an important prerequisite for 3D layered-neuronal implants. iPSCs derived-upper and lower cortical neurons were generated having excitatory glutamatergic and inhibitory GABAergic phenotypes. The efficacy of differentiation was assessed by morphological analysis, gene expression profiling, and immunofluorescence.

Upper and lower cortical neurons were fabricated into the 3D neuronal constructs using either a 3D printer home-modified to print cells using Matrigel-based bio-ink (Zhou et al, 2020) or microfluidic technique. The 3D implants survived for months in vitro and showed proliferation and continuous differentiation of the cells. Further neuronal activity will be assessed by Ca+ imaging and electrophysiological recording.

Implantation of the 3D layered-neuronal implant into TBI immunodeficient C57BL mice will be established and followed by an assessment of the proliferation, differentiation, and of integration of the implant into functional neural circuits.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Jemima Becker**

## Casc15 lncRNA regulates subventricular zone neurogenesis

Supervisors: Professor Zoltán Molnár & Associate Professor Francis Szele

Many long non-coding RNAs (lncRNAs) are expressed in the postnatal ventricular-subventricular zone (V-SVZ) of the mammalian brain. Neural stem cells (NSC) in the V-SVZ have been extensively studied, yet how epigenetic molecular mechanisms regulate their function is unclear. The lncRNA Casc15 (2610307P16Rik) is enriched in neural stem cells and neuroblasts, and is highly conserved between mouse and human.

### Research Aim

To establish whether the lncRNA Casc15 regulates neurogenesis, and by what mechanism it does this.

### Methods and Results

Based on a range of open-access online data, Casc15 is predicted to regulate V-SVZ neurogenesis and stem cell function. Fluorescence in-situ hybridization (FISH) revealed both nuclear and cytoplasmic localization of Casc15 in NSCs isolated in vitro from the V-SVZ. Depletion of Casc15 transcripts using siRNA in vitro leads to altered expression of many genes that regulate neurogenesis such as Ascl1/Mash1, Pax6, and Dcx.

Depletion of Casc15 transcripts using anti-sense oligonucleotides (ASOs) increased SVZ NSCs proliferation and enhanced V-SVZ NSC migration in Matrigel in vitro, but also triggered rapid death of pups in vivo.

RNA-sequencing after Casc15 ASO knockdown suggested that Casc15 regulates several neurodevelopmental genes. Additionally, the expression levels of genes involved in the mevalonate pathway such as Hmgcr, Hmgsc1, and Soat1, were increased after the loss of Casc15.

### Conclusion

These data suggest Casc15 has myriad intersecting gene regulatory network functions and that it regulates neurogenesis via modulation of cell migration, through a currently unclear epigenetic mechanism.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Féodora Bertherat**

## iPSC-derived neurons from sporadic Alzheimer's disease patients reflect their clinical vulnerability

Supervisors: Professor Richard Wade-Martins, Dr Nora Bengoa-Vergniory, Dr Sally Cowley

Most Alzheimer's disease (AD) research has been focused on familial AD (fAD), even though sporadic AD (sAD) accounts for over 90% of cases. This is in part due to the clear genetic causes of fAD, which makes it easier to model. Although useful, these models do not allow us to investigate the mechanisms and pathways leading to the development of sAD. It is therefore essential to develop reliable sAD models.We have generated 14 iPSC lines from sAD patients from the Deep and Frequent Phenotyping study, which has collected a battery of clinical data from early-stage sAD patients.

### Research Aim

The aim of this project is to compare cellular phenotypes observed in iPSC-derived cortical neurons of sAD patients with their clinical data and determine whether they reflect their clinical vulnerability.

### Methods and Results

We have generated 14 iPSC lines from sAD patients from the Deep and Frequent Phenotyping study, which has collected a battery of clinical data from early-stage sAD patients. Phenotypes such as neurite loss or cytotoxicity were measured in iPSC-derived cortical neurons following Amyloid Beta oligomer treatment. We observed that AD patient lines exhibit different levels of neurite loss due to amyloid beta, which correlates with their clinical vulnerability.

### Conclusion

Sporadic AD progression and vulnerability to amyloid beta load varies between patients. Here, we show that the vulnerability rating observed in clinical data correlates with the vulnerability of individual patients lines in response to Amyloid Beta treatment.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Benjamin Breant**

## The short-lasting hybrid state of wakefulness induced by psychedelic compound 5-MeO-DMT

Supervisors: Professor Vladyslav Vyazovskiy, Professor David Banner & Professor Trevor Sharp

### Research Aim

The traditional view that the serotonergic system plays an important role in controlling global sleep-wake states is supported by observations that administration of serotonergic psychedelics has a wake-promoting effect. However, the possibility that potentiating the serotonergic system through psychedelics results in an occurrence of altered states of vigilance has received less attention. Here we tested the hypothesis that serotonergic psychedelics alter fundamental characteristics of sleep and waking, rather than merely affecting the time spent in a specific state of vigilance.

### Methods

We performed chronic EEG and EMG recordings in freely-behaving laboratory mice (n = 7) after injection of a short-lasting psychedelic compound, 5-methoxy-N,N-dimethyltryptamine (5 MeO-DMT, 5 mg/kg) at the beginning of the light period.

### Results

We observed that 5-MeO-DMT administration resulted in an awake state characterised by altered EEG patterns as reflected in increased sleep-like spectral power in slow frequency range (9.11 % to 48.60 %) in the first 30 minutes following the injection. The effects were short-lasting and largely dissipated 1 hour after the injection.

### Conclusion

Our data support the notion that the effects of 5-MeO-DMT are short-lasting. Importantly, this compound did not merely change the amount and distribution of vigilance states but had an observed effect on state-specific brain activity patterns. Reduced theta-activity and increased slow wave activity during waking after administration of 5-MeO-DMT reflect an occurrence of qualitatively different, hybrid and paradoxical vigilance state, having features of both waking and sleep.

This project was supported by a BBSRC Scholarship. The Compound was provided by Beckley Psytech.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Dr Zelie Britton**

## Redox balance and sleep: A deuterated polyunsaturated fatty acid diet in mice

Supervisors: Professor Gero Miesenböck & Professor Vladyslav Vyazovskiy

### Research Aim

Many studies have implicated the role of oxidative stress in sleep. In Drosophila, the redox-sensitive Hyperkinetic-Shaker potassium channel within the outer cell membrane transduces oxidative stress into increased excitability of sleep-active neurons. Administration of deuterated polyunsaturated fatty acids (D-PUFAs), that reduce the propagation of oxidative free radicals within the cell membrane lipid bilayer, reduced total sleep duration in Drosophila. This study examined the effects of D-PUFAs on sleep in mice.

### Methods and Results

Wildtype C57BL/6 mice were fed D-PUFA (n=14) and control (n=17) diet from 3 weeks prior to conception (maternal line) until experimental endpoint. Mice underwent electroencephalography and electromyography probe implantation at 10 weeks and subsequent recordings were obtained during 72 hours of baseline sleep-wake cycle, 6 hours sleep deprivation and a further 66 hours of sleep-wake cycle. Post-experiment, brain samples were flash frozen for mass spectrometry analysis.

There was no difference in baseline or post-sleep-deprivation sleep between the two cohorts (fixed effect first order autoregressive model). Mass spectrometry confirmed 34% incorporation of deuterated polyunsaturated fatty acids into the brain.

### Conclusion

The lack of difference in sleep between the two cohorts may have several explanations: 34% incorporation does not sufficiently reduce lipid peroxide production; the limiting factor in sleep pressure is not the lipid peroxides but number of Hyperkinetic-Shaker complexes; the deuterated fatty acids were located in the incorrect subcellular compartment; or, that the hypothesised mechanism (sleep deprivation leading to increased membrane lipid peroxides that activate the Hyperkinetic-Shaker channel and thus lead to increased sleep) is incorrect.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Marcos Castro Guarda**

## Beyond the Krebs cycle: Fumarate reprogrammes metabolic preferences within human cardiomyocytes

Supervisors: Associate Professor Lisa Heather, Dr Kerstin Timm, Ms Claudia Montes Aparicio

### Research Aim

Intracellular fumarate concentrations increase dramatically in the heart during ischemia. Fumarate can activate Nrf2 (Nuclear factor erythroid 2 [NF-E2]-related factor 2), a transcription factor that regulates antioxidant enzyme expression. However, there is evidence in the liver that Nrf2 can also regulate metabolism. Therefore, the purpose of this study was to evaluate if exogenous fumarate derivatives can influence cardiomyocyte metabolism, repurposing a compound already approved for use in humans for the treatment of autoimmune diseases.

### Methods

Matured human iPSC-derived cardiomyocytes (hiPSC-CM) were treated with dimethyl fumarate or hydrogen peroxide. The effect on gene and protein expression was evaluated using qPCR and western blotting. Palmitate oxidation and glycolytic rates were measured radioactively. Mitochondrial oxygen consumption was measured using a Seahorse analyser.

### Results

In human cardiomyocytes, dimethyl fumarate metabolically reprogrammed cardiac substrate utilisation by decreasing genes involved in fatty acid metabolism, followed by upregulating glucose metabolism genes. This resulted in decreased palmitate oxidation rates and increased glycolytic rates and lactate production. No changes in the mitochondrial enzymes or respiration were detected, indicating a shift in fuel preference within the cardiomyocyte. To understand the mechanism driving this metabolic reprogramming we showed that dimethyl fumarate promoted Nrf2's nuclear translocation. When we exposed cells to hydrogen peroxide to induce oxidative stress, we found the same metabolic shift from fat to glucose metabolic genes.

### Conclusion

Dimethyl fumarate emerges as a key molecule to modulate cardiomyocyte metabolism, decreasing fatty acid oxidation and increasing glycolysis. Our data indicates this is via the same Nrf2 mechanism activated by oxidative stress.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Juliana Choi**

## The role of octopamine in Drosophila melanogaster learning and memory

Supervisors: Professor Scott Waddell & Dr Pedro F. Jacob

The Drosophila melanogaster octopamine receptor oamb has previously been identified as a key receptor in appetitive short-term memory (STM) with only sweet but not nutritive sugars, like arabinose. We hope to characterise the role of other octopamine receptors in mediating memory, and identify whether they only mediate memory with arabinose. We knocked down the 6 octopamine receptors in the majority of dopaminergic neurons (DANs), and we found that RNAi Knockdown of Octβ2R in γ4 DANs impacts appetitive long-term memory (LTM).

**Ríona Devereux**

# Chemical tools to understand fatty acid uptake, trafficking, metabolism and signalling in type 2 diabetes

Supervisors: Associate Professor Lisa Heather, Professor Angela Russell & Dr Josie Gaynord

## Research Aim

Fatty acids are the predominant fuel in the body, with many important functions. Too much fatty acid metabolism can contribute to many diseases including the cardiac complications that occur in type 2 diabetes. Despite the prevalence of clinical data demonstrating this role of fatty acid metabolism in type 2 diabetes, we do not currently understand with which proteins the fatty acids interact within cells and its impact on the disease. Therefore, this project aims to develop chemical probes to begin to look into which long chain fatty acids or metabolites are most prevalent in type 2 diabetes and with which biomolecules do these long chain fatty acids/metabolites interact with.

## Methods and Results

A palmitic acid photoaffinity probe (containing both a diazirine photo-crosslinking group and a pulldown "Click" alkyne handle) and photocatalytic probe (μMap) have been successfully synthesised and fully characterised. The photoaffinity probe has been used in preliminary experiments in MDA-MB-231 cells. A concentration series of probe has been carried out and demonstrated a concentration-dependent increase in proteins interacting with the probe following an 18-hour incubation, as visualised using SDS-PAGE and in-gel fluorescence.

## Conclusion

The proteins captured without UV radiation are likely due to due to metabolic incorporation of probe 1 into the protein structure (e.g., post-translational palmitoylation). UV exposure increased the number of proteins identified as interacting with the fatty acid probe, demonstrating that the inclusion of the photo-crosslinking diazirine group improved our ability to detect lipid interacting partners.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Lucille Duquenoy**

## Exploring how presynaptic cholinergic input modulates dopamine release in mice and Drosophila melanogaster

Supervisors: Professor Scott Waddell & Professor Stephanie Cragg

### Research Aim

Dopamine (DA) is a neuromodulatory transmitter that plays a central role in reward prediction error, decision-making and goal directed behaviours. In the mouse striatum, local cholinergic input to dopaminergic axons is known to alter dopamine release differently depending on the dopaminergic axons' activity. Such modulation is mediated by nicotinic acetylcholine receptors (nAChRs) located on DA axons. However, complex circuitry within the striatum makes it hard to understand the mechanisms underlying these interactions.

The Drosophila mushroom body (MB) is a centre for olfactory learning that also has pertinent ACh-DA interactions which have been shown to be involved in aversive learning through cholinergic modulation of DA release through nAChRs.

This project aims at discovering molecular mechanisms through which this ACh-DA modulation operates and the function it provides.

### Methods and Results

To understand DA and ACh dynamic in behaving flies, we imaged MB neurones expressing GRAB sensors in tethered Drosophila. In parallel, we used FCV and GRAB sensors in mouse brain slices to monitor respectively DA and ACh to investigate molecular mechanisms downstream of nAChR activation on DA axons, including voltage-gated ion channels. We found that blocking voltage gated potassium partially mimics nAChR activation on DA neurons, and that availability of sodium channels could modulate DA release through nAChRs.

### Conclusion

We are exploring the potentially conserved parallels and mechanisms underlying the ACh regulation of DA release in flies and mouse. Our inter-phylum approach could uncover common function of cholinergic modulation of DA release by linking molecular mechanisms to behaviourally relevant signals.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Milda Folkmanaite**

## Modelling and in silico simulation of human induced pluripotent stem cell derived cardiomyocyte electro-mechanical properties

Supervisors: Professor Manuela Zaccolo, Professor Blanca Rodriguez & Dr Xin Zhou

### Background

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) enable accessible human data-based cardiology studies. However, a caveat in hiPSC-CM-based studies is their immaturity. One of the modifications occurring during hiPSC-CM maturation is the change in myofilament calcium sensitivity, an important indicator of cardiac muscle function. In silico hiPSC-CM investigations could help improve understanding of the hiPSC-CM-specific contractile behaviour and its changes during maturation.

### Research Aim

To facilitate hiPSC-CM-based investigations, we develop an electromechanical human data-based iPSC-CM computer model. We use the model to investigate the effects of the changes in myofilament calcium sensitivity.

### Methods

We establish the model by coupling a published hiPSC-CM electrophysiological model with a model of the human adult cardiomyocyte contractile machinery. The model is calibrated using experimental hiPSC-CM data.

### Results

The model successfully reproduces hiPSC-CM contractile phenotype with peak twitch tension of 0.44 kPa which takes 201 ms to peak and 164 ms to achieve 50% relaxation, which all agree with the experimental hiPSC-CM values. The sensitivity analysis of the model shows an increase in active tension amplitude with a decrease in calcium transient peaks upon increased myofilament calcium sensitivity. Large increases in myofilament calcium sensitivity result in depolarization failure with low amplitude fluctuations of membrane voltage, calcium transient and active tension.

### Conclusion

Altogether simulation results demonstrate the usability of the model for simulating and exploring not only physiological, but also pathological cardiac conditions.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Rachel Héon-Roberts**

## A dual genome-wide CRISPRi and CLEM approach to investigate the effects of aggregating proteins in neurodegeneration

Supervisors: Professor Richard Wade-Martins & Dr Brent Ryan

### Research Aim

Alzheimer's disease (AD) and Parkinson's disease (PD) are two of the most common neurodegenerative disorders associated with ageing. Protein aggregation is a hallmark of both diseases, with PD showing α-synuclein inclusions, and AD showing amyloid-β plaques. Despite overall divergent aetiologies and symptomology, both mitochondrial dysfunction and lysosomal-autophagy pathway impairment have been implicated in the cellular pathology of both diseases. However, the mechanisms by which amyloid-β and α-synuclein are involved in these pathological cascades are not yet understood.

### Methods and Results

To investigate the effects of these aggregating proteins on mitochondria and lysosomes, we are using correlative light and electron microscopy (CLEM) to evaluate the ultrastructural morphology of these organelles in the presence and absence of pre-formed fibrils of α-synuclein, or amyloid-β oligomers. Preliminary light microscopy experiments show changes to the overall state of fusion and fission of the mitochondrial network, as well as changes to lysosome abundance, but not morphology, in the presence of α-synuclein aggregation. To obtain unbiased mechanistic information, we will also conduct a genome-wide pooled CRISPR interference (CRISPRi) screen to detect genes and pathways involved in promoting or mitigating the toxicity of α-synuclein and amyloid-β. In preparation, we have verified that single guide RNA expression is still present after a month in culture, and we are conducting pilot flow cytometry optimisation experiments to assess the suitability of candidate screening assays.

### Conclusion

Using the dual CRISPRi and CLEM approach is expected to provide new mechanistic insight into the effects of α-synuclein and amyloid-β in neurons.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Sebastian Klavinskis-Whiting**

## A hierarchical recurrent temporal prediction network captures many aspects of visual cortex

Supervisors: Professor Andrew King, Professor David Dupret & Dr Nicol Harper

### Research Aim

A major goal of neuroscience is to identify whether there are generalised principles that explain the diverse structures and functions of the brain. The principle of temporal prediction provides one approach, arguing that the sensory brain is optimised to represent stimulus features that efficiently predict the immediate future. Previous work which has optimised a hierarchical model for temporal prediction has demonstrated that the hierarchy of model units resembles those of neurons along the visual pathway (Singer et al., 2018). Building on this work, I aimed to better account for the dynamical aspects of visual cortex by incorporating recurrency into a hierarchical temporal prediction model.

### Methods and Results

The model was implemented as a recurrent neural network and was trained on a large dataset of naturalistic video clips to minimise the 'temporal prediction error' – the first group predicted the next video frame in the sequence while higher-order groups predicted the future activity of the preceding group. In addition to capturing the tuning properties of visual neurons, the trained model can account for perceptual effects such as motion optical illusions and can explain aspects of functional connectivity in visual cortex. Moreover, the better the network predicts the future sensory input, the more similar its internal representations are to those of the mouse visual cortex.

### Conclusion

A recurrent network model optimised for temporal prediction can capture many aspects of the mammalian visual system. In this way, temporal prediction shows potential for describing information processing across the sensory hierarchy.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Samuel Cian Liebana Garcia**

## Long-term Learning: Theory meets Experiment

Supervisors: Dr Armin Lak, Dr Andrew Saxe & Professor Rafal Bogacz

### Research Aim

Learning to make decisions underlies many aspects of our behaviour. As such, understanding the neural mechanisms of learning is a fundamental goal in systems neuroscience. However, despite proliferating theories and large datasets, testing theories of learning remains challenging. Experiments often only approximately control the learning epoch, making interpretation of learning trajectories difficult; and modern theories of learning (such as deep RL networks) are often complex and hard to fit to data.

### Methods and Results

Here we address this gap with a three-fold approach: first, we introduce a perceptual decision-making paradigm for mice that remains unaltered across the whole learning epoch, such that the learning period is well-controlled. Second, we record and analyse dopamine release across dorsal striatum to investigate its role in the mice's learning of the paradigm. Third, we describe how to link the behavioural and neural findings through a simple reinforcement learning framework which respects the circuit connectivity of cortico-striatal projections. This last stage is still under development, as we finalise our verification of the behavioural role of the neural signals.

### Conclusion

More broadly, our work takes a step towards linking modern theories of learning to large-scale data being generated by systems neuroscience, offering a potential route to testing theories of learning in the brain and mind.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Jessica Livesey**

## Investigating the regulation of striatal dopamine release by striatal noradrenaline at adrenoreceptors

Supervisors: Professor Stephanie Cragg, Dr Jeffrey Stedehouder & Dr Mark Walton

### Research Aim

Release of dopamine (DA) in the striatum is critical to the regulation of action selection and motivation. DA neurons have extensively branched axons, which form a major site for the regulation of DA release by striatal neuromodulators. The striatum is sparsely innervated by noradrenergic neurons, and evidence suggests noradrenaline (NA) might regulate DA release in the striatum via adrenoreceptors. However, it is unresolved whether the effects of NA on striatal DA release are mediated by direct actions on DA axons or indirectly via other cells. Here, we investigated the regulation of DA transmission by striatal adrenoreceptors.

### Methods and Results

We detected electrically evoked DA release at carbon-fibre microelectrodes using fast-scan cyclic voltammetry in mouse striatal slices following the pharmacological manipulation of adrenoreceptors. The alpha-1 receptor agonist phenylephrine decreased evoked DA release by ~30-40% in the dorsolateral striatum and nucleus accumbens core, while the beta receptor agonist isoproterenol increased evoked DA release by ~5-10% in both regions. Additionally, we are exploring striatal NA dynamics by imaging the genetically-encoded GPCR activation-based NA sensor, GRABNE2h, in striatal slices. GRABNE2h fluorescence reported rapid electrically evoked transients, and was responsive to exogenous NA but not DA. We are characterising the contribution to these signals of endogenous NA versus DA, with a view to further exploring striatal NA signalling and its regulation.

### Conclusion

Overall, we aim to increase understanding of the role of striatal NA in the regulation of DA transmission using novel tools to provide high temporal and spatial resolution of neurotransmitter release.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Elisa Martin Perez**

## Genome-wide CRISPR Screening to Identify Regulators of Hepatic Insulin Signalling

Supervisors: Associate Professor Robin Klemm & Dr Anna Veprik

### Research Aim

Insulin is the central anabolic hormone in the human body. By modulating rates of hepatic glucose output and glucose uptake into neurons, muscle and fat tissue, it is at the core of blood glucose homeostasis, ensuring normoglycemia during fasting and feeding. The liver is one of the three major tissues that acts in sustaining constant blood glucose levels. Hence, hepatic insulin action provides an important layer of regulation in the maintenance of whole-body metabolic homeostasis, and its dysregulation is closely associated with metabolic disease. However, the mechanisms of hepatic insulin signalling and its integration in metabolic regulation are, surprisingly, not completely understood. This project is focused on identifying novel genes involved in hepatic insulin action and aims at a more detailed molecular understanding of how defects in the signalling pathway leads to insulin resistance, which is a major risk factor in the pathogenesis of multiple metabolic diseases.

### Methods, Results and Conclusion

To systematically study the insulin signalling network, I will carry out a genome-wide loss-of-function pooled CRISPR screen using hepatocytes under basal and insulin resistance conditions as a model system. The main read out is based on the nuclear-cytoplasmic shuttling of a fluorescent insulin signalling reporter belonging to the Forkhead Box family of transcription factors called FoxO1-Clover. I have developed and validated an experimental pipeline that uses fluorescent activated nuclear sorting as a novel approach for pooled CRISPR screening with subcellular resolution for hit scoring. Consequently, I am now in the process of analysing and validating experimental results from the screen.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Olivia McGinnis**

## A mating-state dependent physiological switch in the connection between egg-laying circuitry and the mushroom body

Supervisors: Professor Stephen Goodwin, Professor Scott Waddell & Dr Megan Goodwin

After mating, many female animals exhibit marked changes in internal state and behavior. Mating-induced changes in odor preference, for example, bias female choice to accurately reflect reproductive state, including the decision to mate, meeting post-mating nutritional requirements, and where to lay an egg.

### Research Aim

Here, we investigate a mating-state dependent physiological change in the connection between egg-laying circuitry and the mushroom body (MB), a higher order associative learning center, in Drosophila. In females, glutamatergic aDN neurons process olfactory information and are required for olfactory-mediated communal egg-laying. Reward-signaling protocerebral anterior medial (PAM) dopaminergic neurons innervating the MB modulate MB output neurons (MBONs), which encode information about valence critical for action selection. This project aims to reveal (1) whether circuit level phenomena or single synapse level postsynaptic changes, possibly in glutamate receptor profile, underly this switch, (2) whether aDNs respond in a mating-state dependent manner to relevant olfactory stimuli, and (3) the behavioral consequence of this switch for females.

### Methods and Results

Connectomic analyses and anatomy showed aDNs output strongly onto a subset of PAMs and MBON-y5B'2a. Using 2-photon calcium imaging, we found that in virgin females, optogenetic stimulation of aDNs excites y4 PAMs and elicits no response from MBON-y5B'2a while in mated females, this stimulation inhibits y4 PAMs and excites MBON-y5B'2a. Further imaging will reveal whether nodes in this circuit respond differentially to odors before and after mating. RNA sequencing and smFISH will be used to probe possible underlying transcriptional changes.

### Conclusion

These data suggest that the experience of mating drives specific changes in the physiology between egg-laying and MB circuits in female Drosophila. This may represent a neural mechanism by which internal state biases odor preference to ultimately guide adaptive choice.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Bethan O'Connor**

## Convergent regulation of presynaptic short-term plasticity in striatal dopamine release by dopamine transporters and GABA receptors

Supervisors: Professor Stephanie Cragg & Dr Katherine Brimblecombe

### Research Aim

Axonal dopamine (DA) release within the striatum is not an accurate reflection of action potential activity – rather, it exhibits short-term plasticity, ranging from short-term facilitation at short inter-pulse intervals, to short-term depression at longer intervals. Both the DA transporter (DAT) and γ-aminobutyric acid receptors (GABA-Rs) have been shown to modulate this plasticity. The DAT is electrogenic, with transport of DA associated with membrane depolarisation, which limits axonal re-activation and drives release-independent depression, strongly supporting the frequency dependence of DA release. GABA provides an endogenous GABA tone at GABA-Rs that limits DA release, with GABAA-Rs known to limit action potential propagation and spike amplitude via a paradoxical depolarisation inactivation. Despite limiting DA signal amplitude, GABA-Rs also slightly supports the frequency dependence of DA release. We aimed to assess whether there is a co-operation or interdependence between these mechanisms in supporting the dynamics of short-term plasticity in DA release.

### Methods and Results

We used fast-scan cyclic voltammetry to detect dopamine evoked by electrical stimulation in mouse striatal slices. GABA -R antagonists attenuated the effect of DAT inhibition by cocaine on DA release evoked by single pulse stimulation. Using paired-pulse stimulation paradigms, GABA-R agonists limit the effects of cocaine on short-term facilitation. Further, sex differences may exist in the DAT regulation of short-term plasticity.

### Conclusion

These results suggest a convergence between DAT function and GABA tone acting at GABA-Rs in determining the relationship between striatal dopamine axon activity and exocytosis, that together support the frequency dependence over absolute amplitude of DA signalling.

# SECOND YEAR STUDENTS POSTER DAY 2022

# 'A YEAR OF PROGRESS'

## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Jun Yi Ong**

## In vivo effects of OXS-N1 on adult hippocampal neurogenesis

Supervisors: Professor Zoltán Molnár & Associate Professor Francis Szele

### Research Aim

OXS-N1, a novel small molecule, was found to promote neurogenesis in the neurosphere assay. Using the 5xFAD mouse model of Alzheimer's disease (AD) and wild type (WT) mice, we asked if in vivo, OXS-N1 would affect neurogenesis by altering neural stem cell (NSC) division, generation of neuroblasts or density of mature neurons. We also sought to determine if OXS-N1 could be altering neuroinflammation by changing microglial density.

### Methods and Results

Wild type (WT) and 5xFAD Mice were treated with OXS-N1 together with BrdU for a week and behavioural analyses were performed before their brains were collected.

To determine if OXS-N1 changed the density of neuroblasts or mature neurons, we quantified the density of doublecortin+ cells and neuronal nuclei+/BrdU+ cells in the dentate gyrus of the entire cohort of mice and did not find a significant difference between treated and untreated mice in both WT and 5xFAD mice. We then asked if OXS-N1 affected NSC division; examining the density of GFAP+BrdU+ dividing NSCs in a subset of both cohorts, we did not identify any significant difference between treated and untreated mice. Lastly, we quantified the density of Iba1+ microglia in the hippocampus and again did not find a significant difference between treated and untreated mice.

### Conclusion

OXS-N1 did not increase adult hippocampal neurogenesis or alter neuroinflammation in vivo, in both WT mice and a mouse model of AD.

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Julija Rabcuka**

## BAD BLOOD | Metabolic reprogramming under hypoxic storage preserves faster oxygen unloading from stored red blood cells

Supervisors: Professor Pawel Swietach & Dr Noemi Roy

Stored red blood cells (RBCs) incur biochemical and morphological changes, collectively termed "the storage lesion". Functionally, the storage lesion manifests as slower oxygen unloading from RBCs, which may compromise the efficacy of transfusions where the clinical imperative is to rapidly boost oxygen delivery to tissues. Recent analysis of large real-world data linked longer storage with increased recipient mortality. Biochemical rejuvenation with a formulation of adenosine, inosine, and pyruvate can restore gas-handling properties, but its implementation is impractical for most clinical scenarios. We tested whether storage under hypoxia, previously shown to slow biochemical degradation, also preserves gas-handling properties of RBCs. We utilized a purpose-built microfluidic chamber designed to rapidly switch between oxygenated and anoxic superfusates during single-cell oxygen saturation imaging on samples stored for up to 49 days. Aliquots were also analysed flow-cytometrically for side-scatter (a proposed proxy of O2 unloading kinetics), metabolomics, lipidomics and redox proteomics. For benchmarking, units were biochemically rejuvenated at four weeks of standard storage. We were able to show that hypoxic storage hastened O2 unloading in units stored to 35 days, an effect that correlated with side-scatter but was not linked to post-translational modifications of haemoglobin. Although hypoxic storage and rejuvenation produced distinct biochemical changes, a subset of metabolites shared a common signature that correlated with changes in O2 unloading. As such, hypoxic storage of RBCs preserves key metabolic pathways and O2 exchange properties, thereby improving the functional quality of blood products and potentially influencing transfusion outcomes.

# SECOND YEAR STUDENTS POSTER DAY 2022

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## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Aksel Saukko-Paavola**

## VAPB is a negative regulator of mitoguardin-2 Lipid Droplet tethering

Supervisors: Associate Professor Robin Klemm & Associate Professor Samira Lakhal-Littleton

### Research Aim

MIGA2 is a lipid transfer protein that forms contact sites between mitochondria and the endoplasmic reticulum (ER) as well as mitochondria and lipid-droplets (LDs) and is critical for adipocyte differentiation. Work from our group established that MIGA2-ER interactions are mediated through an ER tethering protein, VAP-B via a 'two phenylalanines and an acidic tract' (FFAT) motif. The model proposed suggested ER and LD tethering activities are synergistic and coordinate the supply of metabolic precursors produced by mitochondria to the sites of lipid biosynthesis and storage. My research question was to clarify the relationship between MIGA2's tethering activities to test this model.

### Methods and Results

To test this, I used confocal microscopy of WT MIGA2 and a VAP-B binding deficient mutant (F294A, F295A). Subsequent quantification of MIGA2-LD contacts showed the FFAT motif inhibited MIGA2-LD contacts, suggesting VAPB negatively regulates LD tethering. This observation was recapitulated through co-overexpression of VAP-B blocking MIGA2-LD contacts. I then showed through co-overexpression of VAPB mutants, that VAPB's ability to inhibit MIGA2-LD contacts is dependent on R anchoring and high-affinity FFAT binding.

### Conclusion

These findings suggest that MIGA2's organelle tethering activities are mutually exclusive and suggests that MIGA2-ER tethering dominate over MIGA2-LD tethering. This suggests these two activities could be functionally distinct, refuting the model initially proposed by our group. Considering this, my future avenues of study will probe how MIGA2's distinct organelle tethering activity relates to lipid transfer, and in turn how this is involved in modulating mitochondrial activity in white adipose tissue.

# SECOND YEAR STUDENTS POSTER DAY 2022

# 'A YEAR OF PROGRESS'

## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Esra Sengul**

## Exploring the association between the immune response and heart regeneration in Astyanax mexicanus

Supervisors: Associate Professor Mathilda Mommersteeg & Associate Professor Carolyn Carr

After heart attack, many immune cells are attracted to scar to clear up dead cells and promote cellular recruitment in first few days of post-injury. In humans, the injured tissue is then replaced by a permanent fibrotic scar, reducing heart pump function. Unlike humans, some fish can regenerate their heart and completely replace the scar with new functional heart muscle. We use Astyanax mexicanus as model to understand the underlying mechanisms of regenerative ability and specifically their immune response. The surface-adapted A. mexicanus, can repair their hearts after damage, whereas the cave-adapted ones have lost this ability (Stockdale et al., 2018).

### Research Aim

In this project, I aim to show the role of a prolonged immune response in regenerative ability of surface fish and hypothesized that the suppression of late-stage inflammation can inhibit regeneration and scar maturation.

### Methods and Results

Cryoinjured surface fish were either exposed to dexamethasone or clodronate liposome between 7dpci and 14dpci, hearts were collected at 30dpci and 60dpci and then AFOG, RNAscope and picrosirius red staining were performed.

### Conclusion

I showed a strong prolonged leukocyte response in surface fish at all time points in contrast to cavefish. Upon suppression of immune cells using inhibitors, we observed reduced regeneration in both timepoints and significantly increased collagen deposition at 60dpci with altered alignment. Our findings indicate prolonged present leukocytes might have a leading beneficial role in remodelling scar into a degradable scar. Exploring specific immune cell populations spatially and understanding their role can facilitate new advances in treatment.

# SECOND YEAR STUDENTS POSTER DAY 2022

# 'A YEAR OF PROGRESS'

## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Ayaka Shinozaki**

## Using 23Na sensitivity profiles to accelerate hyperpolarized 13C 2D Chemical Shift Imaging (CSI) with a flexible array coil

Supervisors: Professor Damian Tyler & Dr James Grist

### Research Aim

For 13C metabolic imaging studies, the challenge is to achieve high temporal resolution without decreasing spatial and/or spectral resolution. My work investigated the feasibility of accelerating 13C MRI by combining a 2D Chemical Shift Imaging (CSI) sequence and SENSE reconstruction. SENSE requires a sensitivity profile, but due to the low natural abundance of 13C, this is not possible to pre-acquire. Instead, naturally abundant sodium was chosen for the sensitivity map.

### Methods

SENSE-reconstructed CSI was studied with a bicarbonate ball phantom, an ethylene-glycol head phantom, and a live pig brain. Images were acquired with 2D CSI using a flexible 13C receive coil at 3T. A 4-fold acceleration was obtained by under sampling Cartesian traversal in k-space. SENSE-reconstructed under sampled images and fully sampled images were compared using difference images and signal to noise ratios of the region of interest.

### Results

For the ball and head phantoms, 13C images showed point-spread function artifacts but demonstrated that the coil-weighted and aliasing effects were reversed with SENSE. Visual inspection between full and under sampled images for the pig brain indicated coil-correction and unfolded aliasing.

### Conclusion

The novel approach of using sodium sensitivity profiles with SENSE-reconstructed CSI was demonstrated in phantoms and in vivo, where metabolic information was corrected with a Cartesian k-space reduction.

# SECOND YEAR STUDENTS POSTER DAY 2022

# 'A YEAR OF PROGRESS'

## DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

**Bobby White**

## Harnessing heterogeneity: metabolic reprogramming underpins acid selection in colorectal cancer cell lines

Supervisors: Professor Pawel Swietach & Professor Daniele Dini

### Research Aim

Evolutionary approaches are required to improve non-surgical colorectal cancer (CRC) management. A critical selection pressure in solid tumours like CRC is low extracellular pH (pHe), with a reported median of ~6.8, compared to 7.4 in healthy tissues. Selection requires intratumoural heterogeneity, and CRC cells exhibit heterogeneity in survival at low pHe ("acid-fitness"). For acid-fit cells, low pHe promotes aggressive phenotypes. Oxidative phosphorylation (OXPHOS) is required for acid-fitness, however preliminary data indicate that many OXPHOS-capable CRC cells have low acid-fitness. Consequently, what underlies heterogeneity in acid-fitness among CRC cells remains unclear.

### Methods and Results

Here, I show that acid-fitness heterogeneity relates to variation in acid-induced OXPHOS remodelling. From libraries of >70 CRC cell lines, I selected a panel of five representing the spectrum of acid-fitness. I pre-treated the panel with 48 hr pHe 7.4-6.4, then returned pHe to 7.4 for metabolic profiling, whereby fluorescent pH- and O2-sensitive dyes simultaneously reported glycolytic output and OXPHOS over 17 hr. Relative to pHe 7.4 (control), pHe 6.4 pre-treatment evoked a gradient of OXPHOS responses with induction and suppression in cells with high and low acid-fitness, respectively.

### Conclusion

Persistence of acid-induced metabolic reprogramming during profiling implicates an acidosis-induced gene expression program. To understand the underlying program, I plan to compare transcriptional responses of high and low acid-fitness cells to acid-stress utilising RNA-seq with spike-in normalisation. Ultimately, identification of targets within the selection process for acid-fit cancer cells will provide opportunities to steer evolution in CRC by preventing the expansion of acid-fit, aggressive subclones.

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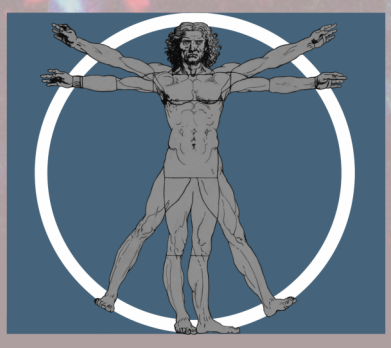

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