

# MSc by Research projects

Please find below the MSc by Research projects (start date of September 2021) available in the School of Physiology, Pharmacology and Neuroscience, University of Bristol. The MSc by Research projects involve 10 months of full-time research in one of our state-of-the-art laboratories followed by 2 months of writing up your thesis (submission deadline 2 years after start date). For more information including fees and how to apply please use the following link: <http://www.bristol.ac.uk/study/postgraduate/2019/life-sciences/phd-physiology-pharmacology/>. Loans are available for UK students (<http://www.bristol.ac.uk/fees-funding/postgraduate/pg-loans/>). If you are interested in one of the projects below or the project area of a particular research group, please feel free to contact the supervisor directly.

## Dissecting principles of long-range neuronal connectivity in mammalian motor circuits

**Supervisor: Dr Paul Chadderton - [p.chadderton@bristol.ac.uk](mailto:p.chadderton@bristol.ac.uk)**

School of Physiology, Pharmacology and Neuroscience

Brain function relies upon specific patterns of wiring between individual neurons. Neurons connect via their axons to other cells in their vicinity (locally) and also to other brain regions (long-range). Both types of connection can be exquisitely precise, but we have much to learn about the logic of neuronal connectivity from one region to another. This issue is particularly pressing in the cerebellum (or 'little brain') which is composed of three discrete structures (cortex, nuclei and inferior olive) that are linked via long-range projections. The aim of this project is to map projection patterns of single cells from the cerebellar cortex to the nuclei using molecular tools (e.g. genetically encoded fluorescent proteins) [1,2] to determine the rules governing connectivity between these two regions [3].

You will join a dynamic and multidisciplinary team who apply a range of approaches (2-photon imaging, high density electrophysiological recording, patch clamp, computational modelling) to understand mechanisms of motor control and learning in the intact brain. During this project, there will be opportunities to collaborate with group members to gain experience of other *in vivo* techniques and to present your work at international conferences.

For more information, please contact Paul Chadderton ([p.chadderton@bristol.ac.uk](mailto:p.chadderton@bristol.ac.uk)).

<http://www.neuralcircuitsinbehaviour.org>

References:

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## The role of protein SUMOylation in Osteoarthritis – a link to Type-2 Diabetes?

**Supervisor: Dr Chrissy Hammond - [Chrissy.Hammond@bristol.ac.uk](mailto:Chrissy.Hammond@bristol.ac.uk)**

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Abstract: In an ageing population, age-associated diseases such as Type-2 Diabetes (T2D) and Osteoarthritis (OA) represent an increasing healthcare and socio-economic burden. Several studies have established that T2D sufferers are more likely to develop OA, suggesting either a causal link or shared risk factors for these diseases. Intriguingly, the post-translational modification, SUMOylation, has been suggested to be involved in both T2D and OA, and studies have demonstrated that exposure of different cell types to T2D dietary risk factors (E.g. saturated fatty acids) increased whole cell protein SUMOylation. Recent genetic association studies have implicated genes involved in the regulation of SUMOylation, such as SENP6, as potential osteoarthritis susceptibility genes but to date we have little understanding of the role they play in the skeletal system. Using zebrafish as an animal model we will test how modulation of SUMOylation affects skeletal cell behaviour *in vivo* and test whether levels of SUMOylation are altered in other mutant lines which develop severe early onset osteoarthritis. We use zebrafish as they develop rapidly, are genetically amenable allowing us to readily generate genetic mutant and are translucent in the larval stages allowing us to follow cell behaviour in the living animal. As zebrafish develop in water, they can readily be drugged by application of compounds of interest to the water in which they are incubated.

In this project the student will use CRISPR-cas9 genome editing to knock out SENP6, they will use live imaging of zebrafish carrying transgenic reporters for chondrocytes, osteoblasts and osteoclasts to visualise the skeletal system. Using biochemical assays (in collaboration with Dr Tim Craig at UWE) they will test levels of SUMOylation in chondrocytes and osteoblasts from wild type and mutant fish at different stages of maturation. Finally using compounds that regulate SUMOylation we will test whether increasing add/or decreasing levels of SUMOylation can delay osteoarthritis progression *in vivo*. For more information on the group see [www.fishosteoarthritis.com](http://www.fishosteoarthritis.com)

## Maturing human stem cell-derived cardiomyocytes to provide improved models of arrhythmia

### Supervisors:

- 1) Dr Stephen Harmer: [s.c.harmer@bristol.ac.uk](mailto:s.c.harmer@bristol.ac.uk)
- 2) Prof. Jules Hancox: [jules.hancox@bristol.ac.uk](mailto:jules.hancox@bristol.ac.uk)

**Abstract:** Cardiac arrhythmias are a major cause of morbidity and mortality. Arrhythmias occur when the normal flow of electricity in the heart becomes disordered. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a unique model system for investigating arrhythmic disorders [1]. As a research model, hiPSC-CMs have a number of advantages in that they are human (and patient specific) in origin, provide an alternative to using cardiomyocytes derived from animals, are genetically editable, and can provide a near renewable supply of cells. However, in comparison to adult cardiomyocytes, hiPSC-CMs are structurally and electrophysiologically immature and exhibit a late foetal phenotype [2,3]. Our view, is that the immaturity of hiPSC-CMs currently hampers their utility as a model system in which to study the mechanisms underlying arrhythmias. Excitingly, a number of protocols have recently been published (for example [4] and [5]) that enable significant and accelerated structural and functional maturation of hiPSC-CMs. However, whether this maturation leads to an improved electrophysiological phenotype has not been determined. In this project the student will explore whether these maturation protocols (and other approaches), when applied singly or in combination, act to improve the electrophysiological phenotype of hiPSC-CMs. To achieve this, the structural maturation of hiPSC-CMs will be determined using immunostaining and confocal laser scanning microscopy (CLSM) and the patch-clamp technique will be used to characterise the electrophysiological phenotype. In summary, if hiPSC-CMs can be matured to achieve an improved electrophysiological phenotype this will greatly enhance the translational utility of this already exciting heart-in-a-dish technology for modelling a wide-range of cardiovascular disorders.

**References:** [1] Mummery, C.L. *Stem Cell Reports* **11**, 1306-1311 (2018). [2] DeLaughter, D. M. *et al. Dev Cell* **39**, 480-490 (2016). [3] Doss, M. X. *et al. PLoS One* **7**, e40288 (2012). [4] Parikh, S. S. *et al. Circ Res* **121**, 1323-1330 (2017). [5] Hu, D. *et al. Circ Res* **123**, 1066-1079 (2018).

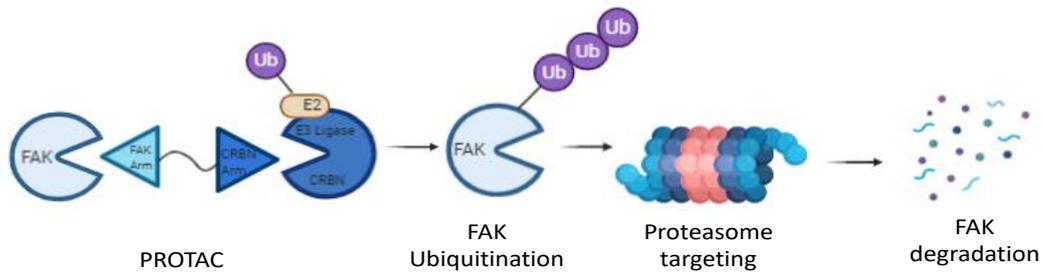
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## Novel approaches to modulate platelet function and thrombosis in cardiovascular disease.

### Supervisors:

Professor Ingeborg Hers (School of Physiology, Pharmacology and Neuroscience)  
Professor Varinder Aggarwal (School of Chemistry)  
Professor Graig Butts (School of Chemistry)

Platelets are small cells in the blood that are that play an important role in stopping a bleeding but when inappropriately activated also contribute to thrombosis and cardiovascular disease. One of the complications with studying how platelets maintain normal haemostatic control and/or promote thrombosis is that they lack a nucleus thereby prohibiting genetic approaches to reduce protein levels. Most of our present knowledge therefore derives from genetic animal models and pharmacological targeting of signaling pathways, each having its own drawbacks associated with species differences and pharmacological non-specificity. In this project aim to optimise a protein degradation approach in human platelets using rationally designed small molecule degraders called PROTACs (PROteolysis TArgeting Chimeras) to investigate signaling pathways involved in their haemostatic and thrombotic function. PROTACs are small heterobifunctional molecules that target proteins for ubiquitination leading to proteasomal degradation (**Figure 1**). We recently made the discovery by proteomic analysis that a generic tyrosine kinase PROTAC leads to potent and specific degradation of six protein tyrosine kinases (BTK/TEC, FAK/PYK2 and FER/FES) within hours, demonstrating that human platelets have the complete proteasome required for degradation.



**Figure 1. The ubiquitin proteasome system and PROTAC mechanism of action.** PROTAC degraders act catalytically by repeated binary target engagement. They put the protein of interest in a spatially favourable position to facilitate its polyubiquitination, thereby effectively inducing selective, intracellular proteolysis of the targeted protein.

These are exciting findings as it is the first time that a human platelet 'knockout' has been generated. In this proposal, we will expand these observations by using newly developed PROTAC molecules that target the tyrosine kinases FAK and PYK2, two tyrosine kinases implicated in platelet function, and develop a highly efficient technology to study platelet signaling pathways and function. We anticipate that the use of PROTACs will be transformative to the platelet research community and be the first step in developing alternative ways to modulate platelet function and thrombosis.

## Underlying mechanism of thrombogenesis in patients with severe coronavirus disease 19 (COVID-19)

### Supervisors:

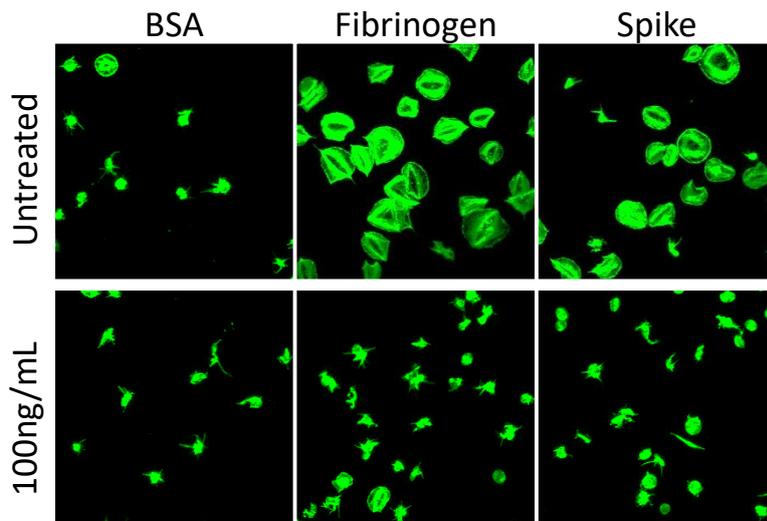
Professor Ingeborg Hers (School of Physiology, Pharmacology and Neuroscience)

Professor Andrew Davidson (School of Cellular and Molecular Medicine)

Professor Imre Berger (School of Biochemistry)

Severe coronavirus disease 19 (COVID-19) is a disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). Patients with COVID-19 are at significantly increased risk of thrombosis, which can be a challenge to manage and is associated with elevated mortality and morbidity. The underlying causes of thrombosis are still largely unknown, although platelet hyperactivity and a procoagulant state are likely to be contributory factors. One of the main mechanisms by which the virus can enter cells and replicate is by binding of the spike protein to the ACE-2 receptor. Although platelets do not express ACE2, SARS-CoV2 is unique amongst coronaviruses that infect humans in that the envelope spike protein of SARS-CoV2 includes an arginine-glutamate-aspartate (RGD) peptide sequence in its receptor-binding domain (RBD). In platelets, RGD sequences are recognised by the main platelet activation and adhesion integrin –  $\alpha_{IIb}\beta_3$  – triggering integrin activation, outside-in signalling, and platelet activation. We recently found that purified SARS-CoV2 spike protein and RBD protein can trigger platelet spreading and that this is blocked by incubating the platelets with the clinically used non peptide RGD mimetic  $\alpha_{IIb}\beta_3$ -integrin blocker, tirofiban (Figure 1).

This MSc project will extend these observations and investigate the effect of the virus/virus particles/proteins on platelet activation and thrombus formation under flow. We will study direct and indirect effects of virus/viral particles/proteins on platelet activation, the latter including contributions from endothelial and immune cells, as well as plasma from COVID19 patients. Techniques that will include a range of cell biology, biochemical and pharmacological techniques, including cell culture,



**Figure 1:** Platelet adhesion to BSA, fibrinogen and spike protein-coated surfaces in the absence (top) and presence of tirofiban (bottom).

transfection and protein expression, immunoblotting, phlebotomy, platelet isolation, platelet aggregation, adhesion assays, *in vitro* thrombosis studies, activation markers and platelet/leukocyte aggregate formation by FACS analysis and confocal microscopy.

## Developing *Drosophila* as a model to study the molecular and behavioural changes underlying Parkinson's disease

**Supervisor: Dr James Hodge** – [james.hodge@bristol.ac.uk](mailto:james.hodge@bristol.ac.uk)  
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The general lab interest is how neural circuit activity underlies behaviour including circadian rhythms, sleep, memory and movement. We study this using *Drosophila* molecular genetics, behavioural paradigms, electrophysiology, imaging and optogenetics. We wish to elucidate the fundamental biological mechanisms underlying behaviour and elucidate how they are affected by ageing, drugs and diseases such as Alzheimer's, Parkinson's, Down's and schizophrenia.

Parkinson's disease (PD) is the 2nd most common neurodegenerative disease and involves dopamine (DA) neurodegeneration leading to motor and non-motor symptoms. Recent Genome Wide Association Studies (GWAS) for PD have identified ~50 risk loci (Chang et al., 2017). To gain molecular insight into how these genes may lead to PD pathology we will perform an in vivo screen of already available stocks of *Drosophila* that mis-express the fly orthologues of these risk loci and human PD genes. These transgenes will be expressed in all neurons, all glia or DA neurons and the effect on lifespan, locomotion, circadian rhythm and sleep compared, while neurodegenerative effects will initially quickly be screened in the eye. Genes that yield PD relevant phenotypes will be further characterized for memory defects. Behavioural phenotypes will be further mapped to the most defined populations of cells using the large number of promoters readily available in flies. This will allow for instance the determination of progressive neurodegeneration of subsets of DA neurons mapped to PD relevant behaviours, this is possible in flies as they have only 200 DA neurons in their 150000-neuron brain and live around 60 days. The most promising candidates will be characterised using electrophysiology, optogenetics and mitochondrial live imaging for changes in dynamics and mitophagy (Julienne et al., 2017).

Chang, D., Nalls, M.A., Hallgrimsdottir, I.B., Hunkapiller, J., van der Brug, M., Cai, F., Kerchner, G.A., Ayalon, G., Bingol, B., Sheng, M., et al. (2017). A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nature genetics* 49, 1511-1516.

Julienne, H., Buhl, E., Leslie, D.S., and Hodge, J.J.L. (2017). *Drosophila* PINK1 and parkin loss-of-function mutants display a range of non-motor Parkinson's disease phenotypes. *Neurobiol Dis* 104, 15-23

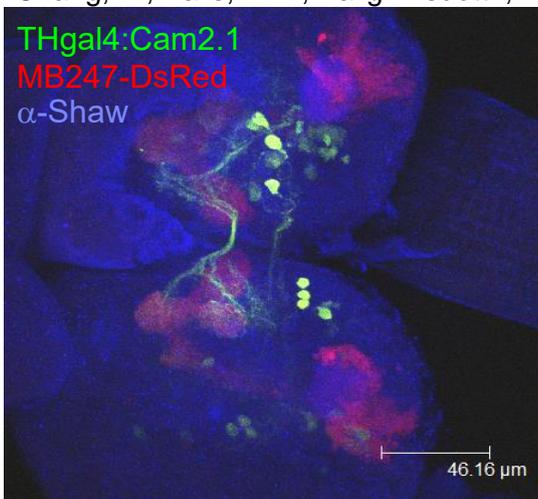


Figure 1 A fly brain showing dopamine neurons expression a calcium reporter in green that innervate the fly memory centre, the mushroom body in red (with Shaw Kv3 channel in blue).

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## Modulation of cyclic AMP level in astrocytes as a novel and alternative mechanism of action of antidepressants

**Supervisor: Prof. Sergey Kasparov** –  
<http://www.bris.ac.uk/phys-pharm/people/sergey-kasparov/index.html>

School of Physiology, Pharmacology and Neuroscience

Modulation of cyclic AMP level in astrocytes as a novel and alternative mechanism of action of antidepressants

Depression is a highly prevalent medical condition which may be life threatening. It also leads to disabilities and has extensive social implications for those affected and the society as a whole.

In spite of many years of research and a vast number of publications on this topic, there is no coherent theory of depression. Most of the current thinking pivots around insufficiency of central

mono-amines, such as serotonin and noradrenalin which seems to be consistent with the fact that the most widely prescribed antidepressants block re-uptake of these monoamines, leading to the increase in their extracellular concentration in the brain. However, there are too many facts which do not fit with this, apparently straightforward theory. For example, antidepressants which block reuptake of monoamines increase their concentration in the brain almost immediately, but it is widely known that it takes weeks for a clinical effect to develop. Why would that be? Moreover, genetically modified mice and rats which almost completely lack central serotonin are not showing obvious signs of depression and, when tested using accepted models of depression, still respond to the drugs, classified as “selective blockers of serotonin re-uptake”. What is then, the substrate of their action? There are many more such controversies. At the same time, long latency of the therapeutic effect is a major problem with the currently available antidepressants.

We are interested in testing a different theory which could explain how antidepressants work. It proposes that the key mechanism leading to depression is reduction of cAMP in brain cells and that antidepressants can increase the level of cyclic AMP in cells, in particular astrocytes, by a direct action on some G-proteins.

The project will use molecular tools and fluorescently tagged G proteins in combination with confocal imaging of living cells to investigate whether the antidepressants of various classes are able to induce translocation of alpha subunits of certain G-proteins. We will also test whether this effect is paralleled by increases in cAMP using FRET-based molecular sensors, viral vectors and other molecular tools.

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## Can we improve patient outcomes by providing a more personalized approach to antiplatelet therapies?

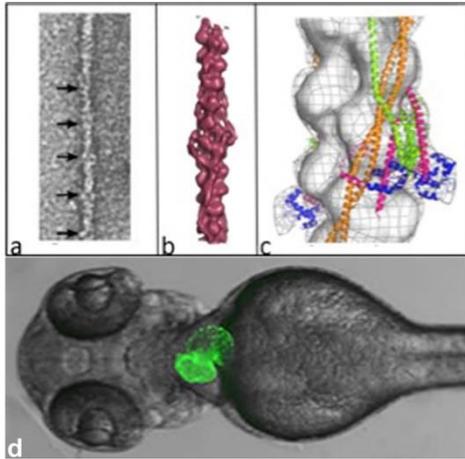
**Supervisor: Professor Stuart Mundell - S.J.Mundell@bristol.ac.uk**

School of Physiology, Pharmacology and Neuroscience

Heart attacks occur when a blockage called a thrombosis develops in the blood vessels of the heart which is a major cause of morbidity and mortality. Platelets play a central role in the development of arterial thrombosis in heart disease, responding to a variety of extracellular stimuli and agonists to undergo a rapid aggregation response, leading to a rapidly growing thrombus. Central amongst these agonists are TxA<sub>2</sub> and ADP which operate through G protein-coupled receptors (GPCRs) on the platelet surface: TP for TxA<sub>2</sub> and P2Y<sub>1</sub> and P2Y<sub>12</sub> for ADP. Current therapeutic strategies for the treatment of arterial thrombosis are largely based on these well characterized receptor systems with aspirin (which reduces TxA<sub>2</sub> generation) and P2Y<sub>12</sub>R antagonists commonly used effective anti-platelet agents with their combination, termed dual antiplatelet therapy (DAPT). Although DAPT offers synergistic benefits in preventing thrombus formation not all patients benefit to the same extent with a marked inter-individual variability in the extent of platelet inhibition. The variability of response to DAPT results in a significant proportion of patients demonstrating either high or low platelet reactivity with an associated risk of thrombotic or bleeding events, respectively. To date, platelet function testing has achieved predominantly negative results in reducing adverse events secondary to DAPT therapy although studies have largely focussed on the entire patient population rather than those at either end of the platelet reactivity spectrum.

Project aims:

- 1) to characterize platelet receptor biology in coronary intervention patients experiencing high and low platelet reactivity and assess the effectiveness of DAPT on these patients.
- 2) characterize the usefulness of existing methods to assess the effect of anti-platelet agents on platelet function.
- 3) develop novel methods of platelet function assessment that may facilitate tailoring of therapy with a reduction in adverse clinical events.



In order to undertake this programme of research you will be supported by an established network of clinical (Bristol Heart Institute) collaborators. This grouping will provide you with both the logistical support and access to patient samples from ongoing clinical trials at the University Hospitals Bristol NHS Foundation Trust. You will receive training in a wide variety of relevant techniques ranging from advanced fluorescent single cell imaging microscopy through to the measurement of cell signalling pathways.

Please do contact me at [S.J.Mundell@bristol.ac.uk](mailto:S.J.Mundell@bristol.ac.uk) if you would like further information about this exciting opportunity.

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## Cryo-EM and High-resolution structural studies of cardiac thin filaments

**Supervisors: Dr Danielle Paul - [Danielle.paul@bristol.ac.uk](mailto:Danielle.paul@bristol.ac.uk)  
Dr Rebecca Richardson - [rebecca.richardson@bristol.ac.uk](mailto:rebecca.richardson@bristol.ac.uk)**

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To understand how mutations in the thin filament regulatory protein troponin cause familial hypertrophic cardiomyopathy (HCM) it is important to know the structure of the cardiac thin filament. Techniques for the genetic manipulation of the zebrafish are well established and it has become a major model for the study of human cardiac disease. Our lab isolates zebrafish thin filaments from adult fish and are working to create a zebrafish model with mutations which cause HCM in humans. We study the 3D structure using Cryo-Electron Microscopy (EM), where the thin filaments are preserved in a frozen hydrated state. Using transmission EM and single particle analysis we can reconstruct a 3D map of the filament.

We now want to study the structure of these filaments in situ and our goal for this project is to look at frozen hydrated cardiomyocytes from zebrafish. After freezing the myocytes, we will perform cryo-sectioning and tomography of the to elucidate the ultrastructure. Some of the techniques that will be learnt in this project include; microdissection, EM and Cryo-EM sample preparation, EM and Cryo-EM imaging, image processing and 3D molecular fitting.

**Figure 1.** (a) A negatively stained transmission electron microscopy image of a gold fish thin filament. (b) A 3D reconstruction revealing the location and shape of the regulatory protein troponin. (c) The known atomic structures are docked into the electron density map and non-crystallised regions modelled. (d) A transgenic zebrafish larvae expressing GFP in the cardiomyocytes of the heart.

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**Circadian Oscillators in Drinking and Feeding Brain Circuits.**  
**Supervisors: Prof. Hugh Piggins - [hugh.piggins@bristol.ac.uk](mailto:hugh.piggins@bristol.ac.uk)**

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Daily or circadian rhythms pervade all aspects of our physiology and behaviour (Hastings et al., 2018). By convention, these rhythms are attributed to the activity of the brain's suprachiasmatic nuclei (SCN). Cells of the SCN contain a molecular clock and the daily cycle in clock genes/protein expression drives 24h variation in SCN neuronal activity. However, clock gene expression is not limited to the SCN and several findings indicate that brain circuits controlling thirst and appetite also contain intrinsic circadian oscillators (Guilding et al., 2009; Northeast et al., 2019). How these oscillators are organised, how they respond to SCN signals, and how they drive rhythms in neuronal activity and behaviour remains unresolved. In this project, live neuronal circuit imaging together with multi-electrode array recordings as well as optogenetic and chemogenetic manipulations will be used to determine how thirst and appetite circuits are organised to initiate, maintain, and terminate ingestive behaviour.

**References**

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Northeast et al., (2019) Keeping time in the lamina terminalis: novel oscillator properties of forebrain sensory circumventricular organs. *FASEB Journal* *in press*.

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**Bioengineering platelets for Blood Transfusions**

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Platelets are essential elements of the blood responsible for primary haemostasis. It is clear, however, that their role is much wider than just this, playing a critical element in aspects of angiogenesis, wound healing, tissue regeneration, tumour growth and metastasis and inflammation. Their loss or dysfunction can therefore impact in a variety of pathophysiological conditions. In humans, a low platelet count (thrombocytopenia) can result from either increased loss (destruction or traumatic) or decreased generation of platelets. In either case therapeutic strategies need to include mechanisms to increase the generation of platelets in the body, and/or to administer exogenous platelets by transfusion. Our current lack of understanding of the molecular and cellular mechanisms of platelet generation are a significant hindrance to achieving these therapeutic strategies efficiently. We have recently made significant progress challenging the current understanding of platelet production from their precursor cells, megakaryocytes, *in vivo* however, using state-of-the-art intravital microscopy and electron microscopy (<http://www.life-science-alliance.org/content/1/2/e201800061>).

Additionally, we have made a breakthrough in generating platelets *in vitro* in a novel microfluidic system. This already produces significant numbers of functional platelets from their precursor megakaryocytes, which is extremely encouraging. A significant bioengineering challenge lies ahead now to optimize the features of this flow system to enhance the numbers and functionality of the platelets that are produced. The work will involve a significant amount of detailed live cell fluorescence microscopy, electron microscopy, some cellular biochemistry and flow channel engineering. The project will use both human and mouse-derived stem cells, engineered to generate megakaryocytes as precursors for platelet production.

The value of the study will be to uncover novel determinants of platelet production in humans, which will provide insight into the causes of thrombocytopenia and pave the way for efficient generation of platelets *in vitro* for blood transfusion purposes. These are major goals for the whole field at present and would make a major advance in our understanding of fundamental mechanisms, but also provide a significant step on the way to clinical transfusion of bioengineered platelets.

## Using Zebrafish to Study Heart Disease and Tissue Regeneration

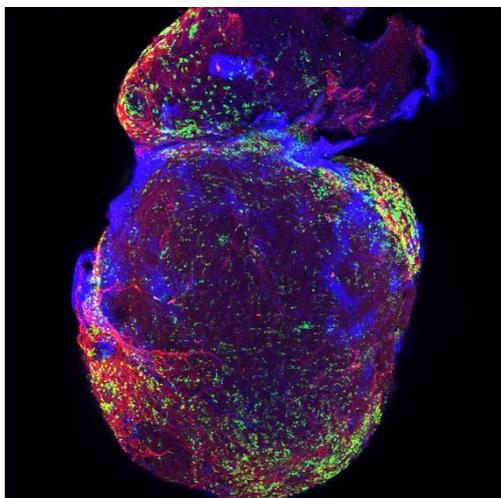
**Supervisor: Dr Beck Richardson - [rebecca.richardson@bristol.ac.uk](mailto:rebecca.richardson@bristol.ac.uk)**

School of Physiology, Pharmacology and Neuroscience

Our Main Interests and Skills:

- *In vivo* animal models
- Live imaging and sophisticated microscopy
- Inflammation
- Tissue repair and regeneration (skin and heart)
- Heart disease models (heart attack, heart failure, arrhythmias)
- Cell-cell communication
- CRISPR technology

Our lab is interested in modelling different aspects of heart disease and tissue repair/regeneration using zebrafish as a model. We are particularly interested in the inflammatory response to tissue injury and the roles this may play in promoting regeneration of damaged organs. The inflammatory response to an acute or ischaemic injury (such as a heart attack) is an inevitable and necessary part of the repair process but directly drives subsequent fibrosis and scar formation in mammals. Adult zebrafish, by contrast, deposit scar tissue following tissue injury but later resolve it entirely allowing for complete regeneration of organs such as the skin and heart. Our studies show that subtle changes in different immune cells are required for scar removal and tissue regeneration in zebrafish, but we still don't fully understand how this is controlled. We have several projects currently available in the lab including: investigating how ageing effects immune cells and tissue repair and regeneration; live imaging the movement of immune cells into the heart; determining which genes are involved in controlling immune cell behaviour during regeneration; using CRISPR technology to introduce specific cardiovascular disease-causing mutations; deciphering the ways cells communicate during repair and regeneration. If you are interested in doing any of these projects for a MSc by Research or would like to hear more or visit the lab, please get in touch.



Immune cells (green) and scar tissue (red) in a zebrafish heart.

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## Investigating the mechanisms of action underlying ketamine's efficacy in major depressive disorder

**Supervisor: Professor Emma Robinson - [Emma.S.J.Robinson@bristol.ac.uk](mailto:Emma.S.J.Robinson@bristol.ac.uk)**

School of Physiology, Pharmacology and Neuroscience

Major depressive disorder is one of the most challenging conditions facing modern society and is associated with significant personal and societal costs. Until recently, treatments for depression were limited and many patients either failed to respond or found side effects too much to tolerate. These treatments also had a delayed onset of action meaning patients did not show improvement for many weeks after initial diagnosis and treatment. This all changed with the discovery that the NMDA antagonists and drug of abuse, ketamine, can reduce symptoms of depression as soon as 1 hr after treatment and a single dose can have effects which last up to 14

days. However, ketamine is not an ideal treatment due to side effects, abuse liability and risks of adverse effects such as bladder and kidney problems (Robinson, 2018). This project will investigate the underlying molecular mechanisms which contribute to ketamine's effects and use this knowledge to test potential alternative treatments which achieve similar efficacy but with reduce risks. The student will have an opportunity to gain hands on in vivo skills in behavioural neuroscience and psychopharmacology.

- 1 Robinson, E. S. J. Translational new approaches for investigating mood disorders in rodents and what they may reveal about the underlying neurobiology of major depressive disorder. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **373**, doi:10.1098/rstb.2017.0036 (2018).

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## **Cystic fibrosis: restoring ion transport with small molecules**

**Supervisor: Prof David N. Sheppard -D.N.Sheppard@bristol.ac.uk**

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Cystic fibrosis (CF) is caused by dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR), a unique ATP-binding cassette transporter that functions as an ATP-gated anion channel, playing a pivotal role in salt and water movement across epithelial tissues (1). To date more than 2,000 mutations have been identified in the CFTR gene, but most are very rare and not all cause disease (1, 2). The most common cause of CF, the F508del mutation, disrupts CFTR transport to and stability at the plasma membrane and interferes with channel gating (2). To overcome these defects, small molecules have been developed called CFTR correctors and potentiators, which rescue the plasma membrane expression and function of F508del-CFTR (3). Other small molecules restore ion transport to CF cells by activating different anion channels expressed in these cells or functioning as artificial anion channels and transporters (4). An MSc by Research project with our group would explore how rare CF mutations cause CFTR dysfunction and small molecules restore ion transport to CF cells by rescuing or bypassing faulty CFTR proteins (5-8).

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## **The role of acute hypoxemia on potentially fatal cardiac ventricular arrhythmias in humans**

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**Study summary.** This exciting project aims to investigate the effect of acute hypoxemia on cardiac repolarization of human subjects with different morbidities. Sufferers of chronic conditions that impair the delivery of oxygen to the heart are at increased risk of fatal ventricular arrhythmias. The QT-interval on an electrocardiogram (ECG) has been studied as a measure of cardiac repolarization. Increases in the duration of this interval are strongly associated with ventricular arrhythmogenesis and sudden cardiac death. Propensity to QT interval prolongation can be inherited or acquired. While inherited long-QT syndromes are rare, it is well established that acquired long-QT can be caused by certain medications. More recently, mounting epidemiological evidence suggests an association between hypoxemia and QT interval prolongation. This is highly relevant from a population health perspective since multiple pathologies can cause hypoxemia or local tissue hypoxia, acutely or chronically, including sleep disordered breathing, atherosclerosis and heart failure. However, few functional studies have investigated the link between acute hypoxemia and long-QT in humans. Moreover, there is little understanding of the role that comorbidities, sex differences and exercise

may play on susceptibility to long-QT induced by acute hypoxemia. Thus, this dry lab project aims to retrospectively analyse the effect of controlled acute hypoxemia on QT-interval in males and females from different study cohorts, including healthy subjects and patients presenting with different morbidities (e.g. hypertension and heart failure).

**The team.** This unique team of supervisors includes two basic scientists and a clinical researcher. Dr Abdala Sheikh is an expert in pre-clinical integrative physiology. In her research, she combines *in vivo* and *ex vivo* physiology, electrophysiology, molecular and computational approaches to study diseases that affect autonomic control. Dr Harmer is an expert electrophysiologist and uses cutting edge molecular and stem cell technologies to investigate inherited cardiac channelopathies. Dr Hart is an expert in clinical physiology studies in humans and has an interest in cardiovascular autonomic control in hypertension and heart failure.

**What you will learn.** During this project you will gain training and experience in clinical physiology studies, governance of human derived data, analysis of cardiovascular parameters, computational methods (coding for data analysis) and statistical analysis of clinical data. There will also be ample opportunities to gain hands-on experience in human experimentation by contributing to ongoing clinical studies from the team.

#### **Starter reading**

May, A. M., D. R. Van Wagoner, and R. Mehra. "Osa and Cardiac Arrhythmogenesis: Mechanistic Insights." *Chest* 151, no. 1 (Jan 2017): 225-41. <https://dx.doi.org/10.1016/j.chest.2016.09.014>.

Shamsuzzaman, A. S., V. K. Somers, T. K. Knilans, M. J. Ackerman, Y. Wang, and R. S. Amin. "Obstructive Sleep Apnea in Patients with Congenital Long Qt Syndrome: Implications for Increased Risk of Sudden Cardiac Death." *Sleep* 38, no. 7 (Jul 1 2015): 1113-9. <https://dx.doi.org/10.5665/sleep.4824>.

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## **Modulation of Brain Energy Metabolism by Astrocytic G-protein-coupled Receptors**

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It has been becoming increasingly clear that 'neuro' science research must consider the roles of non-neuronal cells in the central nervous system if integrated brain function is to be understood. We have by now come to appreciate that astrocytes, the most abundant type of glia cells in the brain, play fundamental roles in neurotransmission, not only by fuelling the underlying cellular processes and by removing waste products, but also by integrating, amplifying, and modulating neuronal signals (Verkhratsky et al., 2015). Astrocytes handle glucose intake across the blood-brain barrier, contain the glycogen stores of the brain and, following glycolysis, release lactate into the extracellular space. This lactate may support neuronal function in states of increased energy demand, for example during memory formation (Alberini et al., 2017). However, beyond energy supply, recent evidence has suggested that lactate also acts as extracellular signalling molecule, for example in context of central arousal to salient stimuli, or in autonomic control (Tang et al., 2014; Teschemacher et al., 2015; Mosienko et al., 2015; Mosienko et al., 2018).

Whilst lacking in electrical excitability, astrocytes express a plethora of G-protein-coupled receptors and highly complex intracellular signalling cascades of which we currently have only limited understanding. Over the recent decade, molecular and imaging tools suitable for investigating these have been developed. This research project will use astrocytes in dissociated and slice cultures, viral vector transgenesis, confocal imaging and biosensor electrode measurements to study the effects of GPCR activation on lactate production and release.

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2. Mosienko V, Rasooli-Nejad S, Kishi K, De Both M, Jane D, Huentelman MJ, Kasparov S, Teschemacher AG (2018) Putative receptors underpinning L-lactate signalling in locus coeruleus. *Neuroglia* 1:365-doi:10.3390/neuroglia1020025.
3. Mosienko V, Teschemacher AG, Kasparov S (2015) Is L-lactate a novel signaling molecule in the brain? *J Cereb Blood Flow Metab* 35:1069-1075.

4. Tang F, Lane S, Korsak A, Paton JF, Gourine AV, Kasparov S, Teschemacher AG (2014) Lactate-mediated glia-neuronal signaling in the mammalian brain. *Nature Communications* 5:3284-DOI: 10.1038/ncomms4284.
  5. Teschemacher AG, Gourine AV, Kasparov S (2015) A Role for Astrocytes in Sensing the Brain Microenvironment and Neuro-Metabolic Integration. *Neurochem Res* 40:2386-2393.
  6. Verkhratsky A, Nedergaard M, Hertz L (2015) Why are astrocytes important? *Neurochem Res* 40:389-401.
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## Determination of pathogenicity of pore mutations in potassium channels linked to Long QT Syndrome

**Proposed supervisor(s): Professor Jules C Hancox and Dr Stephen C Harmer**

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The correct function of the heart depends on an orderly sequence of electrical excitation with each heart-beat. In turn this depends on the coordinated activity of multiple ion channel proteins and electrogenic transporters. Mutations to the underlying genes lead to electrocardiogram (ECG) abnormalities and malignant, potentially fatal ventricular arrhythmias. Two genes, which encode potassium ion channels, that have been strongly linked to malignant ventricular arrhythmias are *KCNQ1* and *KCNH2*, mutations to which can cause repolarization disorders and sudden death [1-3]. Loss-of-function mutations to these genes lead to the LQT1 and LQT2 forms of long QT syndrome (LQTS). Whilst many variants in *KCNQ1* and *KCNH2* have been associated with LQTS, only a relatively small fraction of these has undergone functional characterization. The objective of this project is to elucidate the effects of mutations close to the ion selectivity filter in the pore of *KCNQ1* and hERG (*KCNH2*-encoded) channels. For example, both *KCNQ1* and hERG possess highly conserved proline (P320 and P632 respectively) and threonine residues (T322 and T634 respectively) in this region. Analogous mutations in this region will be made in each channel and studied using an In/On-Cell Western assay to determine effects on channel trafficking to the surface membrane and with whole-cell patch-clamp to evaluate functional expression [4,5]. This comparison of analogous positions in the two channels will establish whether the regions studied are particularly vulnerable to pathological variation.

### References

- [1] Hancox JC, Stuart AG and Harmer SC (2020) Functional evaluation of gene mutations in long QT syndrome: strength of evidence from in vitro assays for deciphering variants of unknown significance. *J Congen Cardiol*, 4(6): <https://doi.org/10.1186/s40949-020-00037-9>
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- [4] Al-Moubarak E, Zhang Y, Dempsey CE, Zhang H, Harmer SC, Hancox JC (2020) Serine mutation of a conserved threonine in the hERG K<sup>+</sup> channel S6-Pore region leads to loss-of-function through trafficking impairment. *BBRC*, 526: 1085-1091.
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## **Cellular mechanisms underlying acetylcholine roles in cerebellar dependent behaviours.**

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### Project description:

The cerebellum is the largest sensorimotor structure in the brain and is crucial for motor coordination and learning. Little is known about how acetylcholine modulates cerebellar circuitry, and until our current work nothing was known about the role of cerebellar acetylcholine receptors in behaviour. We have shown that infusing cholinergic receptor antagonists into the cerebellum of rats caused deficits in a variety of motor tasks and unexpectedly also altered feeding behaviour. This project will investigate the cellular mechanisms underlying these behavioural changes.

The project will take place across two multi-disciplinary research teams, allowing experience of state of the art *in vitro* and *in vivo* techniques. The primary technique will involve recording from single neurons in cerebellar slices combined with use of pharmacological ligands to understand how intrinsic properties and synaptic transmission of these neurons respond to activation of those neurotransmitter systems relevant to the behavioural changes observed (such as those targeting specific acetylcholine receptors and the interactions of these with hormonal modulators of feeding behaviour). In addition, optogenetic stimulation of cholinergic projections to the cerebellum will provide insight into how endogenous acetylcholine release influences cerebellar neurons. This will involve injections of viral vectors into specific brain areas of the rat, including the possibility of using specialist transgenic rat lines, and immunohistochemical processing of brain tissue to examine the resulting viral expression throughout the network. There will be opportunities to get involved with rat behaviour studies which are linked to this work to gain experience of how the different levels of neuroscience research interact and inform each other.