SHERRINGTON TALKS 2022

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

BLAKEMORE LECTURE THEATRE

SHERRINGTON BUILDING

FRIDAY 10TH JUNE AND THURSDAY 16TH JUNE

FROM 1PM

'A Year of Progress' by DPAG Graduate Students in their 3rd year of DPhil research study

Chaired by DGS

Professor Vladyslav Vyazovskiy

UNIVERSITY OF OXFORD

DPAG

SHERRINGTON TALKS DAY ONE 2022

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Judged by Graduate Studies Committee Academics

Prize winners will be notified in the Digest on Monday 20th June

Tea, coffee and biscuits after each session

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Sara Bandiera

Characterization of the earliest thalamocortical interactions in the human fetal brain

Supervisors: Professor Zoltán Molnár & Professor William James

Research Aim

The major source of extrinsic modulation to the development of the cerebral cortex is provided by the early thalamocortical afferents (TCAs). In this study, we aim to understand their role in the human brain, where thalamocortical interactions start at very early stages and continue over a prolonged period, therefore potentially affecting cortical neurogenesis in a crucial manner.

Methods and Results

To this purpose, we traced the early projections from the thalamus in fixed post-mortem human brains at midgestational stages (16 and 17 post-conception weeks, PCW). By this time, TCAs already reached their target cortical area, where they accumulated in the transient subplate, as previously shown in the literature. Surprisingly, they also projected toward the underlying outer subventricular zone (OSVZ), where they were observed in close contact with HOPX-positive outer radial glial cells (oRGC).

Based on previous literature, we selected VGF (non-acronymic) as a candidate thalamic factor that might account for the simultaneous interaction with both progenitor cells of the OSVZ and post-mitotic cortical neurons of the subplate. We are currently validating this molecular candidate by using available bulk and single-cell transcriptomic datasets of the human brain (Brainspan.org and Nemoanalytics.org, respectively) and immunohistochemical analysis of post-mortem human material.

Conclusion

These early thalamocortical interactions might have evolutionary relevance since both the subplate and the OSVZ massively expanded in the fetal human brain, and they are associated with its increased size and complexity. Therefore, a prolonged and finer modulation of cortical neurogenesis by thalamocortical afferents might explain unique aspects of the human cerebral cortex.

Elise Meijer

Investigation of sleep-wake regulation in 'layer 6b silenced' mice

Supervisors: Professor Zoltán Molnár & Professor Vladyslav Vyazovskiy

Research Aim

Cortical layer 6b (L6b) is found in numerous species, implying an evolutionary advantage, yet its function is unknown. It is the only cortical layer activated by orexin, a major neurotransmitter of arousal, and it projects selectively to higher order nuclei of the thalamus, which mediate brain state control. Moreover, L6b receives long-range cortical input, allowing integration of activity in multiple areas. These characteristics could enable L6b to link subcortical and thalamocortical brain state control and I am studying this possible role.

Methods and Results

I investigated the role of L6b in sleep-wake regulation in a mouse model with a Cre-dependent truncation of Synaptosomal Associated Protein of 25 kDa (Snap25) in a subset of L6b (Drd1a-Cre::Snap25fl/fl). In Drd1a -Cre::Snap25fl/fl mice, L6b is present but lacks regulated synaptic vesicle release. By continuous electro-encephalography/electro-myography (EEG/EMG) recording for 24 hours, I found that L6b silenced (n=6) spent 11.32 ± 0.60 vs controls (n=6) 10.75 ± 0.24 hours in in non-rapid eye movement (NREM) sleep, L6b silenced 1.74 ± 0.09 vs controls 2.05 ± 0.11 hours in rapid eye movement (REM) sleep, and L6b silenced 9.92 ± 0.67 vs controls 10.07 ± 0.34 hours in wakefulness (mean ± SEM). Preliminary spectral analysis shows a power reduction in the NREM sigma frequency band in L6b silenced animals in the frontal derivation.

Conclusion

This is the first study where the effect of layer 6b silencing on sleep is investigated. Preliminary analysis suggests a reduction in REM time and a reduction in spindle activity in NREM sleep.

Jean Philippe-Dufour

Slow oscillations and synaptic plasticity

Supervisors: Associate Professor Ed Mann & Professor David Bannerman

Research Aim

Sleep, or sleep-like states have been described in nearly all animals, yet its function remains unclear. The synaptic homeostasis hypothesis proposes that sensory experience and learning during wake results in net strengthening of the associated neocortical synapses, whereas slow-wave sleep leads to a net depression of synaptic weights. A decrease in synaptic strength would concurrently be met with a decrease in slow-wave activity (a well-known substrate of sleep pressure). The synaptic rules potentially responsible remain unclear. Evidence points to an activity-dependent network reorganization during sleep. It is therefore possible that one role of slow wave sleep is to modulate synaptic plasticity rules to promote selective downscaling of synapses during sleep.

Methods and Results

I pair whisker stimulations with UP and DOWN states in vivo using a closed-loop stimulation system while recording in the barrel cortex. I find that pairing sensory inputs with UP states, but not DOWN states selectively induces downscaling of synaptic strength. Furthermore, this synaptic weakening is accompanied with local changes in the slow oscillations, namely a reduction in amplitude and spatial synchronicity across the paired cortical area proportional to the synaptic plasticity. Using a large-scale computational model of slow oscillations, I am testing if a reduction in synaptic strength is sufficient to induce these oscillatory changes.

Conclusion

We present in vivo evidence that UP states bias synapses towards weakening, and that local changes in synaptic strength are accompanied by local changes in slow oscillations. This would lend support to the idea that slow-wave homeostasis can be driven by synaptic weakening.

Sian Wilcox

Fasting-induced torpor in mice: implications for neuroscience research and the 3Rs

Supervisors: Professor Vladyslav Vyazovskiy, Professor Stuart Peirson & Professor David Bannerman

Research Aim

Food restriction is a common strategy used in neuroscience research to facilitate engagement with behavioural tasks when using mouse models. However, research using mouse models is often highly variable and lacks reproducibility. We hypothesised that fasting-induced torpor, a hypometabolic state, may be altering physiology and behaviour in a way that is confounding data generation.

Methods and Results

To this end, I first established that common food restriction paradigms were sufficient to reliably induce torpor in mice, and that torpor timing and characteristics could be altered depending on when food was given. An open field task revealed that torpor significantly impairs locomotor activity and exploratory behaviour, although these effects could be avoided depending on the time the task was conducted relative to torpor bouts. Chronic electroencephalography (EEG) is now being used to determine the effects of torpor on brain activity and sleep dynamics. Preliminary data indicate that sleep architecture is significantly altered in response to food restriction, and that an increase in deep restorative sleep is observed following emergence from torpor.

Conclusion

These results suggest that torpor is readily induced in mice in response to food restriction. Researchers may not be aware of this phenomenon so will be unable to control for torpor induction. Torpor also profoundly alters mouse behaviour, and therefore may be confounding experimental outcomes where behaviour is being assessed. Moreover, the impact of torpor on brain activity, coupled with sleep disturbance, may be further confounding data generated using mouse models.

Alina Krebbers

Sleep as an Antioxident

Supervisors: Professor Gero Miesenböck

Research Aim

Sleep in the fruit fly, Drosophila melanogaster, can be induced by accumulated reactive oxygen species (ROS). This makes two major processes, ROS production and ROS sensing, key regulators of sleep. Artificially inducing ROS initiates sleep within minutes, whereas inhibiting ROS-sensing leads to chronic sleep loss, causing chronic sleep deprivation indicated by a shortened lifespan and defects in learning and memory. Reducing mitochondrial ROS production causes a similar sleep loss. My goal is to investigate whether these short-sleeping flies suffer from chronic sleep deprivation similar to flies with impaired ROS-sensing. If the flies show no signs of sleep deprivation, this indicates clearance of ROS as a function of sleep.

Methods and Results

To reduce mitochondrial ROS production in flies, the alternative oxidase (AOX) was expressed. To impair ROS-sensing in flies, the potassium channel subunit Hyperkinetic (Hk) was knocked out. Sleep of these flies was measured and compared. In both cases, flies slept significantly less than their controls, and sleep loss in both conditions was comparable. To test for consequences resulting from chronic sleep deprivation, learning (using a negative reinforcement paradigm) and lifespan of flies were assessed. Both behaviours were unaffected in short-sleeping flies with reduced ROS production. Contrarily, impaired ROS-sensing caused a learning deficit and shorter lifespan.

Conclusion

Short-sleeping flies with reduced ROS production do not show typical signs of chronic sleep deprivation. Since reducing ROS levels is sufficient to counteract the detrimental effects of chronic sleep loss, clearance of ROS seems to be a function of sleep.

David Oliver

Sensory response properties of the mouse claustrum

Supervisors: Dr Adam Packer & Associate Professor Simon Butt

Research Aim

The claustrum is a long, thin, bilateral structure sequestered beneath the cortex. Despite decades of anatomical research into the exceptional anatomy of the claustrum, studies of the claustrum's function have lagged behind. Previous studies have linked the claustrum to sensory perception and multisensory integration. Moreover, recent research from our lab has shown that individual claustrum cells can integrate inputs from multiple cortical regions. However, it is not yet known how the claustrum responds to sensory stimuli, or whether it can integrate multimodal sensory stimuli.

Methods and Results

We recorded the activity of claustrum axons in the cortex during sensory stimulation. Mice were passively presented with auditory, visual, and tactile stimuli during two-photon calcium recordings. Stimuli were presented either unimodally, or in multimodal combinations. Forty-seven percent of all tested axons displayed significant calcium transients to at least one stimulus modality during passive presentation and all modalities could evoke responses in at least some CLA axons. Interestingly, thirty-five percent of stimulus responsive CLA axons were exclusively responsive to multimodal trial types, while fifteen percent were exclusively responsive to unimodal trial types.

Conclusion

These experiments demonstrate that individual claustrum cells are capable of responding to multiple sensory modalities. Moreover, while half of the recorded axons were responsive to both uni- and multimodal stimuli, smaller fractions were exclusively responsive to either uni- or multimodal stimuli. These results support the possibility that the claustrum may play a role in sensory integration.

Ana Isabel Sánchez Jiménez

Persistence and generalisation of adaptive changes in auditory localisation behaviour following unilateral hearing loss

Supervisors: Professor Andrew King & Dr Fernando Nodal

Research Aim

Sound localisation relies on binaural and monaural spatial cues. Hearing loss in one ear compromises binaural computations, impairing localisation accuracy, but with appropriate training, adult individuals can adapt to this binaural imbalance. However, it remains unclear for how long this learning is retained or whether it generalises beyond the training stimuli.

Methods and Results

We trained ferrets to localise broadband noise bursts in quiet conditions and then measured their performance over repeated periods of monaural earplugging that were interleaved with short or long periods of normal binaural hearing. Localisation accuracy during repeated occlusion of the same ear was retained to a large degree even after months of normal binaural experience, whereas this was not the case if the animals had previously adapted to occlusion of the opposite ear. This supports previous evidence that adaptation is based primarily on increased dependence on the monaural spatial cues available at the intact ear. To explore learning generalisation, we also measured localisation accuracy before and after adaptation using different bandwidth stimuli presented against constant or amplitude-modulated background noise. The degree of adaptation to broadband sounds correlated with the animals' localisation accuracy for other stimulus types, suggesting that training-dependent recovery in spatial hearing can generalise to other sounds and that adaptation and generalisation may occur simultaneously.

Conclusion

Training-induced adaptation to conductive monaural hearing loss persists even after experiencing periods of normal binaural hearing and can generalise beyond the training stimuli to other sounds.

Paul Zimmer-Harwood

Tactile influences on auditory cortical processing

Supervisors: Professor Andrew King & Dr Johannes Dahmen

To evaluate the sounds we hear, our brains must simultaneously identify, analyse and, if necessary, act upon signals from our other senses. While multi-sensory integration is thought to be primarily a cortical function, it was recently demonstrated that somatosensory signals can modulate activity in the auditory cortex via a cortico-collicular projection from the primary somatosensory cortex to inhibitory neurons in the inferior colliculus, which suppress sound-evoked activity in the auditory thalamocortical pathway (Lohse et al., 2021).

Research Aim

As the perceptual implications and functional benefits of somatosensory-auditory suppression are still unclear, my project aims to find out how 1) whisker stimulation affects sound detection and 2) whether this intriguing cortico-colliculo-thalamocortical circuit can operate flexibly under changing task contingencies.

Methods and Results

First, I trained mice on a tone-detection task and recorded neural responses in the auditory cortex using 2-photon calcium imaging. Surprisingly, the inhibition of sound-evoked responses by concurrent whisker stimulation did not interfere with the animals' ability to detect tones. Second, I trained mice on a task-switching paradigm in which they were rewarded either for detecting sounds or whisker deflections. The gain of the suppression changed as a function of stimulus relevance and was strongest when the tactile stimulus was rewarded instead of the sound. Additionally, we could demonstrate the existence of whisker-driven neurons throughout the auditory cortex.

Conclusion

While the function of somatosensory-auditory suppression is still unclear, the top-down projections from the somatosensory cortex seem to allow for flexible, context-dependent but tight control over ascending auditory signals.

Kaitlyn Cramb

Dopamine release defects in iPSC-derived dopamine neurons from PD patients with SNCA-triplication

Supervisors: Professor Richard Wade-Martins & Professor Stephanie Cragg

Research Aim

Parkinson's disease (PD) is a disorder in which the degeneration of dopaminergic neurons (DAns) in the nigrostriatal pathway leads to debilitating motor symptoms. There remains to be a unifying hypothesis for how this degeneration is initiated and because of this, no disease-modifying therapeutics are available. Evidence from several animal models of familial PD indicates that defective dopamine release is an early cardinal feature of PD, preceding both neurodegeneration and symptom onset. Therefore, we aim to address whether dopamine release is dysfunctional in human dopamine neurons from PD-affected individuals and the molecular mechanisms by which this occurs.

Methods and Results

We produced induced pluripotent stem cell (iPSC)-derived dopamine neurons from patients with the PD-associated SNCA-triplication mutation. KCl-evoked dopamine release and total intracellular dopamine content were measured using high performance liquid chromatography electrochemical detection (HPLC-ECD) and synaptic dysfunction was measured using whole-cell patch-clamp electrophysiology. We observed a ninety percent decrease in evoked dopamine release from iPSC-DAns harbouring the SNCA-triplication mutation and found that this coincides with a decrease in total intracellular dopamine content of the same magnitude. Both defective release and content were restored by acute L-DOPA treatment. Further data supports that these defects are not due to defective maturation or dopamine synthesis, but rather alterations in its handling.

Conclusion

Combined, these data provide support from human models that synaptic dysfunction occurs early in PD. Results from this study will be critical to providing novel targets for the development of effective disease-modifying therapeutics.

William McGuinness

TFEB, TFE3 and their role upon lysosomal biogenesis in iPSC-derived dopaminergic neurons

Supervisors: Professor Richard Wade-Martins, Dr Brent Ryan & Dr Warren Hirst

Research Aim

The autophagic-lysosomal pathway (ALP) is a key mechanistic pathway utilised by neurons to remove damaged organelles/proteins. ALP dysfunction has been heavily implicated in Parkinson's disease (PD), and improving lysosomal capacity is considered a promising therapeutic strategy to combat this disease. TFEB and TFE3 are two transcription factors known to upregulate a gene network in which regulates autophagic/ lysosomal function. We aim to assess their ability to restore ALP perturbations in a human-, disease-relevant model of PD.

Methods and Results

We have characterised TFEB/TFE3 biology and are assessing their potential to rescue lysosomal perturbations using iPSC-derived dopaminergic neurons (iPSC-DaNs) from PD patients.
We found TFEB to be minimally expressed and TFE3 to be primarily expressed in human iPSC-derived neurons. This was confirmed through analysing publicly available human DaN RNA-Seq datasets.
We find TFE3 can be activated using an agonist of TRPML1, ML-SA5, in control iPSC-DaNs. Treatment induces small increases in downstream lysosomal genes, suggesting the potential to pharmacologically upregulate ALP in iPSC-DaNs.
We also observe a reduction in lysosomal compartments in iPSC-DaNs harbouring the SNCA-Trp mutation.

Conclusion

These data suggest TFE3 is the primary neuronal therapeutic target, rather than TFEB, for upregulating lysosomal biogenesis. Future work will use ML-SA5 or TFE3 overexpression to further assess the ability of TFE3 to correct the phenotypes seen in patient iPSC-DaNs.

Gosia Cyranka

Glucagon-like peptide 1 (GLP-1) secretion from gut endocrine cells is pH dependent

Supervisors: Associate Professor Heidi de Wet & Associate Professor Lisa Heather

Research Aim

Previous reports showed that secretion of insulin from pancreatic β-cells, as well as glucagon from α-cells is pH sensitive. Here, we investigate the effect of extracellular pH on GLP-1 secretion from gut endocrine cells.

Methods and Results

Utilising two murine models of L cells – GLUTag cells and primary intestinal cultures, we discovered that lowering extracellular pH below 7.0 decreases glucose-induced secretion of active GLP-1 by 50% at pH 6.3, while secretion remains unaltered at pH ranging 7.0-7.6. The effect of low extracellular pH on GLP-1 secretion can be mimicked by the inhibition of intracellular V-ATPase proton pumps, thus suggesting the proton flux into the cytoplasm is responsible for pH dependence of GLP-1 secretion. The proton entry into the L cells is not mediated by electrogenic transport coupled with Na+ as acidic pH lowers GLP-1 release also in the absence of extracellular sodium. Replacing the extracellular chloride completely ceased the exocytosis of GLP-1 irrespective of the secretagogue tested. The whole-cell production of active GLP-1 as well as peptide processing via proteolytic cleavage by pro-hormone convertase 1/3 was not affected by the acidic pH. The secretory granules of the glucose-stimulated cells at acidic pH showed significantly higher active GLP-1 content as compared to the secretory granules at pH 7.4.

Conclusion

Low extracellular pH affects GLP-1 release at the final stages of exocytosis. Further work is required to elucidate the exact mechanism and proton entry route.

Preman Singh

The modelling of amyloidogenesis in live Drosophila cells

Supervisors: Professor Clive Wilson & Professor Adrian Harris

Research Aim

The development of a live *Drosophila* amyloid model to investigate amyloid neurodegenerative diseases.

Methods and Results

The male *Drosophila's* Accessory Glands (AGs) are analogous to the human prostate, and contain large Secondary Cells (SCs) each of which possess 7-10 Dense-Core Granules (DCGs). DCGs are comprised of the *Drosophila* amyloid Midline Fasciclin (MFAS), have physiological roles in reproduction, and in wild-type *Drosophila* are readily visualized as smooth round structures.

MFAS is the *Drosophila* homologue of Transforming Growth Factor Beta-Induced (TGFBI) a potentially amyloidogenic protein which confers structural stability to the human cornea. Mutations in TGFBI cause Corneal Dystrophies (CDs), characterized by amyloid deposits in the corneal stroma. MFAS and TGFBI, as members of a species-spanning family of amyloid proteins, are also related to Amyloid Precursor Protein (APP), the precursor molecule of β-amyloid (Aβ) found in Alzheimer's brain plaques.

My work found that these amyloid proteins influence each other, a finding with clinical implications. Thus, *Drosophila* transgenic for *TGFBI* which carried CD-causing mutations had abnormal biogenesis of MFAS in their DCGs. The introduction of a *TGFBI*-CD mutation into a conserved location in *MFAS* similarly caused MFAS abnormalities.

Familial Alzheimer's Disease (FAD) is caused by autosomal dominant mutations in the membrane-spanning APP, and in the secretases which release it from the membrane to generate Aβ oligomers. Several FAD mutations are clustered within APP's intramembranous Aβ sequence. *Drosophila* transgenic for *Aβ* FAD mutations displayed the reorganization of MFAS into patterns similar to the mutation-specific Aβ plaques. Furthermore, knockdown of *APPL*, the *Drosophila* homologue of *APP* resulted in the non-coalescence of MFAS, giving DCGs a fragmented appearance. Other phenotypic effects on MFAS were seen with manipulation of other amyloid neurodegeneration-associated genes.

Conclusion

Amyloid biogenesis, trafficking, and crosstalk can be modelled in a live *Drosophila* system, a discovery with broad implications for amyloid neurodegenerative diseases. Furthermore, the experimental demonstration of crosstalk occurring between diverse amyloid proteins suggests that they possess fundamentally unique biological properties.

Wiktoria Blaszczak

Metabolic heterogeneity in Pancreatic Ductal Adenocarcinoma

Supervisors: Professor Pawel Swietach

Research Aim

To gain a survival advantage in hypoxic tumour regions, many cancer cells depend on glycolysis as a main source of energy to support growth and proliferation independently of oxygenation. The end-product of glycolysis, lactic acid, is excreted from cells via H+-Monocarboxylate Transporters (MCT). The aim of the project was to assess the potential heterogeneity of cells in terms of their permeability to lactic acid and its metabolic consequences.

Methods and Results

We characterised a panel of Pancreatic Ductal Adenocarcinoma cell lines in terms of membrane permeability to lactic acid. At single-cell resolution, we observed significant variation in the distribution of this parameter. The most profound heterogeneity was noted in MIA PaCa-2 cells, and to explore the underlying mechanisms for variation, we implemented a FAC-sorting technique that separated cells by MCT activity. Functional assay of metabolism revealed that MCT-high cells had increased glycolytic and respiratory rates, compared to MCT-low cells; however, this difference disappeared after 1 week. RNA sequencing of the sub-populations demonstrated striking differences in gene expression patterns, with high expression of the IL6 receptor gene in MCT-high cells. As confirmation for this link, sorting by IL6R expression revealed a higher glycolytic rate of IL6R+ cells. Again, the metabolic difference between IL6R+ and IL6R- cells was transient, disappearing after two weeks.

Conclusion

These findings suggest that cancer cell lines harbour a small population of cells with higher metabolic rates linked to elevated IL6R expression. Since the populations are not maintained, a likely mechanism involves an oscillator of IL6R transcriptional control. Such a desynchronised oscillator could be a survival advantage, as at least some cancer cells would have a higher metabolic rate independently of microenvironmental conditions.

Zaki Alsahafi

Regulation of the carotid body type-1 cell by lipid signalling pathways

Supervisors: Associate Professor Keith Buckler & Professor Jaideep Pandit

Research Aim

Carotid body, the main peripheral arterial chemoreceptor, regulates the respiratory and cardiovascular centres of the brain stem in response to hypoxia and metabolic acidosis. The carotid body responds to hypoxia with an inhibition of background potassium current, which is carried out predominantly by TASK channels leading to type-1 cells depolarization, voltage-dependent calcium influx, and neurosecretion. The mechanisms underlying hypoxia induced TASK channel inhibition are not fully understood. Previous work showed that TASK channels are subject to modulation by GPCRs through lipid signalling. This project investigates the possible role of lipid signalling in regulating TASK channels activity in type-1cell.

Methods and Results

Rat type-1 cells were isolated and calcium signal was measured (spectroflurometry) in response to hypoxia and to other pharmacological agents. 1.3 Results Preliminary results showed that muscarinic agonists evoked voltage gated calcium entry in type-1 cell. U73122, PLC inhibitor, blocked type-1 cell response to hypoxia and muscarine. M-3M3FBS, PLC activator, increased intracellular calcium levels of type-1 cell but had a little effect on type-1 cells muscarinic and hypoxic responses. Dynasore, dynamin inhibitor, blocked hypoxia induced calcium oscillations.

Conclusion

This work suggests a role for lipid signalling in mediating type-1 cell hypoxic and muscarinic inhibition of type-1 cells by either inducing a change in TASK channels gating or by internalizing TASK channels.

Asma Alamoudi

Monitoring the effect of therapeutic interventions in asthma using a novel measure of lung inhomogeneity type

Supervisors: Professor Peter Robbins & Dr Nayia Petousi

Research Aim

In asthma, accurately measuring disease activity within the lung remains a significant challenge that limits early disease detection, management, and objective assessment of therapeutic response. This study explores the utility of a novel measure of lung function for these purposes.

Methods and Results

This was an observational study at a specialist hospital asthma outpatient clinic in patients with type-2 high asthma. The e"ects of a biologic therapy were evaluated on a novel measure of lung inhomogeneity, sigmaCL (a measure of the unevenness of lung inflation or ventilation within the lung), and compared with FEV1% predicted.

A total of 15 participants were recruited. There was a significant correlation (Spearman) ($r = 0.484$, $p < 0.05$) between baseline blood eosinophil count and sigmaCL. There was no correlation between baseline blood eosinophil count and FEV1% predicted ($r = -0.26$, $p > 0.05$).
After 3 months of mepolizumab, there was a strong correlation ($r = 0.673$, $p < 0.005$) between ranked change in eosinophil count and ranked change in sigmaCL. In contrast, there was no correlation ($r = -0.470$, $p > 0.05$) between ranked change in eosinophil count and ranked change in FEV1% predicted.

Conclusion

In this small group of patients with type-2 high asthma, no significant link was found between the blood eosinophil count and disease activity in lung when assessed using FEV1% predicted. In contrast, significant correlations were detected when disease activity in the lung was assessed using sigmaCL.

Clinical Implications: SigmaCL is potentially a sensitive index of disease activity in the lung.

Ni Li

Human iPSC derived cardiac myocytes and sympathetic neurons in disease modelling

Supervisors: Professor David Paterson & Dr Dan Li

Research Aim

Human induced pluripotent stem cells (hiPSCs) offer an unprecedented opportunity to generate a potentially unlimited source of cells to develop model systems that facilitate a mechanistic understanding of human disease. However, the predictive ability of hiPSC derived neurocardiac co-culture systems to recapitulate the human phenotype in diseased modelling is limited. Here, we optimized current methods for induction of cardiac myocytes (hiPSC-CMs) and sympathetic neurons (hiPSC-SNs). The utility of healthy hiPSC-CMs was tested with pressor agents to develop a model of cardiac hypertrophy. Mono-cultures and co-cultures were also made from a patient with a catecholaminergic polymorphic ventricular tachycardia (CPVT) genotype (with isogenic pairing) to generate a model of triggered arrhythmia.

Methods and Results

Healthy hiPSC-SNs possessed neurite outgrowth, stained positive for PHOX2B, tyrosine hydroxylase and peripherin. Derived myocytes showed spontaneous beating, stained positive for cardiac troponin T and a-actinin. Cell surface area and sarcomere length was significantly increased in maturated iPSC-CMs. Healthy hiPSC-CMs exposed to AngII or ET-1 resulted in cell and nuclear enlargement, as well as enhanced proBNP gene expression and proBNP secretion. This overexpression was reversed by losartan in AngII treated cells. Isoprenaline induced cytoplasmic cAMP increase was higher in hypertrophic cells. CMs from the CPVT hiPSC line expressed a higher Ca2+ responsive to isoprenaline, caffeine and KCl stimulation when compared with healthy hiPSC-CMs in calcium imaging. They also displayed spontaneous Ca2+ oscillations after isoprenaline. CPVT hiPSC-SNs had greater Ca2+ transients to nicotinic stimulation, indicating a diseased phenotype also resides in the neuron as well as the myocyte.

Conclusion

We have recapitulated many features of the anatomy and (patho)physiology of SN and CM, where co-culture preparations behave in a manner that mimics key physiological responses seen in other mammalian systems. Whether our cell types have the full transcriptomic atlas of actual human cells remains to be established.

Chloe Tubman

The role of Wilms' tumour 1b in the regenerating zebrafish myocardium

Supervisors: Professor Paul Riley, Professor Tatjana Sauka-Spengler & Dr Filipa Simões

Research Aim

Following a heart attack, adult mammals are unable to regenerate their hearts. In contrast, zebrafish maintain the ability to regenerate their hearts throughout adulthood and this is driven by the dedifferentiation and proliferation of existing cardiomyocytes. However, a contribution of other cardiac progenitors has not been unequivocally excluded, and the mechanisms underlying cardiomyocyte dedifferentiation are unknown.

The epicardium is a single layer of multipotent progenitor cells that covers the outer surface of the heart. In adulthood the epicardium is quiescent, however, it becomes 're-activated' following injury, re-expressing embryonic epicardial genes including wt1b. Our preliminary data suggests that injury-activated wt1b expression may play a role in myocardial regeneration in zebrafish. My project aims to investigate whether there is a cell autonomous wt1b+ epicardial contribution to the regenerating myocardium and/or whether an activation of wt1b in existing cardiomyocytes is required for dedifferentiation prior to cell cycle re-entry and proliferation.

Methods and Results

I have established and validated a number of transgenic zebrafish lines, namely cell-specific inducible Cre recombinases and a system for INTACT nuclear labelling and sequencing, to examine the relative contribution of wt1b+ epicardial cells to the regenerating myocardium and identify the molecular pathways in which wt1b acts to regulate cardiomyocyte dedifferentiation across multiple time points.

Conclusion

During my DPhil, I have developed a number of new in vivo fate mapping and sequencing technologies in zebrafish which will enable me to investigate a role for wt1b in regulating cardiomyocyte dedifferentiation and in parallel to explore whether there is a contribution of wt1+ epicardial progenitor cells to the restored myocardium. Understanding the mechanisms by which the zebrafish heart can regenerate may elucidate potential therapeutic targets for human heart attack patients.

Matthew Lloyd

Identifying factors linking hyperglycaemia-induced metabolic dysregulation to loss of insulin biosynthesis in pancreatic beta cells

Supervisors: Professor Dame Frances Ashcroft

Research Aim

Diabetes/chronic hyperglycaemia alters glucose metabolism and reduces insulin biosynthesis in pancreatic beta cells. However, the underlying molecular mechanism(s) are poorly understood. The aim of this study was to investigate how changes in beta cell metabolism are linked to the downregulation of insulin biogenesis.

Methods and Results

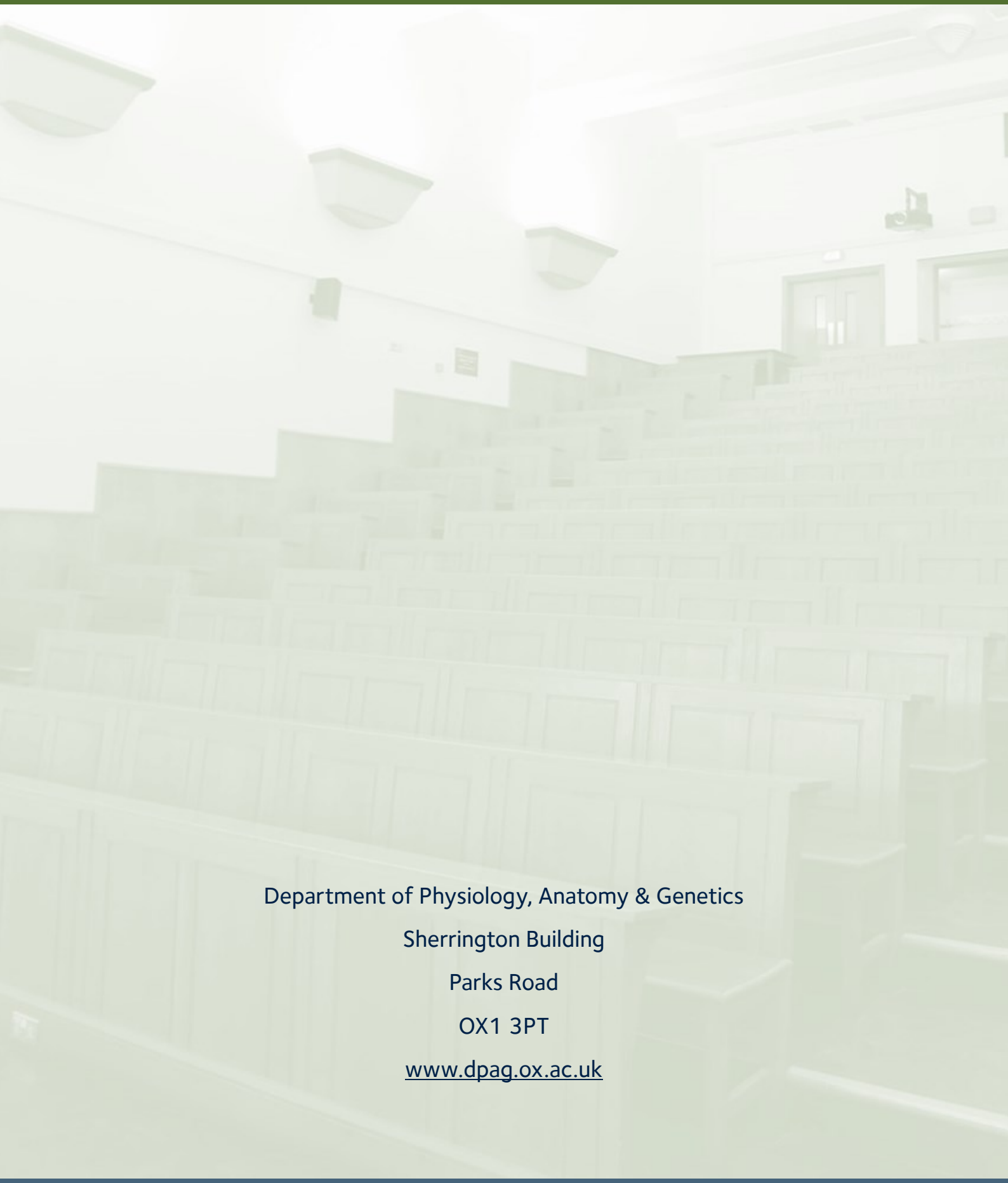
To identify metabolic factors responsible for the loss of insulin biosynthesis during hyperglycaemia, INS-1 cells were cultured at low (5mM) or high (25mM) glucose, ± selective glycolytic enzyme inhibitors. Gene expression was measured by qPCR and cellular insulin content was quantified by ELISA.

Hyperglycaemia reduced mRNA expression of the insulin genes (*Ins1* and *Ins2*) by >80% after 24hrs; however, a subsequent change to low glucose for 24hrs failed to fully restore their expression. 10mM mannoheptulose, a glucokinase inhibitor, prevented downregulation of these genes during 48hrs of hyperglycaemia. 5μM koningic acid, an irreversible inhibitor of GAPDH, reduced *Ins1* and *Ins2* expression by >55% and >80% respectively during 48hrs at low glucose. 24hr low-glucose culture with 25mM 2-deoxyglucose (which is metabolised only to 2-deoxyglucose 6-phosphate) reduced *Ins1* and *Ins2* expression by >60%. In each experiment, changes in mature insulin content were comparable to changes in insulin gene expression and the beta cell identity genes *Mafa, Neurod1, Nkx6-1, Pax6,* and *Pdx1* showed similar trends to the insulin genes.

Conclusion

These results suggest that chronic hyperglycaemia downregulates insulin gene expression in pancreatic beta cells via changes in glycolytic metabolites that lie downstream of glucokinase and upstream of GAPDH. Experiments with 2-deoxyglucose implicate glucose 6-phosphate as an important driver of insulin loss.

SHERRINGTON TALKS 2022



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