SHERRINGTON TALKS 2021 ONLINE DAY TWO

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS
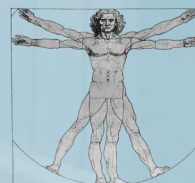
JOIN MICROSOFT TEAMS MEETING

FRIDAY 18TH JUNE

1PM

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

Chaired by Deputy DGS Professor Manuela Zaccolo

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

UNIVERSITY OF OXFORD

SHERRINGTON TALKS 2021

Judges

Graduate Studies Committee Academics and the audience

Prize winners will be notified in the Digest on Monday 21 June

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

Quyen Do

In vitro Modelling of Human Medium Spiny Neurons Using Induced Pluripotent Stem Cells

Supervisors: Dr Nora Bengoa-Vergniory & Professor Richard Wade-Martins

Aim: Medium spiny neurons (MSNs) are GABAergic inhibitory neurons that constitute more than 90% of the striatum and play key role in motor coordination as well as cognitive functions. For example, the selective loss of dopaminergic neurons (DAN) in the substantia nigra and the corresponding changes in MSNs, the major target of DAN axons are central to the pathophysiology of Parkinson's Disease (PD). Here, we optimised differentiation of MSNs from human induced pluripotent stem cells (iPSCs). Subsequent co-culture of iPSC-derived MSNs with iPSC-derived cortical neurons, the major inputs impinging on MSNs *in vivo*, on microfluidic devices with customised connectivity, led to more functionally mature and synaptically active MSNs as compared to those in monocultures. This finding opens up the possibility of establishing an *in vitro* model of an all iPSC-derived cortico-striato-nigral mini-circuit which more faithfully recapitulates the physiological context of MSNs for PD modelling. Such model is critical to enable investigation of early and temporal changes of MSNs in the presence of DAN carrying PD-specific mutations.

Raffaele Sarnataro

Illuminating the cellular underpinning of sleep homeostasis

Supervisors: Professor Gero Miesenböck & A/Professor Vladyslav Vyazovskiy

Aim: Sleep is vital and universal, yet very little is known about its biological function. The need for sleeping more and deeper after loss of sleep, a process called sleep homeostasis, shows how key this state is to organisms.

Neurons innervating the dorsal fan-shaped body (dFB) of fly brain constitute the output arm of its sleep homeostat, and share fundamental features with their mammalian counterpart.

We aim at understanding two aspects of the cellular machinery of sleep homeostasis: if the heterogeneity in this brain region is functionally relevant to the integration of sleep inputs and how sleep history orchestrates molecular changes in dFB neurons.

Methods and Results: Single-cell RNA-sequencing of dFB neurons in conditions of high and low sleep pressure has allowed to assess cellular heterogeneity and analyse dynamic molecular changes.

To functionally validate the *in silico* identity of dFB neurons, we have identified their major fast-acting neurotransmitter according to their transcriptome and tested that experimentally via immunohistochemistry, genetic intersectional approaches and loss-of-function and thermogenetics sleep behavioural assays.

While mitochondrial and metabolic genes appear upregulated in dFB neurons upon sleep deprivation, genes involved in synapse organisation and short-term memory seem downregulated. We synaptically tagged these neurons and observed corresponding reduction in their synaptic active zone proteins.

We have identified a distinct heterogeneity within dFB neurons in transcriptional expression of octopamine receptor subunits, and we will present genetic intersection fluorescence imaging supporting these findings.

Conclusion: This work generated the first full single-cell transcriptome of fly sleep-controlling neurons, in different conditions of sleep pressure.

We have developed multiple evidence supporting that dFB neurons are glutamatergic.

We observed that plastic synaptic changes occur in dFB neurons according their sleep pressure state.

Finally, we found that the monoamine octopamine, implicated in sleep-control, has heterogeneous receptors on dFB neurons, suggesting a functional differentiation of inputs onto this sleep-controlling brain area.

Maria Claudia Caiazza

Ca^{2+} Cellular Compartment Specific Deficits In iPSC-Derived Neuronal Models Of Parkinson's Disease

Supervisors: Professor Richard Wade-Martins & Dr Charmaine Lang

Aim: Parkinson's Disease (PD) is characterised by the loss of dopaminergic neurons (DAns) in the *substantia nigra*. Ca^{2+} is crucial in the regulation of many neuronal cellular processes. In particular, in DAns continuous Ca^{2+} waves occur, placing these neurons in an environment where even small alterations in Ca^{2+} homeostasis might impact on cellular function. We aim at studying the differences in Ca^{2+} dynamics in induced pluripotent stem cells (iPSC)-derived DAns from PD patients.

Methods: Here we utilise iPSC-derived DAns harbouring mutations in the *GBA* gene (*GBA-N370S*) and in the *SNCA* gene (*SNCA*-Trp). We employ Fura-2 to observe gross changes in Ca^{2+} dynamics and genetically encoded Ca^{2+} indicators (GECI) entrapped in the mitochondria and in the endoplasmic reticulum to trace down the source of such changes. Ca^{2+} imaging techniques are used in combination with drugs able to induce Ca^{2+} mobilisation in the cell to study Ca^{2+} dynamics in iPSC-derived DAns.

Results: iPSC-derived DAns harbouring the *GBA-N370S* and the *SNCA*-Trp mutations displayed altered Ca^{2+} dynamics. In particular, ionomycin-induced Ca^{2+} release in the cytoplasm appeared to be reduced. At a closer look, the ER appeared to release less Ca^{2+}. At the same time, mitochondria displayed a decreased Ca^{2+} uptake in response to ionomycin. Finally, CCCP-induced Ca^{2+} release from the mitochondria was reduced in *SNCA*-Triplication neurons.

Further proteomic analysis will be carried out to investigate the origin of this dysfunction.

Conclusions: We believe that studying Ca^{2+} dynamics and deficits in iPSC-derived DAn models of Parkinson's could shed some light on the pathogenic mechanisms associated with neuronal vulnerability and death in Parkinson's. Mass spectrometry analysis will be employed to dig deeper into the causes of the calcium deficits described by studying the levels of Ca^{2+} transporters expressed in the ER and mitochondria of patients cells, with the ultimate goal to identify new therapeutic compounds that can be used to ameliorate early disease phenotypes, to halt the further downstream neurodegeneration as seen in Parkinson's.

Rishi Anand

Striatal glycine inhibits axonal dopamine release in a region-specific manner and partly via regulation of striatal ACh

Supervisors: Dr Katherine Brimblecombe & Professor Stephanie Cragg

Aim: Striatal dopamine (DA) axons are key sites for gating DA release by diverse striatal neurotransmitters and with regional heterogeneity. Microdialysis studies have shown that ligands for glycine receptors (GlyRs) alter extracellular DA concentrations ($[DA]_o$) but report a range of outcomes. This variation might arise from direct or indirect effects via other neurotransmitters, e.g. ACh, and from regional diversity.

Methods and Results: We explored how glycine modulates $[DA]_o$ detected in real-time using fast-scan cyclic voltammetry, evoked electrically in dorsal striatum (unless indicated) in ex vivo slices from C57BL6/J mice. Glycine (5-10 mM) reduced evoked $[DA]_o$ to a greater extent in dorsal than ventral striatum Inhibition of glycine transporter 1 (GLYT1, 500 µM sarcosine) enhanced the effect of glycine, but alone did not alter evoked $[DA]_o$. GlyR agonist taurine (10 mM) and, paradoxically, GlyR antagonist strychnine (10 µM) reduced evoked $[DA]_o$, reflecting either agonist-mediated GlyR desensitisation or non-specific pharmacology of GlyR ligands. Glycine also increased the frequency-dependence of DA release, an effect occluded by prior application of an antagonist for β2-subunit-containing nicotinic ACh receptors (nAChRs) (1 µM DHβE) indicating an action via ACh acting at nAChRs. We tested whether glycine inhibits ACh release by imaging fluorescence of virally expressed $GRAB_{ACh3.0}$ sensor[1] and identified that glycine reduced tonic and evoked levels of dorsal striatal ACh. Glycine modulation of DA release did not involve co-agonism at NMDA receptors or modulation of GABA tone on DA axons, as antagonism of NMDA (50 µM AP-V), $GABA_A$ (10 µM bicuculline) or $GABA_B$ receptors (4 µM CGP-55845) did not alter the effect of glycine.

Conclusion: Glycine reduces DA release particularly in dorsal striatum, partially via inhibition of ACh release and nAChR action, but also partially via a nAChR-independent mechanism that might be mediated directly via putative GlyRs on DA axons.

Zeinab Ali

Reintroducing the 'M323K' mouse as an ALS-FTD model

Supervisors: Dr Silvia Corrochano, Dr Tom Cunningham, A/Professor Peter Oliver & A/Professor Maike Glitch

Aim: Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease characterised by progressive muscle weakness and subsequent motor defects, due to degenerative changes of upper and lower motor neurons. Traditionally, ALS was believed to spare cognitive functions. However, ~50% of ALS patients develop cognitive and behavioural impairments, whilst ~15% of patients develop frontotemporal dementia (FTD). ALS and FTD are now considered parts of the same spectrum disorder.

TDP-43 is an RNA-binding protein that is implicated in both ALS and FTD pathology. This protein is normally localised in the nucleus and is involved in RNA metabolism processes. Cytoplasmic aggregates of this protein are found in >90% of ALS cases and in 45% of FTD cases. Currently, there is no physiological TDP-43 model that exhibits both the motor and cognitive defects seen in ALS-FTD.

The M323K mouse contains a point mutation in the TDP-43 gene (*Tardbp*). Homozygous mice display a mid-to-late life onset neurodegenerative phenotype, mainly motor symptoms (Fratta et al, 2018). However, cognitive testing has not yet been explored.

Methods: A longitudinal study was conducted on M323K female mice, which focused on identifying progressive phenotypic changes in homozygous mice. A range of motor, cognitive and metabolic tests were performed at an early (3-months) and late (1-year) time point. Diffusion and structural MRI scans were done at 1 year.

Results: Homozygous mice display cognitive deficits from 3-months of age. General well-being tests revealed impairments in innate behaviours, indicating non-specific hippocampal dysfunction. Further in-depth cognitive tests revealed learning and memory problems in the homozygous mice at 1-year. MRI scans showed anatomical and structural brain alterations in homozygous mice at 1-year.

Conclusion: The M323K mouse is an excellent physiological model of ALS-FTD. Its ability to mimic clinical motor and cognitive phenotypes make it a unique and invaluable tool to further understand ALS-FTD pathophysiology.

Helen Potts

Exploring the role of the Astyanax mexicanus immune response in heart regeneration

Supervisors: Professor Robin Choudhury & A/Professor Mathilda Mommersteeg

The human heart cannot regenerate following myocardial infarction and instead forms a fibrotic scar that impairs cardiac function. This often leads to heart failure, which is incurable and a major cause of morbidity. Research in the field of cardiac regeneration aims to address this unmet clinical need by stimulating the adult human heart to repair itself, as witnessed in other organisms, like the zebrafish. The Astyanax mexicanus is a uniquely suited model to study cardiac regeneration as it comprises two closely related populations: (1) 'regenerative' surface (SF) populations that efficiently replace lost cardiac tissue after injury and (2) the 'non-regenerative Pachón (PF) cave population that form a scar. The innate immune response is known to be a key regulator of successful regeneration. However, this response differs between successful regeneration and scarring is unknown. To fully characterise the A. mexicanus innate immune response, single cell RNA-sequencing was performed on hearts isolated at uninjured, sham, 1, 3, 7 and 14 days post-cryoinjury (dpci) and validated using in situ hybridisation. We found striking spatiotemporal differences: the PF show a stronger immediate response to injury at 1dpci and 3dpci whereas the SF response is greater at 7dpci ($p<0.01$). The PF show a greater neutrophil response immediately after injury ($p<0.05$) which is then resolved after 3dpci. In contrast, the regenerative SF show a prolonged elevation of neutrophils in the wound at 7dpci and 14dpci. Differential gene expression analysis showed that these late-stage neutrophils upregulate inflammatory TNFα-NFκB signalling. Further studies will aim to determine the role of these late-stage neutrophils in successful regeneration. Understanding how the innate immune response differs between successful regeneration (SF) and scarring (PF) would be a significant step towards identifying novel therapeutic targets for immunomodulation in heart attack patients.

Oliver C. Neely

Non-Classical Hypertension? A neuroimmune pathology underlying a cardiovascular disease

Supervisors: Professor David Paterson & A/Professor Ana Domingos

Hypertension is a global health crisis; the disease kills over nine million people each year, yet its pathophysiology remains enigmatic, and has a multi-faceted aetiology. Medicine needs more effective treatments, and an integrative, systems-level approach is likely required in order to develop these. Looking beyond the cardiovascular system itself, sympathetic nervous system hyperactivity is a key driver of hypertensive disease and its associated co-morbidities. I hypothesised that a neuroimmune pathology may underlie this sympathetic phenotype, and my work examines this using the spontaneously hypertensive rat (SHR) as a model, with some additional data from human hypertensive patients. Using flow cytometry, I discovered a shift proportions of classical and non-classical monocyte subsets within the sympathetic ganglia and blood of the SHR, compared to Wistar rats. SHR rats appear to display a lower classical: non-classical ratio; the non-classical monocytes being known to have pathological pro-inflammatory roles in a number of other diseases. A combination of qPCR and a chemotaxis assay data suggests that this difference is due to an inherently higher production of non-classical rather than classical monocytes in the SHR. Finally, co-culturing bone marrow-derived macrophages with stellate neurons tends to increase the neuronal calcium response to nicotinic stimulation in Wistar neurons, whereas it does not in those of the SHR. From this one could tentatively suggest that the immune cell environment in some way contributes to the SHR's sympathetic hyperactivity phenotype, at the level of the neurons. Further work to confirm this could potentially help reveal novel disease-modifying therapeutics for essential hypertension.

Larissa Goli

RNA splicing in health and in spinal muscular atrophy

Supervisors: Dr Yulia Lomonosova & Professor Matthew Wood

Aim: The goal of my DPhil research is to characterise splicing changes during the early stages of spinal muscular atrophy (SMA), in a time- and treatment-dependent manner, in the two primary tissued affected (muscle and motor neurons), and to apply this knowledge to (1) characterise the efficacy of a newly developed splice-switching therapy; and to (2) identify new molecular targets for therapies that could be used in combination with the existing oligonucleotide antisense therapy.

Methods and Results: SMA is an autosomal recessive neuromuscular disorder caused by the loss of function of the SMN protein. SMN is critical in the formation of the spliceosome and the splicing of pre-mRNA.

I work with a severe mouse model of spinal muscular atrophy. Mice were treated at presymptomatic stages of the disease with Pip6a-PMO, a splice-switching oligonucleotide aiming at increasing the pre-mRNA level of the *SMN2* gene.

Tissue from the tibialis anterior, as well as laser-capture microdissected motor neurons from the spinal cord, were harvested. The extracted RNA was analysed on Affymetrix MTA® exon microarray.

In the first part of the project, I conducted bioinformatics analyses to describe and investigate the pathogenicity of splicing changes in SMA, alongside motoneuron and muscle development.

In the second (ongoing) part of the project, I investigate whether increasing the post-translational stability of the SMN protein, by targeting various atrophy-related molecular actors, can be therapeutically relevant in SMA — with potential applications to other diseases with atrophic symptomatology.

Conclusion: Pip6-PMO therapy is efficacious in restoring gene- and exon-level alterations in both tissues primarily affected in SMA.

In vivo validation in patient-derived cellular models remains to be conducted to confirm the pathogenic role of the identified splicing dysregulations.

This will allow for the design and *in vitro* and *in vivo* assessment of new oligonucleotide-based therapies in neuromuscular diseases.

Gurpreet Kaur Bharj

Insights into Otitis Media: Dissecting the interaction of C-Reactive Protein with *Non-Typeable Haemophilus influenzae*

Supervisors: Dr Derek Hood & Professor Helen Christian

Introduction and aim: Otitis Media (OM) is the inflammation of the middle ear (ME). Non-typeable *Haemophilus influenzae* (NTHi) is one of the leading otopathogens in causing OM. Phosphocholine (PCho) on the NTHi lipopolysaccharide influences host-pathogen interaction. C-Reactive Protein (CRP), an acute phase protein recognizes PCho, and can mediate bacterial killing. However, some strains of NTHi survive even in the presence of CRP and persist to cause OM infection. Currently, there are no OM specific treatments or vaccines against NTHi, thus OM persists to be a successful disease of early childhood globally.

We aim to study the interaction of CRP with NTHi to understand its role in bacterial survival and OM.

Methods and Results: NTHi can efficiently infect the *Junbo* mouse, a characterised model of chronic and acute OM. CRP levels were highest 1 day post-intranasal inoculation in the ME fluid (MEF) and nasal passage (NP) washes. We show CRP is a localized response to NTHi as serum CRP levels were unaffected in NTHi inoculated and non-inoculated mice at 1, 3 and 7-day post intranasal inoculation. Further, we confirm the presence of NTHi influences CRP levels in the MEF and NP washes. We show CRP binding is influenced by the position and expression of PCho on the NTHi surface. Serum bactericidal assays indicate that the expression and position of PCho affects NTHi survival. The removal of CRP from the serum restores NTHi survival. The expression of PCho also influences opsonophagocytosis activity in macrophages, thereby confirming the importance of PCho in NTHi survival.

Discussion: The CRP-NTHi interaction is currently under investigation to advance our understanding of its role in the complex biological processes that influence bacterial killing and the onset, progression and resolution of OM caused by NTHi.

Konstantinos Klaourakis

Investigating the role of cardiac lymphatic vessels in neonatal mouse heart regeneration

Supervisors: Dr Joaquim Vieira & Professor Paul Riley

Aim: Myocardial infarction (MI) triggers an immune response, whereby phagocytic cells remove debris and subsequently function to assist in repair and remodeling of the infarcted heart. In adult mice, MI activates cardiac lymphatics, which function to clear macrophages to the mediastinal lymph nodes (MLNs), reducing inflammatory/fibrotic cell content and improving functional output. Mice at postnatal day 1 (P1) fully regenerate their heart after MI in a pro-regenerative macrophage-dependent manner, whereas at P7 scarring is induced by pro-fibrotic macrophages. We hypothesise that lymphatics respond and function differently following MI during this regenerative window (P1–P7), trafficking subtypes of macrophages depending upon their requirement during regeneration and fibrotic repair.

Methods and Results: Here, we studied the development of postnatal cardiac lymphatics using *Vegfr3$^{lacz/+}$* knock-in mice, and examined the lymphatic response to surgically induced MI by light-sheet and confocal microscopy, as well as immune cell adoptive transfer. We observed that lymphatics expand from base-to-apex and undergo branching morphogenesis until P16. Whole-mount and cryo-section imaging data suggest that lymphatics have limited lymphangiogenic response after P1 MI, compared to P7 MI. To assess trafficking of macrophages from the heart to MLNs, we did adoptive cell transfer by injecting splenic *hCD68-eGFP* monocytes into the myocardium of P1 and P7 recipient mice undergoing MI. Imaging the MLNs 7 days after injury indicated a less efficient macrophage clearance from the heart to the lymph nodes after P1 MI, compared to P7 MI.

Conclusion: Collectively, we show that postnatal cardiac lymphatics continue to grow and mature until late in development and that at P1 cardiac lymphatics respond to MI and clear macrophages to the MLNs less efficiently, compared to P7 in line with a need to retain pro-regenerative macrophages in the neonatal heart. This project will increase our understanding of the functional role of innate immunity and lymphatic vasculature after MI and may lead to novel therapies for patients with heart disease.

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

Filippo Ghezzi

Investigation into early interneuron circuit of the mouse visual cortex

Supervisors: A/Professor Simon Butt, Dr Michael Kohl & Dr Louise Upton

Aim: GABAergic interneurons (INs) play an essential role during the development of the mammalian neocortical network. Somatostatin-expressing (SST+) INs are optimally suited to provide the main source of GABAergic signalling during postnatal development as they integrate early into the cortical network. In mouse primary somatosensory barrel cortex (S1BF), layer (L)5b SST+ INs establish a transient, translaminar, reciprocal circuit with thalamorecipient L4 spiny stellate neurons during the critical period for plasticity (postnatal day (P)5-8). These SST+ interneurons are also engaged by sensory stimulation of the whiskers from early postnatal period and hence play a role in regulating the transition to the mature network architecture.

The aim of this study is to investigate whether the same developmental strategy is employed in the primary visual cortex (V1).

Methods and Results: We set out to compare the function of SST+ INs to emergent visual perception using a combined in vitro circuit mapping and in vivo electrophysiology approach. Mapping of V1 GABAergic networks was performed through laser-scanning photostimulation coupled with glutamate -uncaging and in vitro electrophysiology. Our results show that GABAergic networks of V1 differ from S1BF from early development: indeed, V1 L4 excitatory neurons only receive input from SST+ interneurons located within L4 itself.

We then employed an optogenetic spike-tagging approach during in vivo electrophysiological recordings to study the contribution of genetically defined interneuron subtypes to processing of visual stimuli around the time of eye opening. Remarkably, SST+ interneurons in V1 are recruited by visual stimuli prior to eye opening. In contrast, Nkx2-1+ interneurons, which include parvalbumin-expressing (PV+) interneurons, are primarily recruited by visual stimuli only after eye opening.

Conclusion: This data suggests that SST+ interneurons establish different networks in S1BF and V1. However, they might provide the main source of GABAergic signalling in both primary sensory areas, to drive the maturation of the network.

SHERRINGTON TALKS 2021

Department of Physiology, Anatomy & Genetics

Sherrington Building

Parks Road

OX1 3PT

www.dpag.ox.ac.uk