

# SHERRINGTON TALKS

## 2020 ONLINE

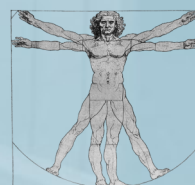
DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

JOIN MICROSOFT TEAMS MEETING

FRIDAY 19TH JUNE

1 PM

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



DEPARTMENT OF  
PHYSIOLOGY,  
ANATOMY &  
GENETICS



# SHERRINGTON TALKS 2020

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1450	Aashika Sekar Hormone-dependent and -independent steroid receptor regulation of prostate-like secondary cells in <i>Drosophila</i>	<i>Professor Clive Wilson &amp; Dr Aaron Leiblich MD</i>	10
1505	Dr Peter Kilfeather Found in Translation: Dopamine Neurons in Age and Disease	<i>Professor Richard Wade-Martins &amp; Dr Natalie Connor-Robson</i>	11
1520	Closing remarks from Professor Helen Christian MD, DPAG DGS		

## Judges

*Graduate Studies Committee Academics*

*Prize winners will be notified in the Digest on Monday 22 June*

# SHERRINGTON TALKS 2020

**Tai-Ying Lee**

## Decoding neural activity of a feedback circuit

Supervisors: Dr Johannes Dahmen & Professor Andrew King

Perception is shaped not only by sensory information but also by our past experiences, indicating sensory processing involves integration of both external inputs and internal information. While much research studies how bottom-up sensory information is represented in sensory areas, comparatively little is known about how top-down internal states influence this processing. To address this question, this study focuses on the mouse auditory circuits. Specifically, the shell of the inferior colliculus (IC), which is the higher-order part of this midbrain structure that receives dense feedback projection from the auditory cortex, as well as substantial local midbrain inputs and ascending projections from auditory brainstem nuclei. Despite this unique position in the auditory system, how this circuit is involved in auditory processing and learning is poorly understood. By utilising an adeno-associated virus-mediated anterograde transsynaptic approach, we selectively targeted neurons in the shell of the IC that receive inputs from the auditory cortex. We used two-photon calcium imaging to monitor the activity of the neurons while the animals learned an auditory detection task. By comparing the neural activity to each task variable, we assess how information is represented in the shell of the IC, and how this representation is involved in sensory processing during learning.

# SHERRINGTON TALKS 2020

**Dr Adrian Soto Mota MD**

## A Ketone Ester as treatment for type 2 diabetes mellitus

Supervisors: Professor Kieran Clarke & Dr Rhys Evans MD

Emerging evidence shows that calorie restriction and ketogenic diets (low carbohydrate, high fat) improve insulin resistance, weight loss and glucose metabolism. It is unclear to what extent these results are secondary to high ketone blood levels or to carbohydrate restriction. Also, both low calorie and ketogenic diets are poorly tolerated.

Additionally, in both animals and humans, the infusion of acetoacetate or D-beta hydroxybutyrate lowers blood glucose levels. Interestingly, this blood sugar lowering effect is greater in people with diabetes than in healthy adults.

Nowadays it is possible to raise blood ketone levels by providing ketones in a drink ( $\Delta G^\circ$ ) without restricting food intake or carbohydrates. Its consumption in healthy volunteers has been proven to be safe and tolerable. If proven safe, tolerable and effective, this intervention could translate into a useful new treatment for chronic diseases such as type 2 diabetes and obesity.

In this study, we induced ketosis in twenty-two type 2 diabetes patients for one month while continuously measuring their blood glucose for six weeks. Blood lipids, blood pressure and acid-base were also monitored during their participation.

Both HbA1c and fructosamine improved after 28 days of exogenous ketosis. There were no differences in blood lipids, hypertension and post-prandial or fasting glucose. Improvement in HbA1c was comparable to other oral diabetes treatments.



# SHERRINGTON TALKS 2020

## Kyung Chan (KC) Park

Propionate anions accumulated systemically in propionic acidaemia produce sustained remodelling of cardiac epigenetics and excitation-contraction coupling

Supervisors: A/Professor Pawel Swietach & A/Professor Nicola Smart

**Background:** Propionic acidaemia (PA) is an inborn error of metabolism caused by defective propionyl-CoA carboxylase. In PA, abnormal catabolism of propiogenic substrates lead to metabolic acidosis and propionate accumulation. We investigated whether these changes relate to cardiac complications (cardiomyopathy, long-QT) common in PA. In other systems, propionate is known to inhibit histone deacetylases, therefore we hypothesised cardiac dysfunction in PA is epigenetically determined.

**Methods and Results:** Experiments were conducted using the hypomorphic mouse model of PA (*Pcca*<sup>-/-</sup> A138T) or wild-type rats. Ventricular myocytes (VMs) isolated from PA mice produced Ca transients (CaTs; FuraRed) from an elevated diastolic level with unchanged systolic Ca, consistent with reduced Ca reuptake and release from the sarcoplasmic reticulum (SR). SR Ca-ATPase flux was reduced, attributable to increased phospholamban (western). In keeping with cellular observations, end-systolic and diastolic volumes were higher in PA mice (cine-MRI), known risk-factors for cardiac dysfunction. Intriguingly, the changes to CaTs were not recapitulated by acute exposure (10-30 minutes) of rat VMs to propionate, indicating propionate is exerting sustained actions, which we attribute to changes in gene expression. In support of this, long-term exposure (48 hours) of rat VMs to propionate produced stable remodelling of action potentials (FluoVolt) and CaTs (Fluo-3), and a dramatic change in gene expression (RNA-seq). Enrichment analysis of differentially expressed genes predicted association with histone 3 lysine 9 and 27 acetylation (H3K9ac/H3K27ac). Immunofluorescence of nuclear H3K9ac/H3K27ac in rat VMs treated with propionate revealed a striking spatial pattern of acetylation (confocal), occurring in regions associated with active chromosomal compartments.

**Conclusion:** We demonstrate in PA sustained derangements in excitation-contraction (E-C) coupling, a common precursor to the development of cardiomyopathy and arrhythmias. Propionate increases H3K9ac and H3K27ac, histone marks previously associated with cardiac pathology. We propose cardiac dysfunction in PA is driven by epigenetic changes in the expression of key proteins involved in E-C coupling.

# SHERRINGTON TALKS 2020

**Dr Lukas Krone**

## Cortical regulation of global sleep homeostasis

Supervisors: A/Professor Vladyslav Vyazovskiy, Professor Colin Akerman & Professor Zoltán Molnár

**Aim:** While sleep-wake states are largely defined by characteristic neocortical and hippocampal oscillations, the transitions between vigilance states are thought to be regulated subcortically. Despite the well-established phenomenon of 'local sleep' – the local and use-dependent regulation of slow waves in neocortex – the possibility that cortical structures contribute to the global control of vigilance states has been overlooked. I set out to test whether specific cortical neurons, layer 5 pyramidal cells, contribute to the generation of cortical sleep slow waves and to the regulation of sleep-wake states.

**Methods and Results:** I chose a three-step approach combining local and global electrophysiological recordings with chronic and acute manipulations of neuronal activity in freely moving and naturally sleeping mice.

First, laminar recordings from primary motor cortex indicated that layer 5 leads the transition from silent 'OFF' to active 'ON' states during the slow oscillation of non-rapid eye movement (NREM) sleep. Second, chronic silencing of a subset of neocortical layer 5 pyramidal cells through cell-specific ablation of the key t-SNARE protein SNAP25 (Rbp4-Cre;Ai14;Snap25<sup>fl/fl</sup>) resulted in increased wake time and attenuated build-up of NREM sleep slow wave activity during spontaneous long wake episodes and sleep deprivation. Recordings of locomotor activity using infrared sensors revealed no obvious alterations in circadian activity patterns. Third, acute chemogenetic manipulations in combination with sleep electrophysiology are ongoing.

**Conclusion:** My results suggest a novel and unexpected role for the cortex in sleep-wake regulation. Cortical structures are not only responsible for the generation of state-specific oscillations, but also exert active control over the homeostatic regulation of sleep without disruption of the circadian clock. Based on these findings, the focus of research into molecular and cellular underpinnings of sleep homeostasis in the mammalian brain should shift from subcortical to cortical structures. Ultimately, cortical manipulations might provide inroads into the understanding and treatment of sleep disorders.

# SHERRINGTON TALKS 2020

## Anna Parsons

### Screening for the exogenous upregulation of the neuroprotective gene OXR1

Supervisors: Professor Roger Cox, A/Professor Peter Oliver, Dr Silvia Corrochano & A/Professor Francis Szele

Oxidative stress is implicated as part of the neurodegenerative process in many neurological disorders such as Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS). Importantly, harnessing the endogenous cellular mechanisms that prevent such damage may be a viable neuroprotective approach. Oxidation Resistance 1 (*OXR1*) was originally identified from a screen for genes that could prevent oxidative damage to bacterial DNA, and most recently, disruption of *OXR1* has been implicated in human neurodevelopmental disorders characterised by epilepsy and cerebellar ataxia. We have since demonstrated that over-expression of *Oxr1* can effectively protect against oxidative stress-induced cell death in neuronal cells, while expressing higher levels of *Oxr1 in vivo* is able to reduce and delay neurodegeneration and neuroinflammation in two independent mouse model of ALS. These findings suggest that *Oxr1* is a viable and safe therapeutic target against OS-related neurodegeneration. We are currently investigating pharmacological upregulation of this neuroprotective gene and studies of the conserved promoter region have resulted in an optimised dual reporter assay in a stable neuronal cell line for large-scale compound screening. Our aim is to identify promising leads for testing in cellular and mouse models of neurodegeneration, while continuing to understand the molecular mechanisms of these protective properties.

Anna Parsons<sup>1,2</sup>, Matthew G Williamson<sup>2</sup>, Mattea J Finelli<sup>2</sup>, Kay E Davies<sup>2</sup>, Peter L Oliver<sup>1,2</sup>

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# SHERRINGTON TALKS 2020

Laura Garmendia Sanchez

## Mapping sleep-control circuits in *Drosophila melanogaster*

Supervisor: Professor Gero Miesenböck

**Aim:** During wake, reactive oxygen species (ROS) accumulate in *Drosophila melanogaster*'s dorsal fan-shaped body (dFB) sleep-promoting neurons; this intracellular increase in ROS alters the kinetics of membrane conductances, leading to increased neuronal firing and, eventually, inducing sleep. While it is widely assumed that the primary function of sleep is in the brain, it has also been proved that sleep loss affects non-neural physiological systems in both rodents and flies. The effects of sleep deprivation outside the brain include changes in oxidative stress in peripheral tissues, such as increased ROS in the gut of mice and flies. The aim of my project is to test the hypothesis that ROS in tissues other than the brain, such as in the gut, have a sleep-regulating function.

**Methods and Results:** I have devised and developed genetic tools to screen for and establish, in an unbiased way, a possible causal link between sleep and oxidative stress in any tissue of the fly, including extraneural tissues. These tools consist of a chemogenetic system that makes it possible to control the intracellular production of ROS in any genetically defined cellular population in the fly. The system is based on a D-amino acid oxidase from yeast which, through the oxidation of D-amino acids, produces H<sub>2</sub>O<sub>2</sub>. Additionally, this chemogenetic system is linked to a H<sub>2</sub>O<sub>2</sub> fluorescent sensor, which makes it possible to easily quantify the induced increases in ROS intracellularly. The validity of the system to generate oxidative stress through chemogenetics has been successfully verified in vitro. I have also begun testing the ROS-inducing chemogenetic tools behaviourally.

**Conclusion:** Having successfully developed these ROS-inducing chemogenetic tools, I now hope to use them to determine if increasing ROS in tissues other than the brain can cause sleep in flies.



# SHERRINGTON TALKS 2020

## Sebastian Birtles

### Modulation of Sexual Behaviour by Neuropeptides in *Drosophila*

Supervisors: Professor Stephen Goodwin & Professor Scott Waddell

**Aim:** To examine the role of neuropeptides in modulating sexual behaviour in the fly. Specifically, by addressing the following questions:

- What is the distribution of neuropeptide *receptors* in the nervous system? Are neuropeptide receptors expressed in the *dsx* expressing neurons underlying sexual behaviour?

(With a focus on the neuropeptide, SIFamide):

- Does SIFamide signalling through *dsx* neurons directly modulate sexual behaviour? In what context does this signalling occur?
- How does signalling affect neuronal and circuit properties?

**Methods and Results:** A combination of genetic-based tools, immunohistochemistry and single cell sequencing data suggests Neuropeptide receptors are expressed broadly in the nervous system, with several expressed in the *dsx* circuits governing sexual behaviours, including the receptor for the neuropeptide SIFamide (SIFaR). Use of a null allele of SIFaR receptor reveals that expression of the receptor is required for normal male courtship behaviour, while knockdown of the receptor in *dsx* neurons also produces abnormal courtship phenotypes.

**Conclusion:** Neuropeptide receptors are expressed in the neurons underlying sexual behaviour and signalling through these neurons likely functions to modulate the performance of these behaviours in various contexts.

# SHERRINGTON TALKS 2020

## Aashika Sekar

### Hormone-dependent and -independent steroid receptor regulation of prostate-like secondary cells in *Drosophila*

Supervisors: Professor Clive Wilson & Dr Aaron Leiblich MD

Castration-resistant prostate cancer (CRPC) is an incurable, androgen-independent form of prostate cancer that emerges in the hormone-depleted environment of androgen deprivation therapy (ADT). However, CRPC growth still frequently depends on Androgen Receptor (AR) signalling. We have found that prostate-like secondary cells (SCs) of the *Drosophila* male accessory gland grow with age in virgin males, and this growth requires the steroid ecdysone and its receptor, the Ecdysone Receptor (EcR), which shares broad structural similarities with the AR. Bone morphogenetic protein (BMP) signalling also stimulates growth by regulating EcR levels post-transcriptionally via its N-terminal domain (NTD). Additional EcR-dependent SC growth occurs after mating, and this does not require ecdysone. This unique hormone-independent mechanism mirrors events in CRPC. Genome endoreplication, a process not normally observed in SCs of adult virgin males, partly accounts for this growth<sup>1</sup>. Several cell cycle regulators implicated in CRPC, such as cyclin D and E, Retinoblastoma and E2F1, are required to induce mating-dependent endoreplication and hormone-independent SC growth. We propose that hormone-independent signalling allows male flies to adjust their allocation of resources, adapting the secretory activity of SCs in response to the presence of females. These parallels with CRPC suggest that there may be an unsuspected physiological basis behind the emergence of this disease in humans. We are continuing to identify other signalling pathways and transcription factors involved in regulating this process in flies that could help dissect out the complex genetic regulation involved in the transition to CRPC.

1. Leiblich A, Hellberg JEEU, Sekar A, et al. Mating induces switch from hormone-dependent to hormone-independent steroid receptor-mediated growth in *Drosophila* secondary cells. *PLoS Biol.* 2019;17(10):e3000145. doi:10.1371/journal.pbio.3000145

# SHERRINGTON TALKS 2020

## Dr Peter Kilfeather MRCVS

### Found in Translation: Dopamine Neurons in Age and Disease

Supervisors: Professor Richard Wade-Martins & Dr Natalie Connor-Robson

#### Aims:

- To demonstrate axonal translation in a highly vulnerable cell type; dopaminergic neurons.
- To compare the axonal and cell body “translatome” during healthy ageing and in a Parkinson’s mouse model.
- To identify markers of vulnerability and protection from degeneration in nigrostriatal dopamine neurons.

**Methods and Results:** Our lab have produced a novel transgenic mouse line by crossing a human alpha-synuclein overexpression model, with a DAT-Cre driven TRAP line. TRAP Mice express an eGFP-tagged ribosomal subunit specifically in dopaminergic neurons, enabling the selective extraction of translating mRNA from these cells. I have optimised a method to purify this mRNA from both the cell body compartment and axons of the same mouse.

I compared axonal and cell body samples from 3 and 18-month wild-type and Parkinsonian mice. Using a series of transcriptomic and genomic analysis methods, I observe differences in how axons and cell bodies respond to age and disease at the gene expression and splicing level. These differences reflect coordinated changes in expression of functionally related genes, that together represent biological pathways with established and novel roles in neuronal function, ageing and Parkinson’s disease.

I have observed differences in the expression profile of two populations of dopaminergic axons that are differentially susceptible to degeneration, providing insight into the components of gene expression that confer susceptibility or resistance.

**Conclusion:** Dopaminergic neurons demonstrate changes in gene expression at both the cell body and axon level with ageing that are perturbed by a model of Parkinsonian ageing. These changes represent interactions not previously known to play a role in age and disease and provide grounds for hypothesis generation for further investigation.



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