SHERRINGTON TALKS 2019

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

LARGE LECTURE THEATRE

SHERRINGTON BUILDING

FRIDAY 14TH JUNE

1PM

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

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

UNIVERSITY OF OXFORD

SHERRINGTON TALKS 2019

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Judges

Graduate Studies Committee Academics

Monzilur Rahman

Investigating the temporal window of sensory representation in the auditory cortex

Supervisors: Professor Andrew King, Dr Nicol Harper & Dr Ben Willmore

In this study, we investigated the temporal window represented by the neural population in auditory cortex; how far into the past does the population represent auditory information, and how far into the future can it predict, and what kind of mechanisms might be involved in this representation. We played 20 natural sound clips, each of 5 s duration, to 6 anaesthetized ferrets and recorded single-unit responses in primary auditory cortical areas A1 and AAF, providing a neural population response for 73 neurons. We then processed the sound clips through a simple cochlear model to provide a time-dependent frequency decomposition (a 'cochleagram'). Then, using linear decoding, we estimated the past and future cochleagram from a 5 ms window of neural population response at the present. We could reconstruct about 0. 7 s into the past, and predict about 0. 3 s into the future, with at least some fidelity. We also performed the same analysis for a neural population response in the inferior colliculus (30 neurons). For the inferior colliculus, we could reconstruct only 0. 1 s into the past and predict 0. 15 s into the future. We investigated if the capacity for prediction and reconstruction could be explained by the linear spectrotemporal response properties of auditory neurons plus a static nonlinearity, i. e. a linear-nonlinear-Poisson (LNP) model. For both the cortical and collicular datasets, neuronal activity estimated low frequency spectral components of future sounds more faithfully than the LNP model, but estimated past sounds and high frequencies in future sounds with less accuracy. This suggests nonlinear mechanisms that increase some capacities for prediction may be involved in auditory processing, potentially at the expense of representation of the past. However, it should be noted that both datasets were dominated by high frequency tuned neurons, and the natural sound clips tended to have most power at low frequencies, which are points to consider in interpretation. We also examined decoding using different time windows of neural response (spans of 1, 5, and 10 time bins of 5 ms duration each). Longer time windows tended to improve the LNP model's capacity to estimate the past relative to that of the real neural responses, but had little effect on prediction of the future.

Jamie Lee

Investigation of Nedd4l, a gene recently associated with hearing loss in the mouse

Supervisors: Associate Professor Victoria Bajo Lorenzana MD & Dr Michael Bowl (MRC MGU)

Discovering and characterising novel genes required for auditory perception will further our understanding of the underlying molecular mechanisms of auditory function. The International Mouse Phenotyping Consortium (IMPC) contributes to this goal, as it aims to characterise every mouse protein-coding gene through a high-throughput, broad-based set of phenotyping tests. Auditory assessment of generated knockout models occurs at 14-weeks of age, via Auditory Brainstem Responses (ABRs). To date, 52 novel candidate deafness-causing genes have been reported, one being the *Neuronal Precursor Cell Expressed, Developmentally Downregulated 4 Like* (*Nedd4l*) gene. Knockout mice (*Nedd4l*$^{tm1b/tm1b}$) were found to be profoundly deaf at all frequencies tested.

As IMPC auditory assessment of *Nedd4l*$^{tm1b/tm1b}$ mice occurred at 14-weeks of age, longitudinal ABRs were performed to determine the onset of hearing loss. ABRs of younger *Nedd4l*$^{tm1b/tm1b}$ mice (P16, 3 - dand 8-weeks of age) revealed an early-onset progressive hearing loss, leading to profound hearing loss by 8-weeks of age. Distortion Product Otoacoustic Emissions (DPOAEs) were also performed on *Nedd4l*$^{tm1b/tm1b}$ mice, which yielded similar results to the longitudinal ABRs; DPOAE response levels progressively decreased from 3- to 8-weeks of age in *Nedd4l*$^{tm1b/tm1b}$ mice. Auditory tests were also performed on hair cell- and cochlear-specific *Nedd4l* knockout lines to investigate *Nedd4l*'s requirement in different cochlear tissues. An early postnatal hair cell-specific *Nedd4l* knockout model showed no loss of auditory function, whereas an embryonic whole cochlea *Nedd4l* knockout model resulted in a variable profound hearing loss.

Assessment of the sensory epithelium and cochlear structures was performed to identify the primary cause of hearing loss in *Nedd4l*$^{tm1b/tm1b}$ mice. At 3-weeks of age, immunolabelling of *Nedd4l*$^{tm1b/tm1b}$ mice inner hair cell afferent ribbon synapses revealed a decrease of matched pre- and post-synaptic membrane proteins. Additionally, a progressive loss of spiral ganglion neurons was observed in *Nedd4l*$^{tm1b/tm1b}$ mice, which first affects the apical turn of the cochlea at P16 but is widespread across all turns of the cochlea by 6-months of age. Loss of basal outer hair cell stereocilia bundles was observed at 8-weeks of age. However, the primary cause of the hearing loss in *Nedd4l*$^{tm1b/tm1b}$ mice is still unknown and the observed deterioration of cochlear tissues are likely secondary effects. This suggestion is supported by *Nedd4l*-regulated *LacZ* expression; X-Gal staining was found only in the interdental cells of neonatal mice and the endolymphatic sac of E13.5 and 16.5 embryos.

Auditory characterisation of *Nedd4l* knockout models has identified a novel deafness-causing gene. Identification of Nedd4l's *in vivo* substrates will help elucidate its role in the mammalian process of hearing.

Séverin Limal

The neural representation of unified percepts: Studying the integration of auditory and tactile stimuli in the somatosensory cortex

Supervisors: Dr Michael Kohl, Dr Kerry Walker & Professor Andrew King

Multisensory integration is the process through which we combine information from different senses in order to better understand our environment and resolve sensory ambiguities. Early anatomical and electrophysiological evidence suggested that a combination of base-level subcortical processing and higher cortical convergence in 'integration areas' was the central process behind multisensory integration. In such a model, primary sensory areas were thought to almost exclusively process information from their preferred sensory modality and contribute only little to the integration process. More recent studies indicate that primary sensory areas may form direct connections with other primary regions. It is however unclear how this contributes to multisensory integration at early cortical stages. In other words, it is not known how the responses of primary sensory neurons to multisensory stimuli are organised nor how these differ from unisensory responses. In the present study, we aim to answer these questions in the primary somatosensory cortex of the mouse. More specifically, we are investigating the effects that auditory-somatosensory training may have on neural responses in the barrel cortex and how this may relate to inputs from the primary auditory cortex. In order to do so, mice are trained on a sensory discrimination task and undergo awake 2-photon imaging while performing that same task both before and after training. Hence, as an animal learns to integrate information from different senses in order to obtain a reward, neural responses to stimuli can be recorded and compared. In the current presentation, I will highlight behavioural data showing the adequacy of a novel vibrotactile, auditory and audio-vibrotactile training paradigm and preliminary 2-photon data. These results, combined with preliminary longitudinal two-photon data, highlight the establishment of an experimental setup better adapted to the study of multisensory integration as well as a first look into changes in neural responses during training.

Aleksandar Ivanov

How the auditory system adapts to different environments: a tale of reverberation

Supervisors: Dr Kerry Walker, Dr Nicol Harper, Dr Ben Willmore & Professor Andrew King

Almost every natural sound is accompanied by many delayed and distorted copies of itself (echoes or reverberation) (Traer and McDermott, 2016). Unless the environment is very echoic, our brains cope effectively with reverberation. In contrast, reverberation can cause severe difficulties for speech recognition algorithms and hearing-impaired people. How might the healthy auditory system cope so well with reverberation? A major feature of auditory neurons is their ability to adapt to the sound statistics such as the mean or variance of the sound level (Dean et al. 2005, Rabinowitz et al. 2011). We posit that such adaptive phenomena could reduce the difficulties in identifying sound sources in reverberant environments. To test this hypothesis, we used a large data set of anechoic natural sounds and a room simulator 'Roomsim' (Campbell et al., 2005) to generate reverberant versions of these sounds. Both anechoic and reverberant sounds were passed through a model of the cochlea to produce 'cochleagrams'. We then trained a linear model to find the best mapping from the reverberant to the anechoic cochleagrams. The transformation learned by the model displayed similar characteristics to the spectro-temporal receptive fields (STRFs) of auditory neurons, such as narrow frequency tuning and lagging inhibition (Dean et al. 2008). For each model unit we measured a time constant of decay for this inhibition which was frequency-dependent, consistent with previous experimental results (Dean et al. 2008). The model also provided new predictions. First, the model's time constants increased with the amount of reverberation. Second, the ratio of inhibition to excitation in model kernels increased with the amount of reverb. We tested these predictions by recording the responses of large numbers of single neurons to these sounds in the auditory cortex of anaesthetised ferrets. Our data show that auditory cortical neurons seem to adapt to reverberation by adjusting their filtering properties in line with the two model predictions described above. Together, our results suggest that cortical neurons respond to reverberation by prolonging the inhibitory component of the receptive fields (increasing the time constants) and increasing the amount of inhibition relative to excitation.

Stewart Humble

Mapping Functional Gene-gene Interaction Networks in FTD/ALS and PD

Supervisor: Professor Richard Wade-Martins, Dr Brent Ryan & Dr Michael Ward MD (NIH)

Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) are adult-onset neurodegenerative diseases that are closely related clinically, pathologically, and genetically. Therefore, it is likely that converging disease pathways exist and can be used both to enhance our understanding of basic FTD/ALS biology and as leads for therapeutic intervention. FTD/ALS is often inherited and causal mutations are highly penetrant, indicating that these mutations have large effects on critical cellular pathways. Approximately half of the genes known to cause FTD/ALS encode proteins that regulate endolysosomal biology or play a crucial role in regulating proteostasis. Our own unpublished data implicates lysosomal dysfunction as a central pathophysiological consequence of mutations in *GRN* due to defects in endolysosomal acidification and/or maturation, building upon previously established work from my mentor demonstrating that lysosomes are a key player in FTD/ALS-related neurodegeneration.

Here, we propose to use our modified i^{3}Neuron platform to facilitate systematic knockdown of large panels of FTD/ALS-associated genes through CRISPR interference (CRISPRi) to interrogate Gene-Drug and Gene-Gene interactions. From this, we will generate genetic interaction maps of pairs of FTD/ALS genes in iPSC-derived neurons, identifying common pathogenic changes elicited by disease genes related to several key cellular processes, including endolysosomal acidification and maturation, lysosomal ion homeostasis, selective autophagy, organelle quality control, and neuronal survival. We will also use drug libraries with well-characterized molecular targets in combination with CRISPRi knockdowns to more precisely define sensitized pathways in FTD/ALS. Through these efforts, we hope to lay the groundwork for improved molecular understanding and therapeutic discovery.

Bradley Roberts

Striatal GABA transporter activity governs dopamine transmission and shows maladaptive downregulation in a mouse model of parkinsonism

Supervisor: Professor Stephanie Cragg

Dopamine (DA) release in the striatum is directly gated by mechanisms operating on striatal axons. We recently demonstrated that DA release is under tonic inhibition by a striatal GABA source. Given the paucity of GABAergic axoaxonic synapses on DA axons, this striatal GABA tone presumably arises from ambient GABA. GABA can provide an ambient tone on GABAergic striatal projection neurons at a level limited by striatal plasma membrane GABA transporters (GATs) but whether GATs determine DA output has been unknown.

We reveal that GAT-1 and GAT-3 strongly regulate DA release in mouse striatum by limiting the GABA tone on DA axons in dorsolateral striatum (DLS) but not nucleus accumbens core (NAcC). We find correspondingly greater GAT-1 and GAT-3 levels in DLS than NAcC. Further, we demonstrate that GAT-1 and GAT-3 located at least in part on astrocytes are critical to the level of GABA inhibition of DA release, as astrocyte inactivation prevented the effects of GAT inhibition. Moreover, in a human alpha-synuclein-overexpressing mouse model of parkinsonism, we find that tonic inhibition of DA release by GABA is augmented in DLS but not NAcC as a consequence of decreased GAT levels. Altogether, these data indicate that striatal GATs determine the level of GABA inhibition of DA release in a region-specific manner that supports DA release in DLS, and that GATs are a site of maladaptive plasticity in a model of Parkinson's that limits DA output.

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