Progress Report

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Title of Project: Characterizing the mechanism of antiseizure drugs with brain organoids and calcium imaging

Summary:

Hypothesis vs findings

The objective of this project is to use optical imaging and brain organoids to understand how antiseizure medications (ASMs) modify the properties of neuronal networks to prevent seizures, and the project was conducted within the Ion Channels & Human Diseases Laboratory (ICHDL) at the Florey Institute. We hypothesise that "treated" organoids would exhibit measurable changes in network properties compared to "untreated" organoids, and that these findings may provide novel biomarkers for ASM drug discovery and enhance the search for newer and more effective treatments.

Our first goal was to use 2-photon Calcium imaging to characterize the neuronal network activity of untreated organoids that have not received ASMs. This step provides us with baseline data to then compare with treated organoids. To achieve this, embryonic stem cells were genetically modified using CRISP-Cas9 to express the calcium fluorescence indicator GCaMP6s, cortical spheroids (organoids) were derived, and organoids were imaged using a 2-photon microscope to record neuronal network activity. After imaging ~30 organoids, we observed marked variability in their activity: many organoids had under 10 active cells, which would preclude detailed analysis of their network properties, and several organoids exhibited non-physiological activity such as global synchrony. These results are problematic for testing our hypothesis for several reasons. First, both the low and variable amount of activity would render it difficult to identify consistent differences between treated and untreated organoids. Second, and perhaps more importantly, the low and at times non-physiological activity may limit the validity of this system when drawing conclusions about drug mechanism-of-action, since studies using *in-vivo* imaging to examine cortical networks demonstrate higher levels of neuronal activity. Since our observations are nevertheless of importance to the field, they are currently being prepared as a manuscript for publication.

Changes to project

Considering these findings, we have decided to shift our experimental approach from using brain organoids to instead using *in-vivo* 2-photon imaging in mice to characterize the impact of ASMs on neuronal network properties. Although *in-vivo* imaging has the drawback of involving a more time-consuming imaging workflow, and hence lower "throughput" when screening for drug effects, it also holds several important advantages. First, it avoids issues associated with batch-to-batch variability in organoid generation and the low (and non-physiological) activity that we encountered. Importantly, *in-vivo* imaging, currently, likely provides a closer approximation to the cellular composition and connectivity of human cortex, and greater validity when assessing the biological impact of ASMs.

Progress and future directions

We have begun establishing an *in-vivo* 2-photon imaging platform within the ICHDL at the Florey Institute which has involved modifying our existing microscope to incorporate a head fixation and locomotion system. We have also begun optimizing our experimental workflow which involves procedures to genetically express calcium fluorescent indicators within cortical networks *in-vivo* and inserting long-term cranial imaging windows for optical access to the brain. Finally, we have commenced 2-photon imaging of the visual cortex to characterize neuronal network activity in "untreated" mice for baseline data. The next step will be to perform follow-up imaging in both treated and untreated mice. This will then allow us to examine within-subject changes in cortical activity induced by ASMs, and to address the original aim of this project.