Scientific Summary

Medical treatments for epilepsy remain non-curative, and about a third of patients are medically refractory. The development of effective therapies requires novel experimental systems to model seizure development. One very promising new platform is human brain organoids (or simply organoids), 3D cultures where specific brain-like structures are created from either human embryonic or induced pluripotent stem cells (hESCs or hiPSCs). Organoids recapitulate many structural features of the human brain and have provided unique insights into various neurological diseases. We generated "fusion" organoid structures in which excitatory neuron-predominant cortex (Cx) and inhibitory interneuron-predominant ganglionic eminence (GE) populations integrate, yielding an ideal platform for modeling neural circuit assembly and seizure development. Using this technique, I found that hESC-derived fusion organoids display interneuron-modulated spontaneous neural network activities including complex oscillations. Single cell RNA sequencing that I performed demonstrated that fusion was also critical for interneuron cell survival, as unfused GE organoids showed progressive loss of interneuron clusters with increasing age, unlike fusions. I also showed that hiPSC-derived fusion organoids from a patient with Rett syndrome, a genetic disorder highly associated with epilepsy, have epileptiform-like activity and altered network oscillations vs. isogenic control organoids. I rescued some of these abnormalities by treatment with the anti-seizure medication sodium valproate or the p53 inhibitor pifithrin-α. These data demonstrated that ***the fusion organoid model enhances interneuron survival, recapitulates epilepsy-associated abnormalities in vitro, and offers a novel platform for therapeutic validation and discovery.***

Based on these data and more recent preliminary findings, I propose to expand on this approach to model brain region specific cellular changes and the physiologic phenotype for a severe developmental and epileptic encephalopathy (DEE). I have recently generated fusion Cx+GE and hippocampus+GE (H+GE) organoids from a patient harboring an epilepsy-associated mutation in the *SCN8A* gene. *SCN8A* encodes the voltage gated sodium channel $Na_V1.6$ and gain of function mutations in *SCN8A* result in a devastating DEE called early infantile epileptic encephalopathy 13 (EIEE13). Reports of fetal seizure make brain organoids particularly well suited to model EIEE13. My preliminary data suggests a highly hyperexcitable phenotype, characterized by hypersynchronized calcium activity by live organoid two photon imaging and bursts of high amplitude local field potentials (LFPs), in SCN8A mutant Cx+GE fusions. Interestingly, *SCN8A* mutant H+GE fusions do not show the same hyperexcitable phenotype, and instead lack sharp wave ripple (SWR) oscillations. SWRs, which I frequently observe in non-mutant H+GE fusions, are believed to be interneuron dependent oscillations associated with hippocampal memory consolidation. Based on these data, *I hypothesize that the SCN8A mutant brain hyperexcitability is driven by cortical excitatory neurons, whereas SCN8A mutations in hippocampus result in interneuron dependent defects in SWR oscillatory activity.*

Aim 1: Determine the role of GE-derived inhibitory versus Cx-derived excitatory neurons in the *SCN8A* mutant cortical hyperexcitability phenotype. *The hypothesis that cortical excitability results from SCN8A GOF mutations selectively in Cx excitatory neurons will be tested by performing calcium imaging and LFP recordings on "unmixed" vs. "mixed" fusions. In mixed fusions either Cx or GE will be SCN8A mutant and the other half will be non-mutant.*

Aim 2: Determine the role of GE-derived inhibitory interneurons in hippocampal sharp wave ripples. *The hypothesis that SCN8A GOF mutation restricted to GE-derived interneurons will be sufficient to abrogate SWR oscillations in H+GE fusion organoids will be tested by performing mixed fusion experiments on H+GE organoids as in Aim 1.*

In harnessing an emerging, highly promising, and human cell based technology to model epilepsy, this proposal has the potential to provide groundbreaking insights into the pathophysiology of epilepsy. In addition, these studies are focused on the pathophysiologic changes in EIEE13, which is consistent with the CURE mission to cure epilepsy. The use of epilepsy patient iPSC derived organoids, with its potential for individualized and patient specific disease modeling, is aligned with the CURE mission of patient-focused care.

Future Directions

The Aims in this proposal are intended to lay the groundwork for a number of future studies. One broad future research objective is to establish the relationship between brain organoid single cell activity, the cell type involved, and the neuronal population activity seen in the LFP recording. In future studies I will develop methods to simultaneously record 2P calcium activity together with LFP recordings (currently these recordings are done separately). Simultaneous recordings will be followed by post-hoc whole organoid clearing, immunohistochemistry (IHC), and acquisition of stacked images in order to efficiently match the GFP/calcium recording region with the IHC stained region. The goal will be to decipher the specific single cell activities (by 2P calcium imaging) and the cell-types (by IHC) that contribute to particular LFP oscillations. In addition, I hope to perform inhibitor experiments, initially using drugs targeted at specific inhibitory or excitatory channels (i.e., gabazine for $GABA_A$ or CNQX for the kainate receptor etc.) to determine the cell types involved in specific oscillations. This can be followed by more specific targeting approaches using chemogenetic or optogenetic manipulations. For example, I can use designer receptor exclusively activated by designer drug (DREADD) experiments to selectively inhibit excitatory neurons by utilizing the CAMKII promoter to target this neuronal cell type. Another broad future objective is to use brain organoids in the design of drug/therapeutic screening tools. Once I have established patterns of physiological activity using relatively low throughput but high sensitivity tests such as single electrode LFPs, I intend to use higher throughput technology such as multi-electrode arrays that will allow me to simultaneously screen several dozen organoids at once. Using the LFP changes I have found by traditional recording methods, I will screen the MEA output for specific changes. For example, if I discover from the current experiments that a loss of SWR activity is a consistent physiologic phenotype in *SCN8A* mutant H+GE organoids, I will look for this in the MEA output. This method can then be used for efficient drug screening to rescue the physiological change and could potentially be a method for patient specific drug testing.

Award Relevance

This research is distinct from the work of my mentor, Dr. Bennett Novitch. Ben is a developmental neurobiologist with a primary interest is in the molecular pathways that determine neuronal cell differentiation and circuit assembly. Ben's work in brain organoids is based largely on his interest in neural differentiation and neurodevelopment. He is not a physiologist by training. Until his relatively more recent work with organoids, Ben was primarily known for his work in spinal cord development where his focus has been on understanding the circuits needed for breathing and motor activity. This is work that he continues in his laboratory. In contrast to Ben, I am a clinician-scientist with subspecialty training in epilepsy, and my interest is in neural network activity and physiology, and more specifically, in how epilepsy affects neural network formation and function. Our shared interest in utilizing and developing the brain organoid as a model system for neurological disease and brain development has made him an invaluable mentor during my training in his laboratory. However, given my distinct research, I have also collaborated with a number of other scientist-mentors at UCLA, such as Dr. Istvan Mody and Dr. Peyman Golshani, to establish the calcium imaging and LFP recording methods that are described in this proposal. The proposed work, in which I will delve more deeply into epilepsy and basic physiology, will be critically important to future work (as described above) and to further establish my independence from Ben.