II. Technical Description of Research

A) Introduction

Neurodevelopmental disorders (NDDs) such as epilepsy, autism spectrum disorder, and developmental delays vary from mild to life-threatening and often impair social interaction, speech, and cognitive development (1,2 Review article, 3). Despite these variations, NDDs share common neurophysiological characteristics: the hallmark of these disorders is imbalanced excitatory inhibitory input (E/I), which during development leads to dysfunction in neuronal circuits (4 Review article, 5). In most cases multiple factors affect the E/I balance, such as genetic expression, environmental factors, and complex compensatory mechanisms (6,7). Even when the disorder is monogenetic, the effects and identification of the causative mechanism and subsequent treatment are often difficult to understand (8–10). Brain channelopathies—disorders in which the activity of neuronal ion channels become altered—are particularly attractive for studying the E/I imbalance mechanism since their function (or malfunction) is directly linked to neuronal excitability (9, 11-14,17,19 Review articles) (9,11-17). Neuronal ion channels play a critical role in generating the electrical activity in all neurons, and disruptions in their normal activity have been highly associated with NDDs (11,17–19). In epilepsy, for example, channelopathies account for more than 30% of genetic screens (20), and several ion channel genes have been highly associated with autism spectrum disorders (4,8,21).

The current method for studying channelopathies is to express the mutated channel in a HEK cell and characterize its biophysical properties with electrophysiological recordings. This is a relatively simple procedure and it is relative simple to mathematically model channel activity in isolation (22); however, deciphering how a specific channel mutation affects the neuronal circuit requires careful experiments using designed animal models and can take years to execute (16,23–25). This need for highly-specific and time-consuming research limits our efforts to understand how different channelopathies cause individual expressions of NDDs and how to tailor the optimal therapy for children suffering from the disorders.

Here, I propose an alternative approach: developing a computational tool that can simulate both the effects of ion channel mutations on both individual neurons and on network activity. This tool would allow me to evaluate the therapeutic benefits of different drugs and interventions by modeling their effects on the cellular level without having to perform expensive and time-consuming animal studies. My approach utilizes a large-scale, highly-detailed biophysical neuronal simulation of a neocortical column that is reconfigurable and can mimic the neuronal activity observed empirically for healthy and disease states (26). The simulations provide a detailed mechanistic understanding of the role channelopathies play in the imbalanced E/I (Aim #1) and allow us to develop therapeutic targets that specifically counteract the disease symptoms (Aim #2). Finally in Aim #3, I will tailor the simulation network properties to match data from empirical studies to increase the simulation's predictive power for tailored therapies. This approach will allow me to study a wide variety of channelopathies in a fast and cost-effective manner and, if successful, could be applied later for a wide variety of NDDs. The goal is to develop a tool that accurately models the disease state and can be tailored to a child's genetic diagnosis, allowing clinicians to assess the effectiveness of different therapeutics on a given mutation with minimal time and resources.

B) Context

Currently, the common approach to studying NDDs (including channelopathies) is to design

animal models that replicate the causative mutation found in patients. The goal is to understand how these mutations cause altered brain function and how they can be targeted for therapy (27 Review article). This approach takes years and cannot always be generalized to other patients. Alternatively, using computational simulation of cortical column, I can integrate our knowledge about a specific channelopathy and fine tune some parameters of the model to simulate a different channelopathy (28). Similarly, I can study the interaction between a channelopathy and specific therapy to identify new therapeutic avenues for the treatment of NDD.

- **Competitive Analysis**: In this proposal, I combine different computational techniques into a neocortical circuit model that considers electrophysiological data and mathematical modeling to study the mechanisms underlying channel-related NDDs. I rely on two technologies to create a detailed and robust model: first, I use a biophysically-detailed model of a Primary motor cortex (M1) column (26); second, I use a state-of-the-art fitting method to generate ion channel models that are based on empirical voltage-clamp recording data from channels expressed in HEK (29-31) (provided by Dr. George). Several studies have been done using computational models to study epilepsy (32, Review article); however, these studies do not use highly-detailed, large-scale simulations that can provide cellular and even subcellular resolution of the neuronal circuit. Here I propose to incorporate the biophysical characterization of ion channel mutations into a large-scale, highly-detailed simulation that can simulate NDDs and potential therapies, including considering side effects and interactions with other drugs. These kinds of simulations were not available until recently and are now accessible to labs with standard computational resources. Utilizing these simulations will lead to new discoveries and insights regarding the mechanisms underlying NDDs, as well as accelerate the delivery of effective individualized treatments to patients, which is the goal of precision medicine. Additionally, this approach can be broadly applicable to other neurological disorders.

- **Supporting Evidence**: My previous studies showed how detailed simulations of single neurons constitute a revolutionary tool for studying NDDs (15,16,28-31); here I will expand this project to simulate large cortical networks to apply the method to developing therapies for NDDs.

C) Aims

My team has previously shown that neuronal biophysical models of single neurons can accurately simulate excitability changes due to channelopathies (15,16,28-31). Recent advances in neuronal simulations enable the generation of neuronal circuit and cortical column simulations using highly-detailed, single neuron models (26,33,34). I hypothesize that highly-detailed biophysical simulations of neuronal circuitry will provide a platform to study how different therapies can reverse the altered neuronal activity arising from channelopathies. I will study this hypothesis and generate a platform to simulate the effects of channelopathies on neuronal activity in Aim #1, test different therapeutic avenues in Aim #2, and tailor the column simulation to specific channelopathy cases in Aim #3.

Aim #1: Study *in-silico* how different channelopathies affect the excitability of cortical circuits and Layer 5 pyramidal neurons.

Rationale: The rationale for Aim #1 is that accurately simulating how channelopathies affect the excitability of Layer 5 pyramidal neurons (L5PNs) in a large-scale neocortical simulation will help us better understand the root cause of NDDs and develop more effective treatments. L5PNs are the main output of cortical networks and have a relatively high expression of NDD-associated genes (14,17,36). L5PN dendrites receive inputs from all cortical layers and long-range projections

from distal brain regions. These connections make them particularly sensitive to E/I imbalance. In my previous studies, I showed that the excitability of L5PN is an excellent marker for the behavior of the whole circuit (15,16,29).

Approach: In this aim, my team and I would utilize a model of M1 (primary motor) cortex (26) and simulate how channelopathies affect individual and network neuronal activity. Channelopathies can cause an increase or decrease in the excitability of neurons, and these changes can be due to either a change in the number of functional channels or to a change in the biophysics of the channel. These changes can be measured empirically by expressing the specific channel variant in the heterologous system (e.g., HEK cells) and recording the channel (15,31). From these recordings, I can characterize the unique biophysical changes of each ion channel variant. A major gap in the field of brain channelopathies is how to translate the biophysical measurements into changes in overall neuronal activity. Here, I will first modulate the channel biophysics in our model based on empirical data recorded in HEK cells and then incorporate the biophysical measurements of the channel into the *in silico* neurons that express it. Next, I will use a cortical column simulation to measure how these changes affect the overall excitability of the network and specifically see how L5PN change their firing patterns to better understand the pathophysiology of the simulated channelopathy.

Research Strategy

Experiment 1.1 Simulate ion channel-altered biophysical activity rising from channelopathies: I developed a pipeline that fits a detailed numerical ion channel model to a biophysically-characterized ion channel (15,28,31). Using these highly-detailed ion channel models (see description of Hidden Markov Models (HMM) in experiment 1.2) I can simulate the altered biophysics caused by each mutation and generate a numerical model for it. The ion channel models will be fitted to empirical data using a genetic algorithm (GA) (37,38). Most of the empirical data will come from my collaborator, Prof. Al George at Northwestern University. In his lab they use syncroPatch, a high throughput automated patch-clamp system to measure biophysical properties of channel variants. They have characterized the biophysical properties of 30-120 variants from each of the following channelopathies (SCN1A, SCN2A, KCNQ2), all of which are highly associated with NDDs (8,21). This empirical data will be complemented with data from databases that characterized these ion channels (SCN1A, CACNA1H). Each channelopathy variant (I will choose 20 mutants from each channelopathy) will require 24 node hours to generate an ion channel model: 480 core hours. The termination criteria for fitting an ion channel model to empirical data would typically be that the biophysical properties of the model converge to an RMS error of <5%.

Experiment 1.2: Simulate altered channel activity in different neuronal subtypes: Here I will use biophysically-detailed models of different neuronal subtypes from the M1 circuit simulation. However, these ion channel models are based on the Hodgkin & Huxley (HH) formalism (39), which allows a more simplified simulation of the channel but lacks flexibility. Generally speaking, HH channels cannot recapitulate all the different biophysical properties that more complex models offer (40,41). The Hidden Markov Models (HMM) are much more complex and require more computational resources, but allow the capture of all the biophysical properties seen in voltage clamp experiments (42). In the past, my team has been able to replace HH channel models with HMM models within the neuronal model and keep the original behavior. In this aim, I will change those channels for each of the neuronal subtypes that express the channels we study.

I will incorporate the altered channel from experiment 1 into the relevant neuronal model to simulate a specific channelopathy variant and study the firing patterns of individual neurons. This will allow me to generate neuron models for the different neuronal subtypes affected by the specific channelopathy. The completion of this experiment will yield two different models for the neuron sub-types affected by the channelopathy: a wild-type and a channelopathy model. These models will serve as the building blocks for the cortical column simulations in experiment 1.3.

Experiment 1.3: Simulate the effect of channelopathies on the cortical microcircuit: Recent advances in neuronal simulations (26,43,44) allow me to use wiring diagrams for connecting detailed single neurons to form a neuronal microcircuit. Using the neuronal models from experiment 1.2, I will simulate how channelopathies alter the activity of the M1 cortical circuit. The baseline M1 cortical circuit will be based on the paper from my collaborator the Dura-Bernal lab (26). Neurons will be simulated using multicompartment HH conductance-based models (except those replaced in Experiment 1.2 that will be HMM models) with multiple ionic channels and synaptic types. Neuron densities, distribution, morphology, and biophysics, as well as connectivity at the long-range, local, and dendritic scales, will be constrained by experimental data. The NetPyNE tool includes automated parameter optimization using genetic and Bayesian methods (45). My collaborator, Dr. Dura-Bernal, has developed models of macaque A1, mouse M1, and rat S1 thalamocortical circuits (26,46-48). The model includes over 10,000 neurons and 30 million synaptic connections, as well as long-range inputs from seven cortical and thalamic regions (Fig. 1). Neuron density, distribution, morphology, biophysics, and connectivity were derived from years of experimental data (26) . The model is tuned to reproduce layer- and cell-type specific behavior-dependent responses recorded from mice *in vivo* (26,49).

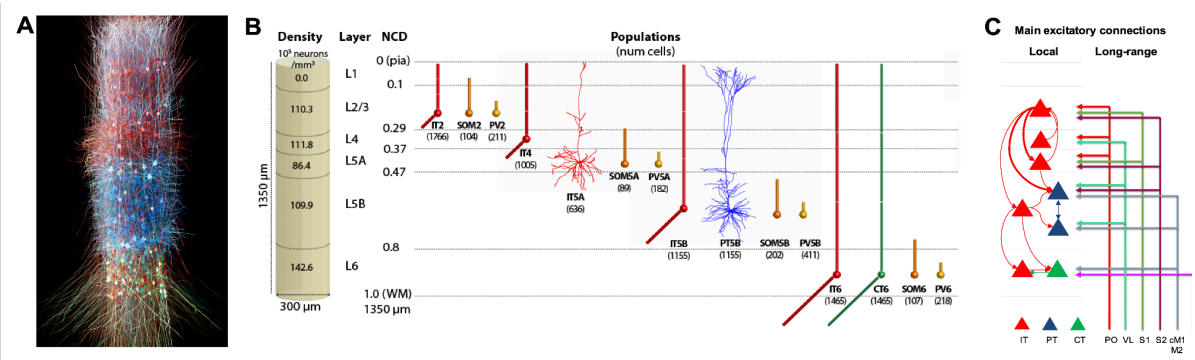

Fig. 1: Biophysical cortical circuit model. A. 3D representation. B. Size of simulated column with laminar cell densities, layer boundaries, cell morphologies and populations. C. Major local and long-range excitatory connections.

By studying the firing patterns of the circuit and the individual neurons, I can better understand the pathophysiology of each channelopathy. To characterize the impact of the disease on individual cells, I will compare the effect of mutant channels on the firing properties of the model cells to that of wild-type cells in terms of action potential (AP) height & width, threshold, inter-spike interval, and rheobase current. To characterize the network-level impact we will measure the spike rate and burstiness in the network. Spike rate is defined as the number of spikes fired per second in the neuronal population. Burstiness is defined as the fraction of the time a neuron is active (i.e., when its firing rate exceeds some low threshold). From these metrics I will investigate how the pathological changes alter the dynamics of the network. After changing the channels of the neuronal models (Experiment 1.2), my team will need to adjust the parameters of the microcircuit to maintain the same neuronal activity with the HH models. This will require running the

simulation ~100-1000 times; each simulation cost 80 node hours, so 8000 – 80K node hours.

Risks vs gain: In this aim I am choosing to use Markov models to make sure the mutated channel is simulated in high details and mimics the empirical data. However, this type of complex modeling does come with certain risks and challenges. The main risk is that these changes will modify the single neuron and network dynamics in a way that may not be physiological. To minimize this risk, I will first validate the models outside the circuit and limit changes in the network that do not reflect the pathologies of the diseases. I will also test if the modeled neurons and networks are robust against variations by conducting parameter sensitivity studies.

Overall, in this aim I will simulate the effects of different types of channelopathies on a neocortical microcircuit and measure both circuit and individual neuron activity. My team will first generate the channel model from empirical recordings (Experiment 1), then incorporate those changes in neurons expressing the channel (Experiment 2) and measure how those changes affect the L5PNs and the overall network (Experiment 3).

The completion of this aim will yield a detailed simulation for each channelopathy, so that I can investigate how the disorder alters the intrinsic excitability of each neuron. This will not only provide a tool to investigate the underlying neuronal mechanisms of disorders affecting many children in the US, it will also enable me to simulate how novel therapeutics can return excitability to neurotypical levels and ultimately be translated to the clinic.

Aim #2: Utilizing neuronal simulations to develop novel therapies for channelopathies

Rationale: The rationale for Aim #2 is that cortical column simulations can help us understand the mechanism of action of different therapies for channelopathies.

Approach: A common characteristic among NDDs is excitatory inhibitory imbalance (4,9,14,50 Review articles) and many successful therapeutic strategies are aimed at restoring this balance. In this aim I will simulate the effect of existing therapeutic avenues and identify targets for novel treatments. There are several therapeutic strategies that have been successful in treating NDDs (4,9,51) and the most common treatments for channelopathies is anti-seizure drugs (52-56 Review articles,56,57). In Experiment 2.1, I will simulate how known anti-seizure drugs (and drug cocktails) can normalize the E/I balance and restore function. Then in Experiment 2.2, I will iteratively modulate the biophysical properties of different ion channels to simulate new drugs that have not been developed yet and identify targets for drug development. Finally, in Experiment 2.3, I will simulate different aspects of gene therapy to study what the necessary levels of transcription to recover the E/I balance in the circuit would be. I hypothesize that focusing on therapies that restore the E/I balance of the circuit will optimize current therapies and identify new therapeutic targets to improve the health of children suffering from NDDs.

Research Strategy

Experiment 2.1: Simulate how different anti-seizure drug can normalize the E/I balance of the M1 Microcircuit: The most common treatment for NDDs is anti-seizure drugs. These drugs can normalize the E/I balance and restore function (52). However, predicting how a specific drug would affect any given disorder is often impossible, as these are complex systems that are affected by many different factors: the interaction of the drug with the specific mutation, channel expression levels, and interaction with other drugs (55, Review article). Simulating the changes in the E/I balance of the circuit can be used to predict the effects of a drug or a mixture of drugs and thus guide treatment.

Anti-seizure drugs can be divided into two categories depending on their target: those that act on synaptic function, and those that act on the channel either to block the channel or alter its biophysical properties (Table 1 adapted from (52-53, Review article)). There are numerous studies that use voltage-clamp recordings to characterize which biophysical properties of the channel are affected in the presence of different drugs (55 Review article,56,57). My team will incorporate how the drugs change channel function into our fitting method to create a "treated" version of the channel model. For anti-seizure drugs that act on the synapse, I will alter the synaptic mechanisms to simulate the drug's effects (e.g., simulating Clobazam will increase the synaptic activity of inhibitory inputs). I will select examples from each category (Table 1) that have been studied rigorously and have sufficient empirical data, and I will simulate them in high detail. Finally, after having a "treated" version for individual drug targets, I can simulate the effect of a combined treatment, i.e., a combination of drugs acting on different targets. To test the efficacy of a specific therapy, I will compare individual neuron firing patterns and network activity in 3 different simulation conditions: healthy, channelopathy only (Aim #1), and channelopathy with treatment. A treatment will be considered to have potential if it exhibits a reversal of the neuron firing pattern and restoration of the network dynamics to the healthy state.

Table 1: Anti-seizure drugs

Mechanism of Action	Simulation implementation	Anti-seizure drugs
Sodium channel blocker	Alter the biophysical properties of sodium channels	Phenytoin Carbamazepine Lamotrigine Oxcarbazepine Zonisamide Rufinamide Lacosamide Eslicarbazepine-acetate
T-type calcium channel blocker	Alter the biophyiscal properties of T-Type calcium channel	Trimethadione Ethosuximide
Calcium channel blocker α2δ subunit	Alter the biophyiscal properties of calcium channels	Gabapentin Pregabalin
Potassium channel activator	Alter the biophyiscal properties of potassium channels	Retigabine
GABA potentiation	Modify GABA synaptic properties	Phenobarbital Primidone Diazepam Clonazepam Clobazam Progabide Vigabatrin Tiagabine
AMPA inhibition	Modify AMPA synaptic properties	Perampanel
Multiple actions	May alter both network and channel properties	Valporate Felbamate Topiramate Levetiracetam Stiripentol

Experiment 2.2: Identify biophysical targets for new drug development: While in Experiment 2.1 I tested FDA-approved drugs, in this experiment I will look for targets for drugs that have not been developed yet. In the circuit simulation, I can modulate the biophysics of any channel or the synaptic function of any synapses; therefore, I can change their properties and identify potential targets for new drugs. By iteratively scanning the biophysical properties of the neurons and their synapses, I can target relevant ionic currents. For example, most SCN2A cases have hypoexcitable L5PNs due to decreased sodium current (15,16,31). This can be reversed by several methods, such as decreasing potassium current, increasing sodium or calcium

current, or increasing excitatory synaptic input. By monitoring the different ionic currents in the L5PN (Na, K, Ca), I can map biophysical property changes to the effect on the different current. This will yield the first line of candidates to drug targets, then I will discuss with pharmaceutical companies (e.g. Praxis) which biophysical properties are easiest to pursue and simulate them on channelopathies from Aim #1.

Experiment 2.3: Simulate the efficacy and safety of gene therapies for channelopathies: Finally, in the third experiment I will simulate gene therapy in the circuit, including CRISPR-based therapies and antisense oligonucleotides. While the ideal gene therapy will reverse the action of the channelopathy and recover excitability, in most cases it is not feasible. For example, in a case in which the mutation causes gain of function (GoF) of a channel, two independent gene therapies would be necessary: one to silence the GoF gene and the other to increase the expression of the healthy allele. For haploinsufficient channelopathies in which transcription is affected, my team and I will simulate what the necessary amount of transcription would be that is sufficient to recover excitability, as well as the consequences of enhancing transcription over biophysiological levels similar to what we have done in single neurons (58). For other GoF or mixed function channelopathies, I will test if there is a different channel that, by enhancing its expression, may recover excitability. Finally, I will see if we need to combine anti-seizure drugs with gene therapy to reach the best outcome.

Risk vs. gain: The main risk in this aim is that there are many drugs that do not have a clear mechanistic understanding of their action. Even if I find an efficacious drug for a specific channelopathy, it is possible that over time the drug may show side effects that I cannot simulate. However, the goal of this proposal is to develop a framework that allows us to study the mechanisms of NDDs and their treatments.

Aim #3: Study how developmental changes in cortical circuits alter neuronal activity in specific channelopathy cases.

Rationale: The rationale of Aim #3 is that disruption of synaptogenesis during neurodevelopment will alter the cortical circuit; incorporating these changes, as observed from experimental data, into simulations will increase the predictive power of our model.

Neurons and the electrical activity of neuronal precursors are critical for circuit formation early in brain development (2,6). These processes are based on Hebb 's rule - "neurons that fire together wire together" (59). Channelopathies disrupt this critical activity during circuit formation and lead to abnormal neuronal circuits in the mature brain. Although these alterations manifest differently in each channelopathy, there are common characteristics between different groups of channelopathies. For example, in SCN2A, GoF mutations lead to hyperexcitable L5PNs that generate stronger connections with their neighboring neurons (9). On the other hand, when L5PN are hypoexcitable, SCN2A loss of function (LoF), these neurons have weaker connections (15,16,18,31). In this aim, I will incorporate these changes to tailor a highly-detailed simulation for specific sodium channelopathies and their therapies. Two marks of NDDs that can be measured with electrophysiology methods are postsynaptic currents and AMPA-to-NMDA ratios (16). Postsynaptic currents measured on individual neurons are an indicator of the network properties and can be used to evaluate the state of the circuit in terms of the amount and amplitude of excitatory or inhibitory currents that are delivered to the neuron, termed excitatory or inhibitory postsynaptic currents (e/iPSCs). By comparing AMPA-to-NMDA ratios, I can infer the densities of those receptors in the synapse of the recorded neurons. AMPA and NMDA are implemented as

different synaptic mechanisms in the circuit simulation and changing their ratios will change the amplitude and duration of postsynaptic currents. These two metrics will allow me to identify the number and strength of connections onto L5PN from its surrounding neurons. Several studies use these two techniques empirically to study the channelopathy-driven changes that occur in brain development. I hypothesize that by modulating the synaptic connections of these circuits to mimic the conditions observed in channelopathies, I will increase the predictive power of our simulations and the potential to find relevant therapeutics.

Approach: With each different experiment in this aim, I will measure e/iPSC and the AMPA-to-NMDA ratio of L5PNs (and for other neuronal subtypes where empirical data is available from the literature) in the circuit and modulate them to simulate the specific state of the circuit observed in studies relevant to the specific channelopathy.

Research Strategy

Experiment 3.1: Simulate LoF and GoF SCN2A mutations: The current working paradigm in SCN2A research is that LoF of the channel causes hypoexcitable L5PN, and patients suffer from developmental delays and autism without early onset seizures. On the other hand, GoF of the channel is thought to cause hyperexcitable L5PN, and children suffer from early onset epilepsy (9 Review article,15). To recapitulate those neurodevelopmental changes in the mature circuit, I will rewire our network to match the current experimental observations, (16,29) and study how these changes affect the network activity and L5PN's excitability. Then I will *in silico* find which treatment may work best (Aim #2) for these two specific cases.

Experiment 3.2: Simulate LoF and GoF SCN1A mutations: SCN1A, a gene encoding the sodium channel NaV1.1, is predominantly expressed in interneurons and not L5PNs (60). Its effects are somewhat opposite to NaV1.2; SCN1A LoF mutations cause hyperexcitable interneurons, which makes their inhibited targets, L5PNs, hyperexcitable. Similarly, GoF mutations result in hyperexcitable interneurons, which then lead to hypoexcitable L5PN. However, the phenotypes are not similar to the SCN2A case: LoF leads to Dravet syndrome (severe childhood epilepsy), and GoF is mostly characterized by migraines (61). I will use the same method as in the first experiment to model these genetic mutations and study their impact on network activity, as well as L5PN excitability. I will rewire our network to match the current experimental observations and study how these changes affect the network activity (23-25). Then, I will also try to find treatments that are effective for these syndromes *in silico*.

I will use the same statistical analysis used in the previous aims to validate the accuracy of the cortical model and make predictions based on the simulations results. I will modify the model iteratively, initially comparing the activity (single neurons and network) just by manipulating the network connections (Experiment 3.1 & 3.2) and then applying the effects of the mutated channel in the circuit (Experiment 1.3). This will enable me to validate the correction of the model in two independent steps before simulating therapies (Aim #2).

Risk vs Gain: The main risk in this aim is the significant changes in the network properties due to developmental changes. The challenge will be that we do not have accurate data for all the neurons to validate that the cortical simulation is indeed replicating the diseased state. However, examining the network properties onto L5PN and how firing patters are changing should be a reasonable method of validation at this stage and can be compared to several empirical studies. In future projects, I will test this empirically using mouse models and neurons derived from patients with

SCN1A and SCN2A mutations. Completion of this aim will produce a model with desired specifications that will allow me to more accurately test the effectiveness of different therapies.

Overall, this aim will allow me to tailor the simulation framework to specific cases and better understand the changes that occur in these neurodevelopmental disorders. This will be a proof-of-concept to tailor these kinds of simulations to specific cases of NDDs and explore new therapies. Ultimately, I will use these simulations for mutations that have a known carrier in the US and find the optimal therapy to recover the E/I imbalance. Then Dr. Brooks-Kayal, Dr. Rogawski, and I will consult with the patient's neurologist to suggest future treatment.

Statistical methods: All comparison of network and individual neuron activity across all aims will be done using Bonferroni correction for multiple comparisons and two-sided rank based nonparametric tests. Significance will be set as alpha<0.05.

Future directions: My lab aims to develop new therapies for children with NDDs. The proposed project is central to the lab, but there are several other projects that will enhance the outcomes of this project. The first is to develop more biologically-accurate models. I have developed a deep-learning algorithm that fits a neuronal model to experimental data in order to generate models that mimic neuronal activity in higher detail (62). In another project, my lab uses high-definition multi-electrode arrays (HD-MEAs) to record activity of individual neurons and neuronal networks and then simulate their activity in high detail. The goal of this project is to derive neurons from patients with NDDs and use our modeling pipeline to simulate patients' neuronal activity then identify therapies *in silico* and test those *in vitro* before suggesting them to clinicians. I am currently working on this platform with Dr. Kyle Fink, who provides neural stem cells derived from patients with a wide variety of NDDs. Using neurons derived from patients will allow me to simulate neuronal circuits at different developmental stages, which will give insight into the treatment window and which treatment would be ideal at the different stages of neurodevelopment.

D) Backup Strategy

One of the main technical challenges will be to update the cortical circuit model with relevant data for the channelopathy or the simulated therapy. This is challenging since there are many free parameters in these kinds of simulations, and they need to be fine-tuned to get physiological neuronal activity from the simulations. For this reason, I am putting a lot of our resources into the collaboration with NetPyNE to support us in this process. The alternative approach would be to use smaller networks that simulate only the surrounding circuit around L5PNs. This would ease the update of the circuit model but would limit the scale of our simulations as well. Another challenge is to simulate how different drugs alter the excitability properties of neurons. In many cases the mechanisms are not fully understood, so it will be challenging to incorporate those into the simulation. To address this, I will focus initially on drugs that have biophysical data supporting their mechanisms of actions. For drugs that seem promising, I will empirically test how they alter neuronal activity using our HD-MEA system.

E) Timeline

Timeline	Year 1				Year 2				Year 3			
Project	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Aim 1												
Experiment 1.1: Fitting ion channels												
Experiment 1.2: Updating neuron models												
Experiment 1.3: Simulating cortical colum												
Aim 2												
Experiment 2.1: Simulating anti-seizure drugs (Channels)												
Experiment 2.1: Simulating anti-seizure drugs (Synaptics)												
Experiment 2.2: Identifying new target for drugs												
Experiment 2.3: Simulating Gene therapy												
Aim 3												
Experiment 2.1: Simulating SCN2A channelopathies												
Experiment 2.1: Simulating SCN1A channelopathies												
Analysis and Publications												

Milestones:

Aim #1: Experiment 1.1 (Fitting ion channels): I will first replace the HH ion channel for all the channelopathies with Markov models. Then fit the updated ion channels to experimental data of channelopathies. Experiment 1.2 (Updating neuron models) After replacing all the HMM channels I can integrate them in the neurons comprising the cortical column. Experiment 1.3 (Simulating the cortical column) will be done in parallel. While the post-doc is working on updating neuronal models, Dura-Bernal lab will start implementing the M1 cortical column in our local cluster and incorporate the models into the simulation and fine tune the network.

Aim #2: Preferably Aim #2 would be done after Aim #1 when I already have cortical simulations of channelopathies. Experiment 2.1 Simulating anti-seizure drugs: Simulating how drugs effect the channel does not require the circuit. The post-doc would work on simulating different drugs by modulating the channel while the Dura-Bernal lab will work on simulating synaptic effects that do require the circuit. Experiment 2.2 Simulating new target for drugs: This experiment is dependent on completing Experiment 2.1 so I would have the different component that drugs are changing. Then I would apply the optimization algorithm that would find best target for drugs. Experiment 2.3 Simulating gene therapy: This experiment is only dependent on the completion of Aim 1 so can be done in a parallel.

Aim #3: Builds on simulations I created in Aim #1 and extends them to make them more realistic. Both experiments are independent of each other and can be done in parallel. Then I will apply drug simulations as done in Aim #2 to seek the optimal treatment.

III. Budget

A) Budget Table

Budget Expense Category	Year 1	% Effort	Year 2	% Effort	Year 3	% Effort
Personnel						
Roy Ben-Shalom, Ph.D., PI	$ -	20%	$ -	20%	$ -	20%
To Be Named, Ph.D., Post Doc	$ 66,823	100.0%	$ 67,663	100%	$ 68,550	100%
Mandar Patil, M.Sc., Junior Specialist	$ -	25%	$ -	25%	$ -	25%
Supplies						
General Computing Supplies	$ 349		$ 331		$ 54	
Cloud Computing Services	$ 1,700		$ 2,500		$ 1,300	
Post Doc lap	$ 2,200		$ -		$ -	
SUNY subaward	$ 28,928		$ 29,506		$ 30,096	
Total	$ 100,000	145.0%	$ 100,000	145%	$ 100,000	145%

B) Justification of Expenses

Nominee: Roy Ben-Shalom (20% effort)- I have been working more than 15 years on neuronal modeling, I will use my experience to train a new postdoc on how to run the models and the biophysical details that go into simulating channelopathies. In the first year, I will do much of the work with the Dura-Bernal lab in order to get our lab up to speed using their simulations. After the postdoc is trained, I will meet with him weekly to track the results and provide mentorship. For the second year when we will start simulating drugs (Aim #2), I will meet regularly with Dr. Rogawski (anti-seizure drugs expert) and Dr. Fink (gene-therapy expert) to explain how we decided to simulate different therapies and get their feedback. The third year will include much of fine tuning of our simulations to meet the experimental results found in the literature. I will spend as much time as necessary to make sure our simulations are in-line with the experimental data either from Dr. George lab and from the literature. Furthermore, I predict that each aim of this proposal can be published as an independent peer review paper. I will provide the mentorship needed to publish papers that will help dissemination of the information generated by the simulation studies.

Benefits were calculated to only include Hartwell Foundation-approved benefit categories. Benefits that are not government-mandated have been removed from the budget and will be provided by my host department.

- Key Laboratory Support Personnel:

<u>To be named - Postdoctoral fellow:</u> 100% percent effort. I will recruit a postdoc to fully focus on this project. The postdoctoral fellow will have a Ph.D. in physics/computer science/neuroscience or a related field with experience in simulations of ionic channels and/or computational neuroscience. They will also be proficient in computer programming in one or more languages such as Python, NEURON, and preferably NetPyNE. My postdoctoral fellow will work closely with me and the Dura-Bernal lab to ensure the successful completion and interpretation of the results of this work. During this postdoc appointment, he will gain the experimental skills necessary to perform the experiments needed to confirm the results generated by our simulations. He will be involved in the planning and execution of several experiments, testing the predictions of our models. Moreover, the postdoc will take a role in writing the papers and reports required by this research.

Mandar Patil – Computational Junior Specialist – Mandar is a junior specialist in my lab with a master's degree in computational neuroscience. Mandar supports all the computational needs in my lab. For this project, Mandar will build the Graphical User Interface for the ion channel model optimization algorithm and the cortical column simulations. Also, Mandar will help with packaging the code and keep documentation updated.

- **Collaborators Integral to Research Success:** These are described in the SUNY subaward section.

Equipment: During the first year, I will purchase a GPU cluster (Using my startup funds) for testing and validating our simulations. Most of the computation will be done at the National Energy Research Scientific Center at Lawrence National Berkeley Lab, but the development of the simulations and their testing will be done in house on our GPU cluster. This will save time on waiting for shared computational resources, and we will only use them after simulations are ready to launch. Also, during the first year, I will purchase a laptop and all necessary accessories for the postdoc.

General Computing Supplies: This item is for office supplies and other parts necessary for the GPU cluster.

Cloud Computing Services: In the second and third year of the proposal we will be running large scale simulations that might not be suited for our computing cluster. Most of those will be ran on the NERSC supercomputer but we might need to use cloud computing in cases where the NERSC computer is under maintenance or when NERSC job queues are full and the wait is limiting our progress. Examples of how cost was calculated can be found in Aim #1 and #2.

- **Publication Costs** – Publication costs will be covered by other sources of funding.

SUBAWARD to SUNY: The SUNY Downstate Health Sciences University (DHSU) team, led by Dr. Dura-Bernal, will contribute to Aims 1-3 of the Project, which aims to create accurate cortical computational models of channelopathies and help develop targeted therapies for these neurodevelopmental diseases. We will provide support using the NetPyNE multiscale modeling tool, which has been developed in the Dura-Bernal lab, as well as the NEURON and CoreNEURON simulation engines, which Dr. Dura-Bernal helps develop. The team will help adapt a biophysically detailed of primary motor cortex (M1), also developed in the lab, to include the modified ion channel models simulating channelopathies, and simulate the effect of these on the cortical microcircuit. Including the new channels will likely require parameter optimization of the cortical model to obtain a baseline activity after incorporating the new channels, but prior to simulating the channelopathies – the DHSU team has expertise in automated parameter optimization methods for large-scale biological networks. The team will also provide support in developing novel therapies for channelopathies, by performing automated exploration of parameters in the cortical microcircuit model, and simulating how developmental alter cortical circuit dynamics in specific channelopathies

E) Student Expense: The funds will not be used to cover any students' costs either for research or tuition.

F) Statement Regarding Sufficient Funding

The funds describe here will be sufficient to achieve our proposal. I assume I will have access to computational resources from NERSC (see letter of support) and back those up with cloud computing budget. Other assumptions including funds for travel and publication costs that will be from my startup funds and other awards.

IV. Existing Sources of Research Funding

Title: Utilizing computational models to simulate the effects of SCN2A variants on neuronal excitability and testing potential therapeutics

The FamilieSCN2A Foundation - https://www.scn2a.org/professionals.html

Performance period: 11/01/2021 to 3/31/2023

Principal Investigator: Roy Ben-Shalom

Committed Effort: 2.4 calendar months annually

$100,000

Major Goals: In this award I am utilizing computational neuronal models to simulate how different variants in the SCN2A (sodium channel protein) gene affect neuronal excitability. We incorporate the biophysics of the channel into detailed neuronal models and study the effects of different drugs to tailor the best drug for each patient.

Title: An Academic Center for Interventional Genetics (CIG)

UC Davis School of Medicine Cultivating Team Science Award - https://health.ucdavis.edu/news/headlines/school-of-medicine-announces-2022-cultivating-team-science-awards/2022/04

Performance period: 05/01/2022 to 4/30/2024

Principal Investigator: Dr. David J. Segal

Committed Effort: 1.2 calendar months annually

$100,000 (out of $200,000) for Roy Ben-Shalom

Major Goals: Provide initial funds for my lab to build up our experimental setup to characterize the functional neuronal deficiencies in NDDs and evaluate the efficacy of treatments. Another major goal of this award is to hire a coordinator for the CIG (including four labs) to apply for large NIH grants.