Biophysical Simulations for Effective Neuropharmacology Treatment:

A Market Analysis and Commercialization Strategy for the Human Neocortical Neurosolver

By

Adrianna Hohil

B.S. Cognitive Science with a Specialization in Machine Learning and Neural Computation,

University of California at San Diego

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Date _____ _____

Dr. Stephanie R. Jones, Advisor

Date _____ _____

Eric Huang, Reader

Date _____ _____

Daniel Holmander, Reader

Approved by the Graduate Council

Date _____ _____

Dr. Thomas A. Lewis,

Dean of the Graduate School

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Chapter 1: Introduction

Drug development within the pharmaceutical industry has historically proven to be a challenging feat. Investigational drug trials have a 90% failure rate due to adverse events, dosing, and the drug candidate itself (McKenzie, 2024). When evaluating drug development success in the central nervous system (CNS) space specifically, this rate increases with the majority of failures landing in the mid to late stages of development (McKenzie, 2024). In fact, "one study found that between 1995 and 2007, CNS drugs achieved FDA approval at less than half the rate of non-CNS drugs" (McKenzie, 2024).

Part of this problem stems from the fact that the mechanisms behind neurological disorders are poorly understood (NCBI, 2014). Neuropharmaceutical companies face challenges with identifying and validating targets, understanding mechanisms of disease, predictive modeling, utilizing biomarkers, identifying clear regulatory pathways, and relying on and reproducing published data during pre-clinical development (Pankevich, 2014). This poses challenges for translational neuroscience research that aims to use fundamental neuroscience concepts for clinical applications and drug development.

For neurological disorders where little is known regarding the brain circuit mechanisms of disease, current drug treatment is largely symptom-focused. This is evident in prescriptions for schizophrenia, Alzheimer's Disease, Parkinson's Disease, and Fragile X Syndrome. Subjective behavioral endpoints, particularly those assessing symptom severity and patient-reported outcomes, are considered the gold standard for measuring drug treatment success in neurological disorders like schizophrenia (FDA, 2020). This approach aligns with the FDA's emphasis on clinical outcomes that reflect meaningful changes in patient well-being. Although symptom-focused treatment can help to alleviate the symptoms of mental illness, this type of

treatment strategy does not permanently improve patient conditions nor does it inhibit further cognitive decline. Furthermore, objective measures often fail to capture the full spectrum of these symptoms (FDA, 2020). Although it is the current gold standard utilized by the FDA, symptom-focused drug treatment is only a temporary solution to these chronic conditions, demanding more innovative, alternative approaches to neurological drug development.

Recent, novel solutions to existing treatment challenges have included use of electroencephalography (EEG) and computational modeling. Electroencephalography (EEG) is a technique used to record the brain's electrical activity which results in a printout known as an electroencephalogram (EEG) (Mayo Clinic, 2025). Electroencephalograms (EEG) are valuable for neurological disease treatment because they are a non-invasive method for directly measuring brain electrical activity and detecting normal and abnormal brain wave patterns (Figure 1). EEG can identify the location and type of abnormal brain activity, which is crucial for pinpointing the source of neurological symptoms and developing targeted treatment strategies (Mayo Clinic, 2025).

The Jones lab at Brown University has developed a software, known as Human Neocortical Neursolver (HNN), that utilizes EEG signatures and EEG biomarkers to simulate a biophysical model of a cortical column which can be used to study the cell and circuit generators of these biomarkers in neurological disorders. Through an ongoing proof-of-concept project funded by the Brown Innovations to Impact Award, a healthy state EEG and a disease-state EEG for schizophrenia and Fragile X disorder will be simulated while the effects of a mechanistic, circuit-level based treatment will be evaluated to determine if an administered pharmaceutical treatment brings the patient's disease state closer to the healthy state. In doing so, the HNN can be used to help with lead selection and dosing of drug candidates. This thesis report aims to

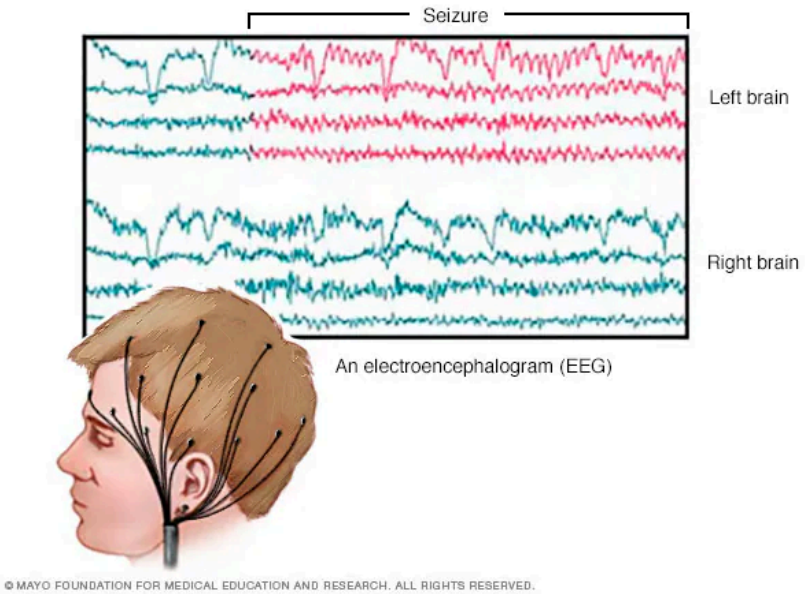
highlight all current proof-of-concept progress and map out a complete commercialization plan to employ the HNN as a tool to help decision making in neuropharmaceutical drug development.

NOTE: Throughout the duration of this thesis, I will be referring to electroencephalograms and electroencephalography as EEG, as this is the abbreviation for both concepts.

Chapter 2: Electroencephalography (EEG)

What is an EEG?

As stated before, an electroencephalogram (EEG) is a test that measures electrical activity in the brain. An EEG test is performed by using small, metal discs known as electrodes that attach to the scalp and record the electrical signals that the brain produces (Mayo Clinic, 2025). When multiple neurons fire electrical impulses simultaneously, known as neural activation, changes in the electrical potential of the brain are detected by electrodes and result in waveforms on an EEG readout (Figure 1). “EEG relies on the placement of electrodes on the person’s scalp, measuring the postsynaptic potentials of pyramidal neurons” (Glomb, 2022). “EEG does not directly measure the action potentials of neurons, though there are some indications of high-frequency oscillations being linked to spiking activity” (Glomb 2022).



<u>Figure 1:</u> EEG Brain Activity

An EEG records the electrical activity of the brain through electrodes put on the scalp. EEG results show changes in brain activity through positive and negative changes in signal amplitude and frequency. In addition to recording normal brain activity (shown in green), EEG helps to diagnose brain disorders like epilepsy because the signals produced by a seizure can be recorded and monitored in real-time (shown in red) (Mayo Clinic, 2025).

EEG Event Related Potentials

Researchers have been analyzing how EEG data correlates to cognitive functioning through spontaneous or natural brain wave occurrence, and in response to a sensory stimulus, such as a visual or auditory stimulus (Abhang, 2016). When studying brain signals in response to a particular stimulus, researchers average the data across hundreds of stimulations to obtain a reliable measure of an individual's brain activity known as averaged evoked potentials (Abhang, 2016). This is because evoked potential amplitudes tend to be low compared to spontaneous brain waves, requiring signal averaging to resolve any potential artifacts or other low amplitude noise in the background of the EEG (Abhang, 2016). "The signal is time-locked to the stimulus and most of the noise [or spontaneous activity] occurs randomly, allowing the noise [or spontaneous activity] to be averaged out by averaging repeated responses" (Teplan, 2002).

Studying sensory evoked potentials helps to discern between cognitive processes in both normal and abnormal states (Abhang, 2016). This is particularly helpful when trying to identify key differences between healthy subjects vs. subjects afflicted with a particular neurological disorder. Specifically for the research discussed in this work, the differences in auditory evoked potentials and evoked gamma activity in response to an auditory stimulus have proven helpful when characterizing Fragile X disorder vs. healthy subjects, and making connections to neuronal hyperexcitability.

EEG Local Field Potentials

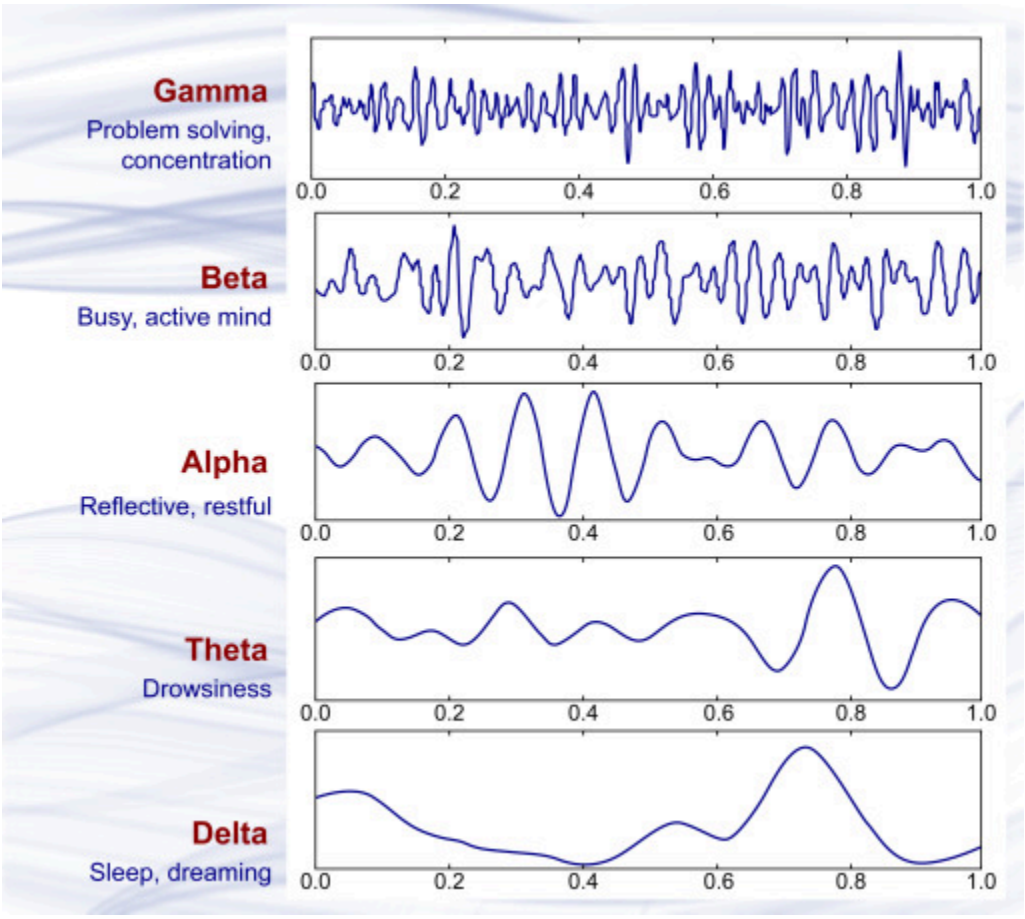
In parallel to event related potentials, researchers have been studying the intracranial local field potentials that correspond to extracranially measured EEG to better understand one's brain state. "Local field potentials (LFPs) are recorded with electrodes inside the brain that pick up the electrical signals generated by the synchronous electrical activity of populations of neurons near the electrode" (Glomb, 2022). LFPs have high spatial resolution, allowing for the detection of neural activity in specific brain regions, and good temporal resolution, capturing rapid changes in neural activity (Montgomery, 2013). The strongest attribute of LFP measurements over extracranial EEGs are in their ability to provide detailed information about neural activity in specific brain regions, which is crucial for understanding the localized effects of pharmaceutical compounds and their relationship to extracranially measured EEG. This precision aids in identifying region-specific biomarkers and understanding the neural circuits involved in disease and treatment (Sasegbon, 2020). Although extracranial EEG measures of ERPs have great temporal resolution, they tend to have lower spatial resolution due to signal attenuation and distortion as electrical activity passes through the skull and scalp (Burle et. al, 2015).

However, the advantages of LFPs over EEG recordings comes at a cost. LFPs require surgical implantation of electrodes into a subject's scalp, limiting its use to animal models or specific clinical situations where invasive monitoring is justified. Additionally, LFPs often pose translational challenges because findings from LFP studies in animals may not always directly translate to humans due to species differences and the invasive nature of the technique.

Overall, LFPs offer greater spatial precision and direct neural access, making them superior for preclinical biomarker validation in drug development. EEG, while useful for later-stage clinical assessments in humans, are less informative for early mechanistic insights.

EEG Brain Rhythms

EEG brain waves have been classified into 5 categories based on their amplitude and frequency, known as Delta, Theta, Alpha, Beta, and Gamma (Figure 2).



<u>Figure 2:</u> EEG Frequency Chart

The brain produces a wide range of signals including Delta, Theta, Alpha, Beta, and Gamma waves (Abhang, 2016).

Delta waves are the slowest but highest amplitude brain waves, and measure between 0.5 to 4 Hz (Abhang, 2016). These types of waves are generally produced in the brain during meditation and deep sleep (Neeuro, 2025). Theta waves are the second slowest wave produced by the brain, measuring between 4 to 8 Hz (Abhang, 2016). Theta waves are generated when the

brain is extremely relaxed and often through periods of creativity, intuition, and spiritual awareness, making them a repository for memories and emotions (Neeuro, 2025). Alpha waves measure between 8 and 12 Hz, sitting at the middle of the frequency spectrum (Abhang, 2016). Alpha waves are generally produced when your brain doesn't require focus or concentration to complete a task, but is still in a state of calm and relaxation (Neeuro, 2025). Beta waves measure between 12 and 35 Hz, initiating and sustaining the brain's state of alertness or normal consciousness (Abhang, 2016). Beta waves are also capable of eliciting a stimulating effect that allows our brains to focus, generating logical thinking and conscious thought. The more focused and alert you are and the more decisions you make, the higher the beta wave frequency that your brain generates (Neeuro, 2025). Lastly, gamma waves measure greater than 35 Hz, and are actively involved in cognitive functioning, information processing, learning, solving problems, and memory (Neeuro, 2025). It is important to note that frequency bounds for each wave type may vary between labs, but the bounds defined here are generally accepted across academia and industry.

EEG in Disease Diagnosis

EEG is frequently used in the neuroscience field as a diagnostic tool to evaluate a patient's cognitive state, often in conjunction with other neuroimaging tools (e.g., fMRI). Initially, EEG was mostly utilized for epilepsy diagnoses, but more recently it's been utilized for diagnosing and studying a wide array of disorders (Amer, 2023), albeit for disorders other than epilepsy and sleep pathology EEG not FDA approved as a diagnostic tool. EEG biomarker identification includes sleep disorders, Alzheimer's and other dementias, Schizophrenia , ADHD, autism, brain death, strokes, and comas (Appaji, 2022), (Amer, 2023). "EEG is recognized as one of the most efficient imaging methods for detecting brain electrical currents due to coordinated actions of hundreds of neurons. This approach offers high temporal resolution, which may be viewed on the screen as a digital representation of a continuous voltage flow. This technique can determine cortical activity even at the lowest time intervals" (Amer 2023). However, it can be challenging for healthcare professionals to read and interpret EEG signals and diagnose patients accurately (Amer, 2024). To correct this, different methods have been developed to better utilize EEG such as time series analysis, signal processing, frequency band analysis, and biomarker validation (Amer, 2024).

Processing EEG signals comes with several challenges that can affect the accuracy of analysis. The signal-to-noise ratio (SNR) is particularly sensitive to external factors like light, smells, blinking, movement, and temperature (Amer 2024). These influences can introduce random noise, or 'artifacts,' which complicate signal processing (Amer 2024). However, various techniques can help mitigate these issues, such as using high-quality electrodes, optimizing electrode placement, applying advanced filtering and denoising algorithms, and refining experimental setups and stimulation methods (Amer 2024).

The use of EEG in Pharmacology

In addition to its uses as a diagnostic tool for patient health, EEG is being used in the pre-clinical and clinical stages of pharmaceutical drug development. More specifically, translational biomarkers in EEG are being used for pre-clinical validation through in-vivo local field potential recordings (SynapCell, 2024).

Pharmaceutical companies spend about $1.8 billion dollars attempting to push a drug to market (Zurdo, 2013). However, it is common for clinical targets in the drug discovery space to fail in "the Valley of Death," which is anywhere from initial target validation and lead selection to Phase II clinical trials (see Figure 3 below) (Zurdo, 2013). In fact, only one in 250 compounds that enter the preclinical stage will progress to the point of receiving regulatory approval (Zurdo, 2013).

The challenge with screening drug candidates succumbing to the Valley of Death is that many targets fail at later stages, amplifying the monetary loss and risk associated with lead selection (Zurdo, 2013). In fact, many prospective targets will make it past pre-clinical trials, showing promise and positive results in vivo (rodents, primates, canines), but instead fail in later stage clinical trials because the anticipated molecular, pharmacokinetic, or mechanistic changes don't take place (Zurdo, 2013). This vicious cycle has inspired a new method known as a "developability assessment" to reduce risks associated with the Valley of Death and improve drug candidate chances which has found the siloed approach to drug discovery is in urgent need of innovation (Zurdo, 2013).

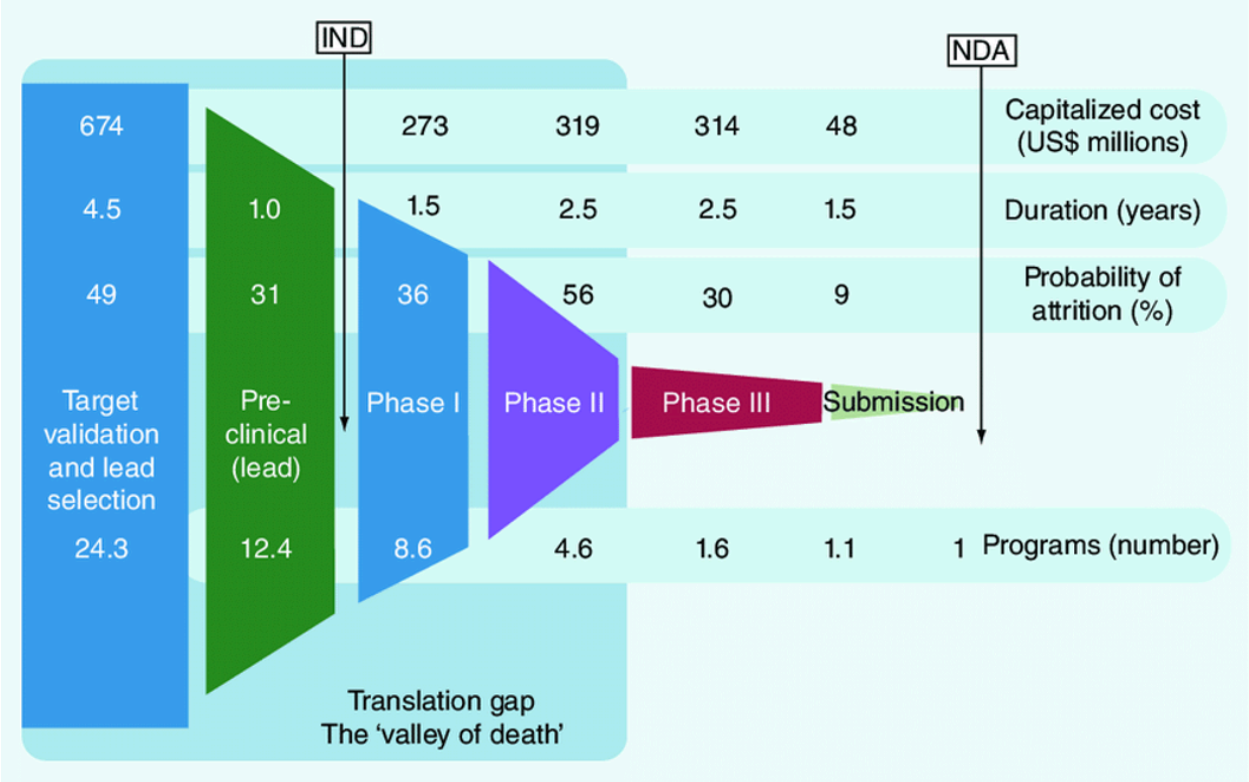


Figure 3: Drug Development and the Valley of Death (Zurdo, 2013).

This is where EEG has proven to be a valuable tool during preclinical in-vivo studies of therapeutic targets. Pharmaceutical companies developing neurotherapeutics utilize EEG to study pharmacokinetic effects of drugs on animals (in both healthy controls and disease state) to gauge their safety and efficacy before testing any drug compounds in humans (Synmeres, 2024).

However, EEG has been used as a supplemental tool to traditional R&D methods. This is because the FDA and other regulatory agencies' current standard for assessing a CNS drug candidate's success relies on evaluating subjective behavioral endpoints, so most pharmaceutical companies focus on operating within the current bounds of drug development criteria (Zaragoza Domingo, 2024). From our team's customer discovery interviews of directors and c-suite executives in the CNS drug development space, it appears that many companies can't afford to invest in experimental methods, even if developing a new method with objective endpoints would improve their chances of bringing drugs to market and reduce R&D costs. This is because pharmaceutical companies must ensure a return on investment of the products in their pipelines, and already face significant challenges related to high cost, risk, and regulation (Zurdo, 2013). To be deemed viable, all therapies must meet adequate quality, manufacturability, efficacy, and safety requirement standards (Zurdo, 2013).

Because of the decrease in throughput of effective CNS drugs over the last decade, start-up biotechnology companies have been experimenting with different methods to aid pharmaceutical companies in drug development. Despite its novelty, EEG biomarker validation has been gaining a lot of traction and popularity within the past 5 years, specifically during the preclinical stage of research and development (SynapCell, 2024). Additionally, there has been a growing demand for innovation using EEG. EEG is driving up to 50% year-over-year increases

in R&D spending, showing our team that there is promise for use of the HNN in the pharmaceutical industry (TBRC, 2025).

Chapter 3: The Human Neocortical Neurosolver

What is HNN?

The Human Neocortical Neurosolver (HNN) is a software tool designed to help researchers and clinicians explore the neural circuit mechanisms that produce EEG and MEG signals. Built on a biologically accurate model of a neocortical column, HNN simulates the electrical activity of cortical cells and networks responsible for generating these brain signals. Additionally, HNN provides a biophysical interpretation of brain activity that translates from animals to humans through neural simulations of cortical networks, creating a cross-species bridge that can be used to enrich R&D research. It provides built-in workflows to simulate inputs from both thalamic and intracortical sources, making it easier to investigate the origins of event-related potentials and low-frequency oscillations. HNN is accessible to users of all experience levels, offering an interactive graphical interface (HNN-GUI) for beginners and a Python-based command-line interface (HNN-Core) for more advanced users. The software includes tutorials to help users import their own data and begin testing hypotheses about the neural basis of their EEG/MEG findings (Neymotin, 2020).

What makes the HNN competitive within the neuromodeling industry?

Central nervous system (CNS) drug development continues to face major challenges, particularly in the translational gap between preclinical animal models and clinical outcomes in humans. Many therapeutics fail in late-stage clinical trials despite showing promise in early preclinical studies, largely due to the lack of alignment between biological mechanisms across species and measurable endpoints of efficacy. Our goal and value proposition are to develop the HNN into a proprietary software that forms a cross-species bridge and helps significantly de-risk drug development by enabling in silico target validation at the circuit level. Using this, we can simulate the complex circuit mechanisms of a disease, predict therapeutic outcomes, and provide quantitative endpoints of efficacy. By providing mechanistic insights and offering quantitative biomarkers of efficacy, this approach has the potential to improve translational success and advance the development of more effective CNS therapeutics.

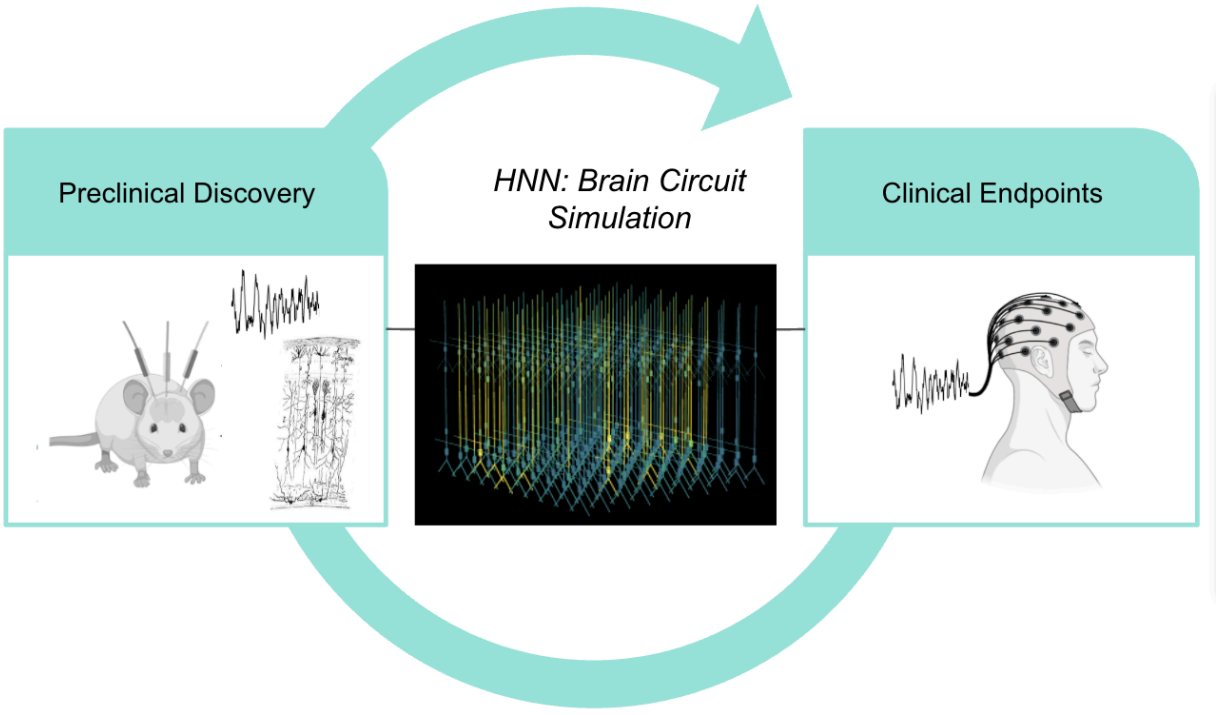


Figure 4: Visualization of the HNN's value proposition (Jones Lab, 2024).

Proof-of-Concept

Below is a proof-of-concept demonstrating how our analyses and simulation tools can be applied to (1) identify novel EEG biomarkers of the disease process, (2) a cell and circuit mechanistic interpretation of these biomarkers, (3) help with drug lead selection and dosing, and (4) ultimately be linked to genetic profiling and personalized precision medicine. The example is focused on a schizophrenia indication, which is the initial target for our proof-of-concept development.

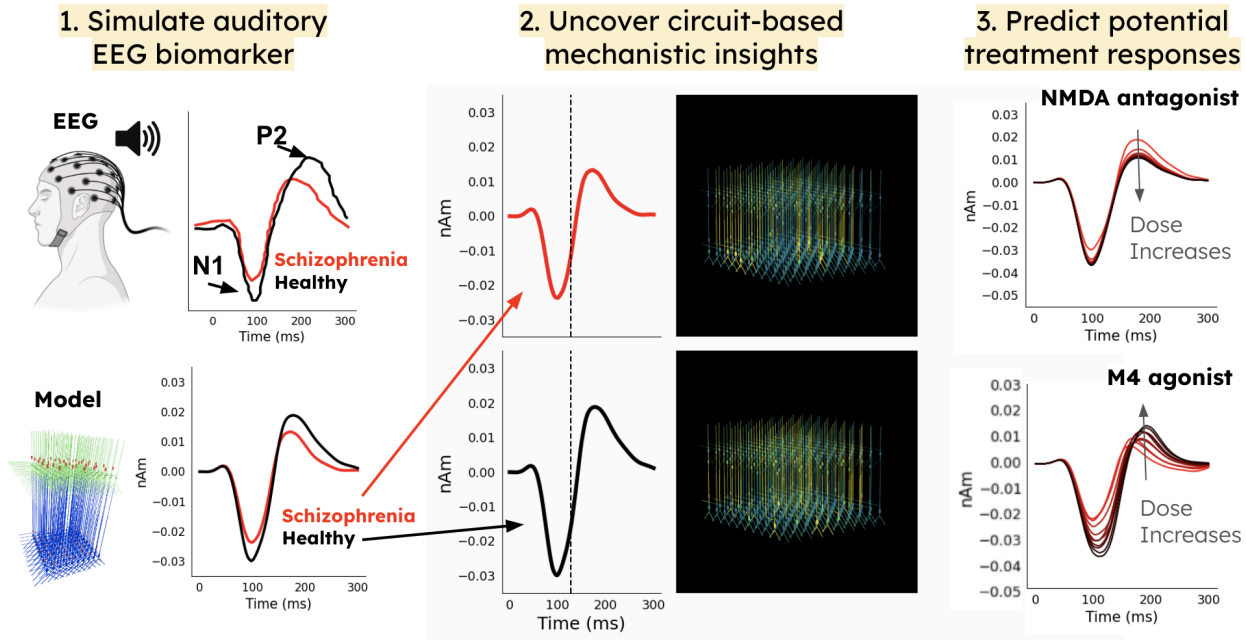


Figure 5: HNN Proof-of-Concept Model (Jones Lab, 2024).

To demonstrate the HNN's capabilities, **Figure 5** presents two simulation studies. In the first step, EEG data showing auditory evoked response potentials (ERPs) from both healthy individuals and schizophrenia patients reveal differences in peak brain activity across groups. While traditional analyses rely on averaged signals, HNN's tools reveal that these group differences can arise from multiple features in the unaveraged (single-trial) data, enabling more

precise, individualized interpretation. The second set of figures illustrate how HNN simulations can reproduce these macroscale EEG patterns, offering detailed insight into the underlying cellular and circuit-level mechanisms that may drive disease-related changes in brain activity. This approach allows researchers to directly link surface-level EEG signals to specific changes in neural circuitry, and even validate these interpretations across species, including rodents. The HNN platform facilitates cross-species translation and can provide data that is often costly or impractical to collect in animal models alone.

In the last step, the HNN was used to simulate the effects of pharmacological interventions on the schizophrenia model under conditions mimicking drug treatment. Simulations tested two major classes of schizophrenia drugs across various dosages. Results showed that one compound (an M4 agonist) was able to normalize the brain response at adequate dosage, while the other (Ketamine) failed to do so. These simulations highlight how HNN can identify both disease-related pathways and potential treatment mechanisms at the circuit level, providing early-stage decision support for pharmaceutical development. Applications include optimizing lead drug selection, determining appropriate doses, selecting clinical indications, and validating neural targets. Additionally, HNN allows researchers to bridge genetic findings—such as disease-linked mutations—with their effects on neuronal dynamics and resulting EEG signals. By transforming EEG interpretation from correlation-based inference to causal understanding rooted in neural circuitry, HNN offers a powerful platform to accelerate and de-risk CNS drug discovery, ultimately improving therapeutic success rates and patient outcomes.

What makes the HNN competitive in the Neuromodeling Industry?

Human Neocortical Neurosolver (HNN) distinguishes itself from other neuromodeling software through its specialized focus on bridging human EEG and MEG data with biophysical neural mechanisms.

When comparing the HNN to other existing neuromodeling softwares designed to simulate the neural mechanisms of EEG such as LFPy, the Virtual Brain, and NetPyne, we found that HNN was the only software that offered biophysical realism, translational workflows for neurotechnology recordings, multiscale visualizations, and open source modularity, providing us with another competitive advantage in this market (LFPy Developers, 2020; The Virtual Brain, 2013; NetPyNE, 2016).

Features	HNN	LFPy	The Virtual Brain	NetPyne
Biophysical realism	✓	✓	✗	✗
Translational workflows for neurotechnology recordings	✓	✗	✗	✗
Multiscale visualization	✓	✗	✗	✓
Open source modularity	✓	✓	✓	✓

Table 1: HNN compared to the neuromodeling software landscape (Jones Lab, 2025). The table shown depicts the features unique to HNN, highlighting that it is the only neuromodeling software available that incorporates all 4 characteristics, giving our technology a competitive edge in the neuromodeling industry.

Chapter 4: Fragile X Syndrome and Beginning of Proof-of-Concept

Introduction

Fragile X syndrome (FXS) is a condition characterized by developmental and intellectual deficits that closely resemble autism spectrum disorder (ASD). Common Fragile X symptoms include (1) intellectual disabilities that affect the ability to think, reason, and learn, (2) learning disabilities (attention disorders, hyperactivity, anxiety, and language-processing problems), (3) sensory processing issues, (4) distinct physical features (narrow face, large head, large ears, flexible joints, flat feet, and prominent forehead, (5) speech deficits (challenges with speaking clearly or at all, stuttering), and (6) behavioral, social, and emotional challenges (anxiety, trouble making eye contact, attention challenges, aggression) (NICHD, 2024). Disease onset has been linked to mutations in the FMR1 (Fragile X Messenger 1) gene found on the X chromosome (NICHD, 2024). This mutation leads to a deficiency in FMRP (Fragile X Messenger Protein) production, which is crucial for regulating synaptic function and neuronal excitability (NICHD, 2024). Loss of this protein disrupts the nervous system and causes auditory processing deficits, neural dynamic dysfunctions to occur at the circuit level, and changes in functional connectivity (Ethridge, 2016).

Notable auditory processing deficits in Fragile X Syndrome (FXS) include auditory hypersensitivity, sound localization difficulties, impaired auditory processing and discrimination, altered neural responses, deficits in sensory gating, and neuroanatomical changes (McCullagh et al., 2020; Razak et al., 2021). Individuals with FXS who exhibit auditory hypersensitivity often experience discomfort or distress in response to everyday auditory stimuli, a phenomenon thought to reflect cortical hyperexcitability (Ethridge et al., 2016). Sound localization difficulties are believed to result from an imbalance of excitatory and inhibitory synaptic inputs in auditory

processing regions (Ferraguto et al., 2023). Impaired auditory discrimination is associated with abnormalities in early sensory cortical processing, which can contribute to challenges in distinguishing phonemes or complex sounds (Razak et al., 2021).

Electrophysiological studies have documented altered neural responses to auditory stimuli in FXS, such as increased amplitudes of the N1 and P2 event-related potential (ERP) components, reflecting exaggerated cortical responses (Ethridge et al., 2016). At the circuit level, FXS is characterized by altered ion channel activity, abnormal firing patterns, and excitation-inhibition imbalances (McCullagh et al., 2020; Routh et al., 2013). Potassium channel dysfunction, particularly reduced BK channel activity, has been implicated in increased neuronal excitability (Ferraguto et al., 2023). Dysregulation of calcium channels, including T-type calcium channels, is also observed and contributes to excessive neuronal firing and excitatory neurotransmission (McCullagh et al., 2020).

The absence of FMRP leads to increased neuronal hyperexcitability and exaggerated firing rates in various brain regions (Razak et al., 2021). Electroencephalography (EEG) studies show increased gamma power and altered theta and alpha rhythms, indicating disrupted oscillatory dynamics (Ethridge et al., 2016). The imbalance in the excitation/inhibition (E/I) ratio has been associated with dysregulated GABAergic signaling, which contributes to excessive excitation (McCullagh et al., 2020). This imbalance may also impair synaptic plasticity mechanisms such as long-term potentiation (LTP) and long-term depression (LTD), affecting learning and memory in individuals with FXS (Razak et al., 2021).

It is important to note that not every person with a mutated FMR1 gene has symptoms of Fragile X syndrome. Depending on the size of a patient's FMR1 gene mutation, their body may

still be able to generate some of the FMRP protein in some of their cells, causing only mild symptom onset (NICHD, 2022).

The goal of this work is to investigate the circuit level disruptions linked to Fragile X disorder, specifically to test the hypothesis that extrinsic inputs and changes in calcium and potassium conductance are related to decreased habituation observed in auditory steady state responses, ASSR (Figure 6). If true, these results will lead to more targeted predictions on effective pharmaceutical interventions. To explore this, I attempted to simulate the ASSR paradigm modeled in the Ethridge 2016 paper that will be discussed further in the methods section. This paradigm is an interesting observation of electrophysiological differences in healthy vs. Fragile X patients and has never been modeled in-silico before. I was excited to take on this challenge to generate a proof-of-concept that HNN is useful to identify disease mechanisms and potential therapeutic targets.

Methods

In order to measure the auditory processing deficits that occur between healthy controls and FXS patients, a common paradigm studied within literature is auditory steady state response (ASSR). The inspiration for this project stems from the Ethridge et. al 2016 paper, which uses ASSR to observe habituation differences in healthy controls vs. Fragile X patients. Each study participant was presented with 150 trials; each trial consisted of a train of four 1000 Hz tones (50 ms duration), with tones separated by a 500 ms interstimulus interval and trials separated by a 4000 ms interval. The results show altered gamma oscillations and neural hyperexcitability are a potential biomarker of sensory issues in Fragile X (Ethridge et. al). More specifically, increased auditory N1 ERP amplitude, decreased gamma phase locking to an auditory stimulus, and increased gamma single power during the auditory stimulus task were all previously reported (data not shown). The figure below (Figure 6) shows the ASSR paradigm that Ethridge used to measure habituation in both healthy controls and Fragile X patients. When brainstorming how to model this in HNN, I noticed that ERPs simulated in HNN are reported in units of nanoamperes (nAm) consistent with the units of source localized signals, and the 2016 Ethridge study measured ERPs only at the sensor level in units of microvolts. I was unsure if I could utilize HNN to model the ASSR without accommodating for this difference, but my advisor assured me that source level waveform shapes simulated in HNN, carries a negligible difference to those measured at the sensor level and as such that wouldn't alter the interpretation of my results for the purposes of this project.

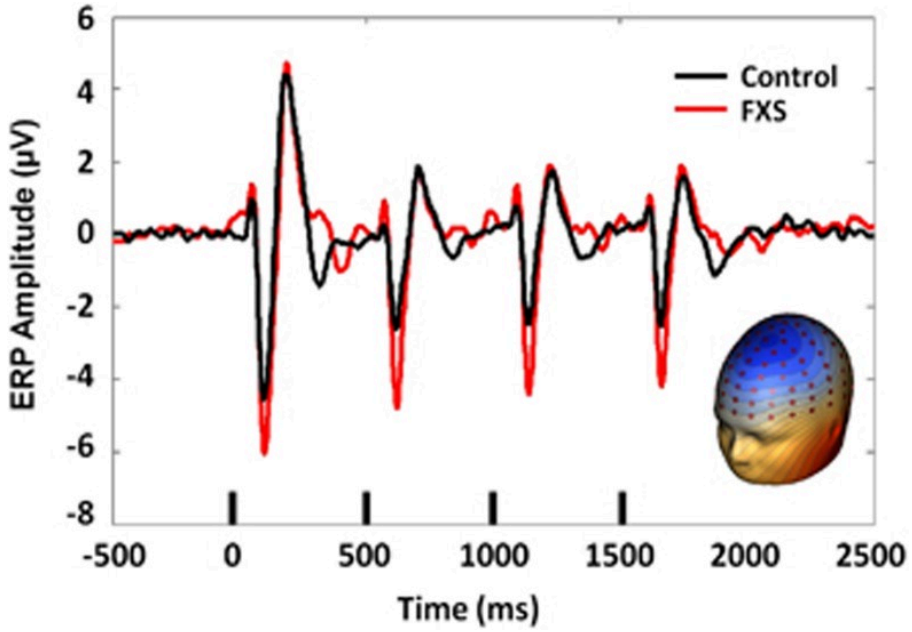


Figure 6: Auditory Steady-State Response in Healthy Controls vs. Fragile X Patients "ERP grand average PCA-weighted virtual channel plot for FXS and matched controls, with inset PCA spatial component topography. Small black bars indicate presentation of the auditory stimulus. ERP, event-related potential; FXS, Fragile X Syndrome; PCA, principal components analysis" (Ethridge, 2016).

Description of HNN Frameworks: Proximal and Distal Drives in Neural Modeling

(Neymotin et. al., 2020)

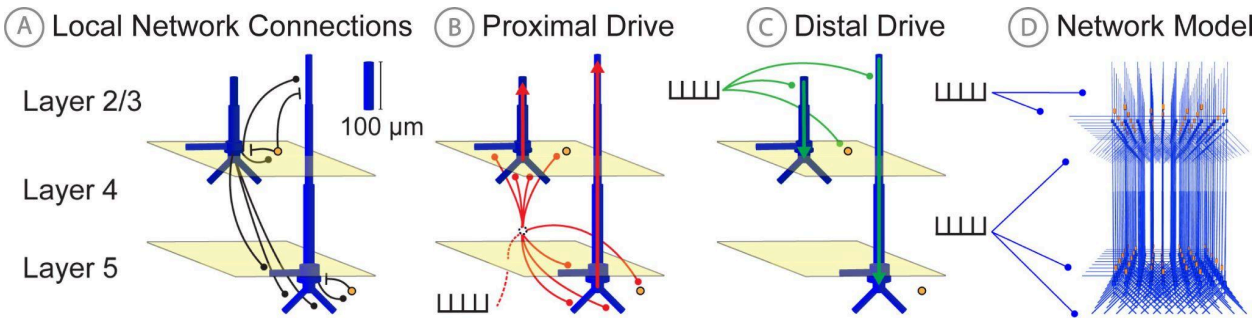


Figure 21: Cortical Circuit Architecture and Activation Pathways in the Human Neocortical Neurosolver (Neymotin et. al., 2020).

The image shows a detailed representation of neuronal connectivity and activation pathways in the Human Neocortical Neurosolver (HNN) framework. The figure effectively illustrates how local network connections (A) interact with extrinsic proximal (B) and distal (C) drives to generate the neural responses shown in the network model (D).

Proximal vs Distal Drives:

Proximal drives (Panel B) represent feedforward inputs from lemniscal/core thalamus that target Layer 4 (not explicitly modeled) and then relay to inhibitory neurons and pyramidal neuron basal dendrites in Layers 2/3 and 5. Distal drives (Panel C) represent feedback inputs from either higher-order cortex or non-lemniscal thalamus, targeting inhibitory neurons in Layer 2/3 and the distal dendrites of pyramidal neurons (Neymotin et. al., 2020).

Relationship to Extrinsic/Intrinsic Activity:

Both proximal and distal drives represent extrinsic (external) inputs to the modeled cortical column. These exogenous drives induce excitatory post-synaptic currents that generate current flow up and down the pyramidal neuron dendrites. The combination of extrinsic inputs and the resulting intrinsic network activity produces the current dipoles measured in EEG/MEG (Neymotin et. al., 2020).

Components of HNN:

HNN consists of a biophysically detailed neocortical column model with:

- A 3-layered structure with pyramidal neurons in supragranular and infragranular layers
- Pyramidal neurons whose dendrites span across layers
- Inhibitory interneurons in a 3:1 ratio with pyramidal cells
- Mechanisms for proximal and distal drives
- A graphical user interface for parameter adjustment and visualization

ERP Simulation:

ERPs are simulated by activating the neural network with specific sequences of exogenous drives:

1. Initial proximal drive creates upward current flow (positive peak)
2. Subsequent distal drive generates downward current flow (negative peak)
3. Second proximal drive produces another upward current (second positive peak)

The timing, strength, and variability of these drives can be adjusted to match experimental data. Automated parameter optimization helps find optimal parameter values.

When first attempting to model the ASSR in HNN, I utilized the ERP HNN tutorial distributed with the HNN software to better understand how ERP modeling works in HNN, then added to it accordingly to formulate my model. I had initially planned to model the entire ASSR for both the healthy and Fragile X conditions, but was advised to start small and focus on the N1 and P1 peaks in healthy conditions. To simulate the N1 and P1 peaks, I used the parameters from a prior study (Jones 2009 model) to first generate a network, added an evoked distal drive and 2 evoked proximal drives, and ran 2 trials over 2 jobs. There was a lot of trial and error throughout this process as I explored the HNN model (data shown in Figures 7 to 20). For hours, I manipulated the synaptic weights of the L2 and L5 pyramidal neurons in both evoked drives and manipulated the standard deviation and mu variables in each drive to explore their effects on the healthy network. After I discussed my findings with my advisor and built a foundation of understanding how each variable contributed to certain changes in the HNN ERP model, I worked on testing my hypotheses.

My first hypothesis was that differences in extrinsic inputs can explain the differences in habituation responses between Fragile X patients and healthy controls in the ASSR paradigm. Depending on the results of my first hypothesis, I planned to test my second hypothesis which was that either lower potassium conductance or higher calcium conductance can explain the differences in habituation responses between Fragile X patients and healthy controls in the ASSR paradigm.

To test my first hypothesis, I first changed the time scale of the default ERP tutorial from the 170 ms time window to 500 ms to match the 2016 Ethridge paper. Next, I altered the mu variable of one of the proximal drives. The mu variable in HNN represents the mean start time of the proximal drive. This was to ensure that the onset of each drive in the ERP would match the

Ethridge paper, but after further discussion and realizing this was an unrealistic representation of the dynamics of the ASSR, I omitted this change and kept the mu variable consistent with the default values in the ERP tutorial. To test my extrinsic inputs hypothesis, I focused on changing the synaptic weights of the distal drive of the network, primarily focusing on increasing the values of the L2 and L5 pyramidal neurons. This is because these values drive network excitation and would potentially increase the amplitude of the distal drive to more closely match the P1 ERP in the ASSR healthy patient response. Through this process, I disproved and invalidated my first hypothesis, which will be explained in more detail in the results section.

Unfortunately, because of the invalidation of my first hypothesis, I was unable to simulate the healthy ASSR state, which then prevented me from experimenting with the healthy ASSR to potentially model the Fragile X ASSR. This also prevented me from testing my second hypothesis that also depended on a successful simulation of the healthy state. In the future, I'd revise my hypotheses to ensure that they could be tested independently of one another.

Results

As stated before, I started with the ERP simulation from the HNN tutorial (shown below).

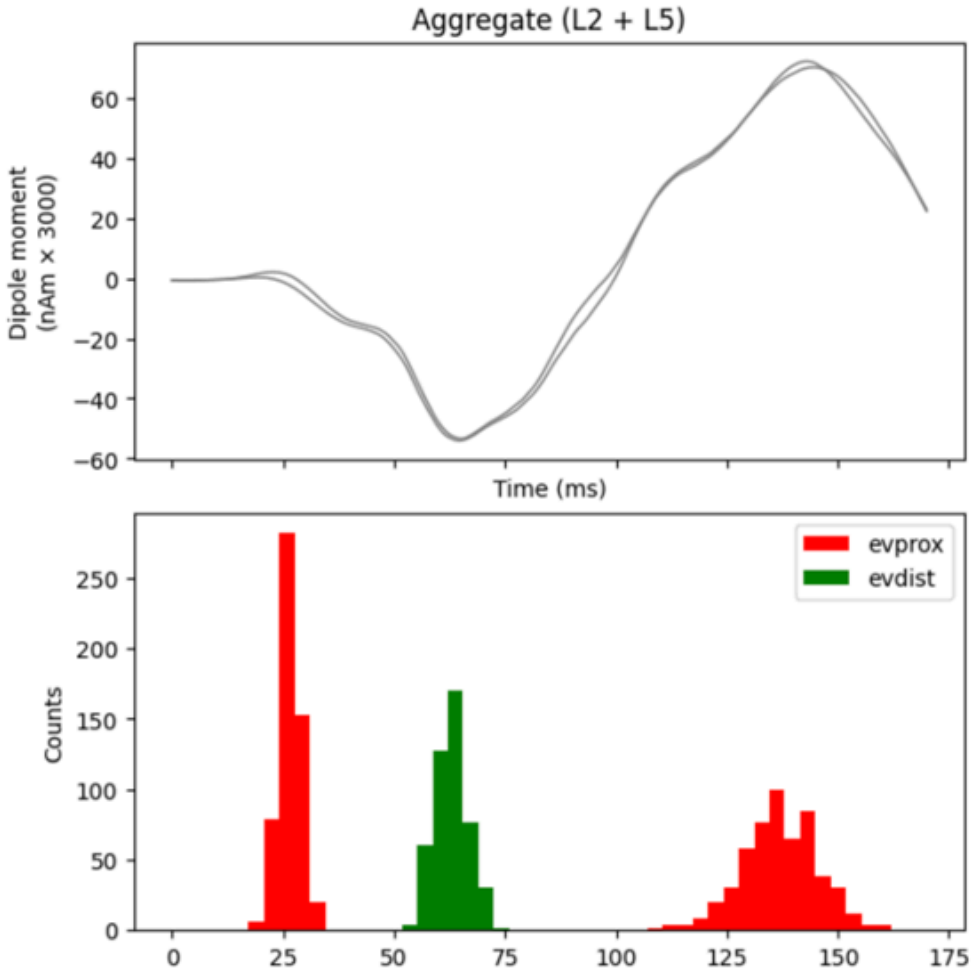


Figure 7 (above): ERP simulation from HNN ERP tutorial. The default parameters from the ERP tutorial were used to simulate this plot representing the first peaks in an auditory evoked response.

The first change I made to the tutorial to match a healthy ASSR from the 2016 Ethridge paper was to change the time window (tstop) from 170 to 500 seconds. I also decreased the mu variable in the proximal drive to 80ms in an attempt to make both the proximal and distal drive occur within a similar distance of one another like the ASSR example. My advisor had initially recommended this change , but after discussing this with him again, he advised against this

change because this wouldn't reflect accurate extrinsic inputs of the ASSR model. Because of this, in successive simulations, I made sure to switch back to the default mean start time values from the HNN ERP tutorial.

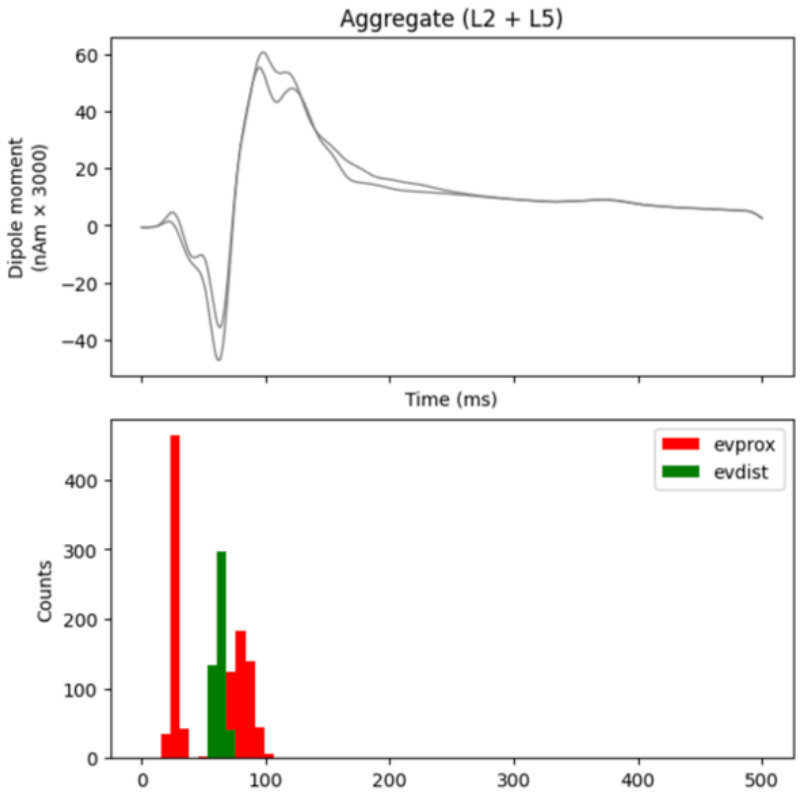


Figure 8 (above): The ERP simulation above shows the initial changes made from the ERP tutorial to start simulating the Ethridge ASSR of a healthy patient. 2 trials were simulated, and the time window (tstop) was changed from 170 ms to 500 ms because each ERP in the ASSR was recorded within a 500 ms time window.

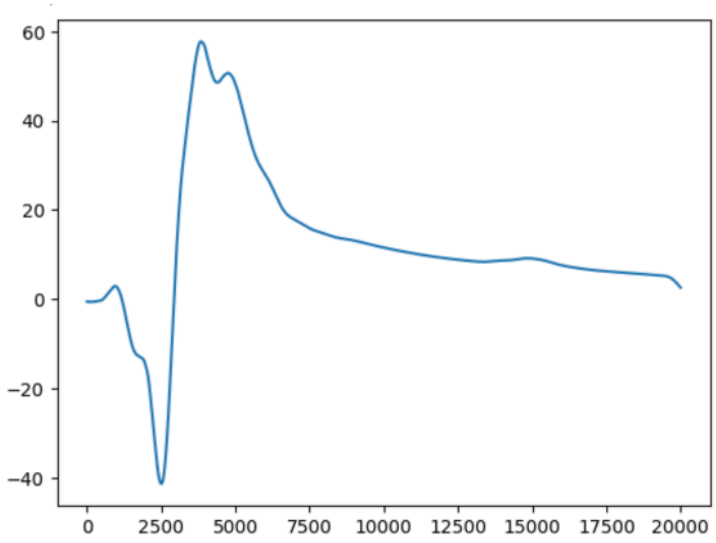


Figure 9 (above): This is the average of both ERP trials shown in figure 8. I averaged the trials of each simulation to stay consistent with the Ethridge 2016 study.

After changing the altered mu value in the second proximal drive back to its default value of 137.12, I focused on changing the synaptic weights of the distal drive since the P1 peak of the ASSR has a significantly greater amplitude than what my simulation was showing. To achieve this, I increased the synaptic weights of the distal drive by a factor of 10 to honor the logarithmic nature of these values. The results of this change to the distal drive led to the invalidation of my first hypothesis. If you look at the figure 10 below, you can see increased synaptic spiking that was not present in my previous simulation.

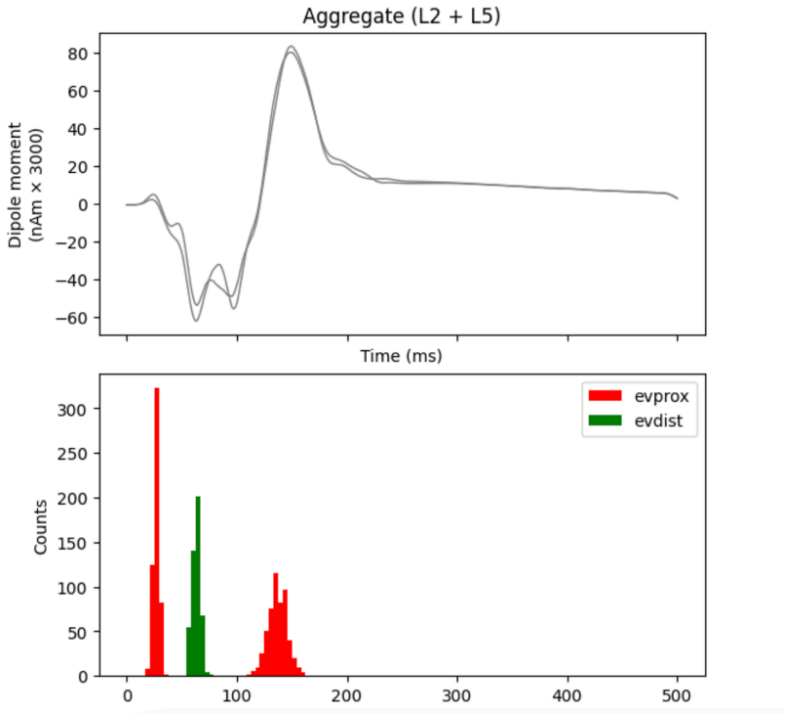


Figure 10 (above): The ERP simulation above shows the results of an increase in the synaptic weights of the distal drive by a factor of 10 from the original ERP tutorial values. In the AMPA weights of the distal drive, the L2 pyramidal neuron value was increased from .000007 to .00007 and the L5 pyramidal neuron value was increased from 0.142300 to 1.423. In the NMDA weights of the distal drive, the L2 pyramidal neuron value was increased from 0.004317 to 0.04317 and the L5 pyramidal neuron value was increased from 0.080074 to 0.80074.

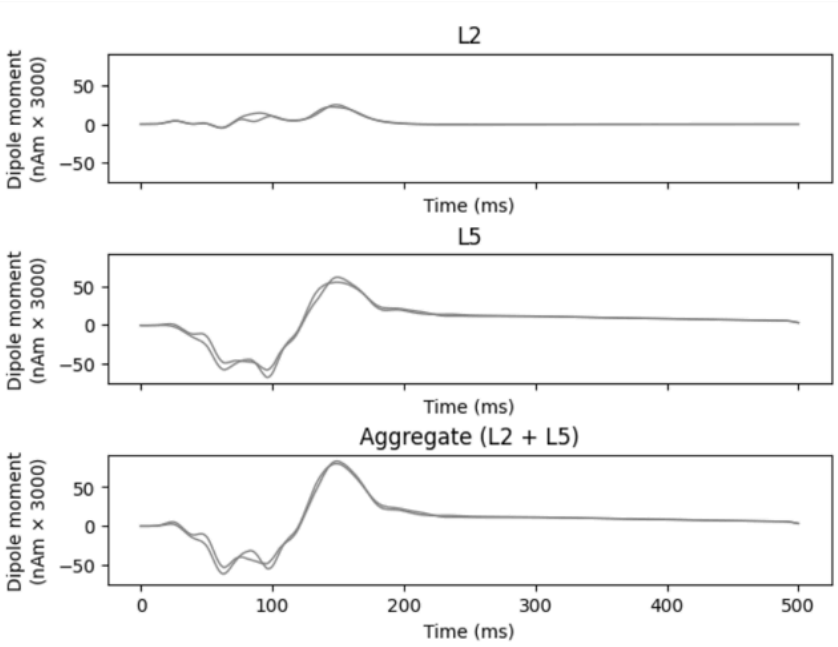


Figure 11 (above): This figure analyzes the individual dipole moments of each layer individually and in the aggregate L2 + L5 time series.

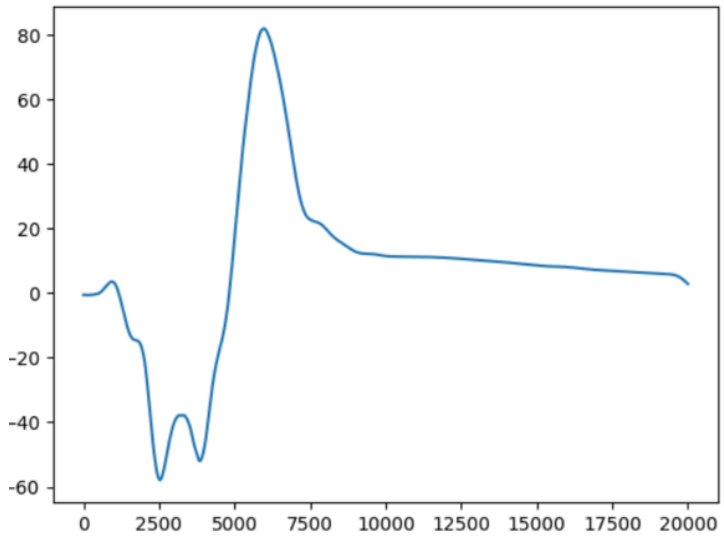


Figure 12 (above): This is the average of both ERP trials shown in figure 10. I averaged the trials of each simulation to stay consistent with the Ethridge 2016 study.

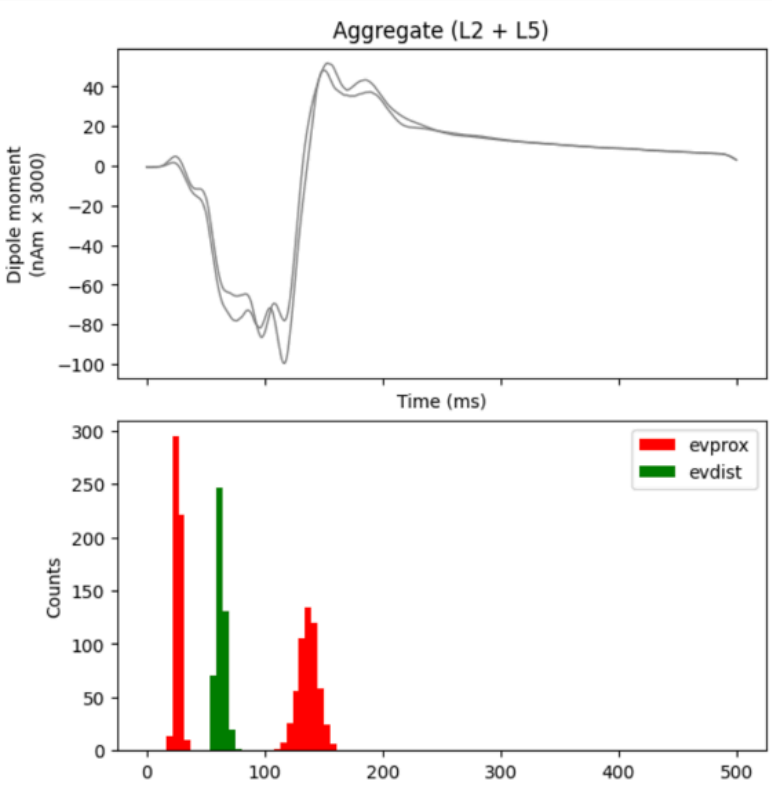


Figure 13 (above): The ERP simulation above shows the results of an increase in the synaptic weights of the distal drive by a factor of 100 from the original ERP tutorial values. In the AMPA weights of the distal drive, the L2 pyramidal neuron value was increased from .00007 to .0007 and the L5 pyramidal neuron value was increased from 1.423 to 14.23. In the NMDA weights of the distal drive, the L2 pyramidal neuron value was increased from 0.04317 to 0.4317 and the L5 pyramidal neuron value was increased from 0.80074 to 8.0074.

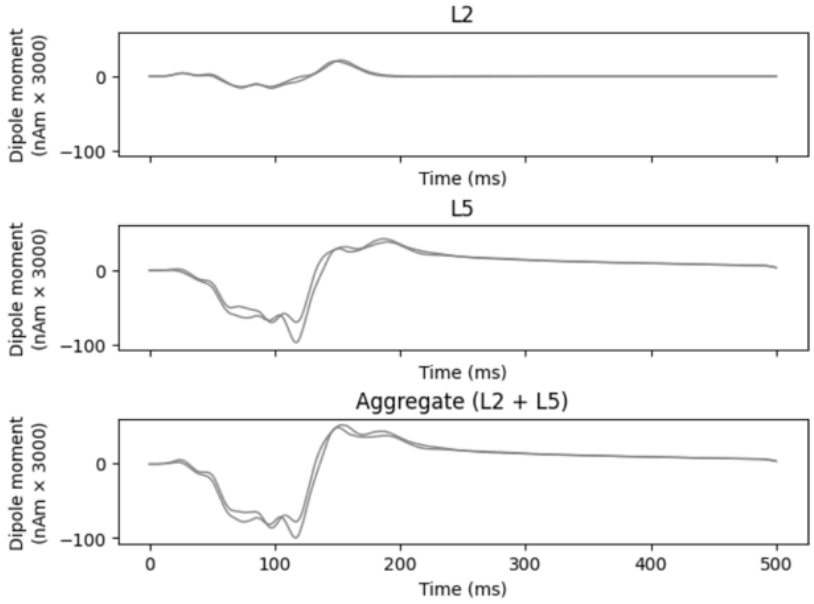


Figure 14 (above): This figure analyzes the individual dipole moments of each layer individually and in the aggregate L2 + L5 time series.

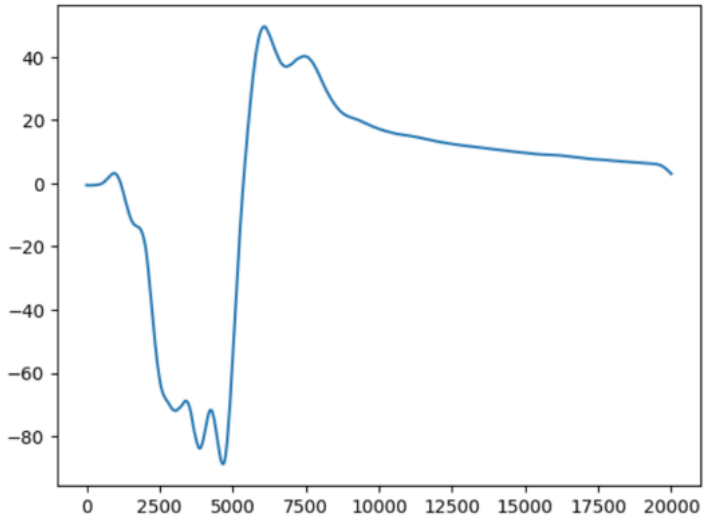


Figure 15 (above): This is the average of both ERP trials shown in figure 13. I averaged the trials of each simulation to stay consistent with the Ethridge 2016 study.

The third and final time I increased the distal drive inputs by a factor of 10, I continued to see an increase in neural spiking in the L5 layer as seen in the 2 previous simulations, thoroughly invalidating my hypothesis.

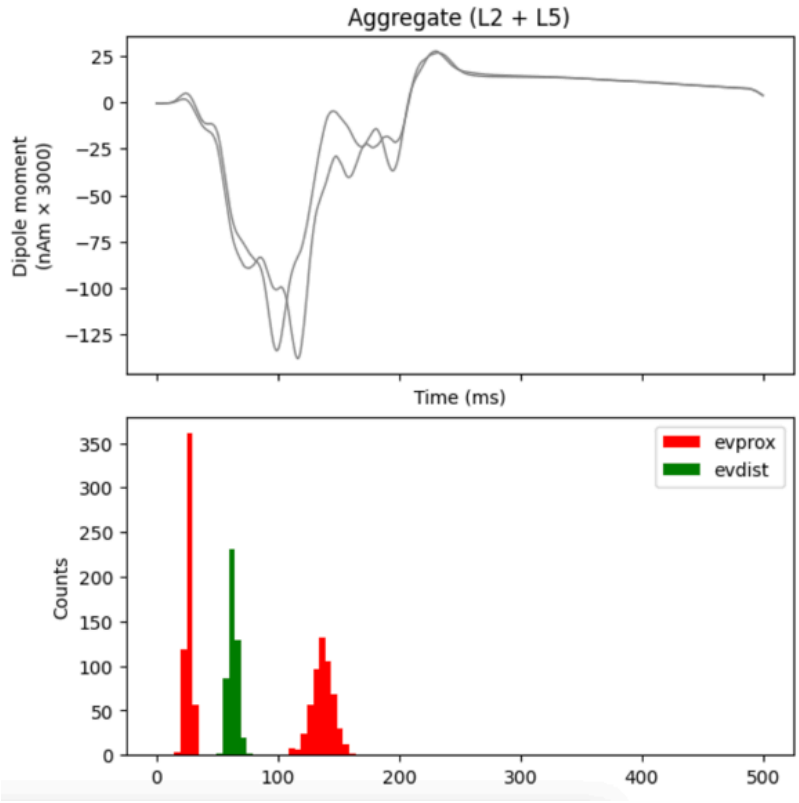


Figure 16 (above): The ERP simulation above shows the results of an increase in the synaptic weights of the distal drive by a factor of 1000 from the original ERP tutorial values. In the AMPA weights of the distal drive, the L2 pyramidal neuron value was increased from .0007 to .007 and the L5 pyramidal neuron value was increased from 14.23 to 142.3. In the NMDA weights of the distal drive, the L2 pyramidal neuron value was increased from 0.4317 to 4.317 and the L5 pyramidal neuron value was increased from 8.0074 to 80.074.

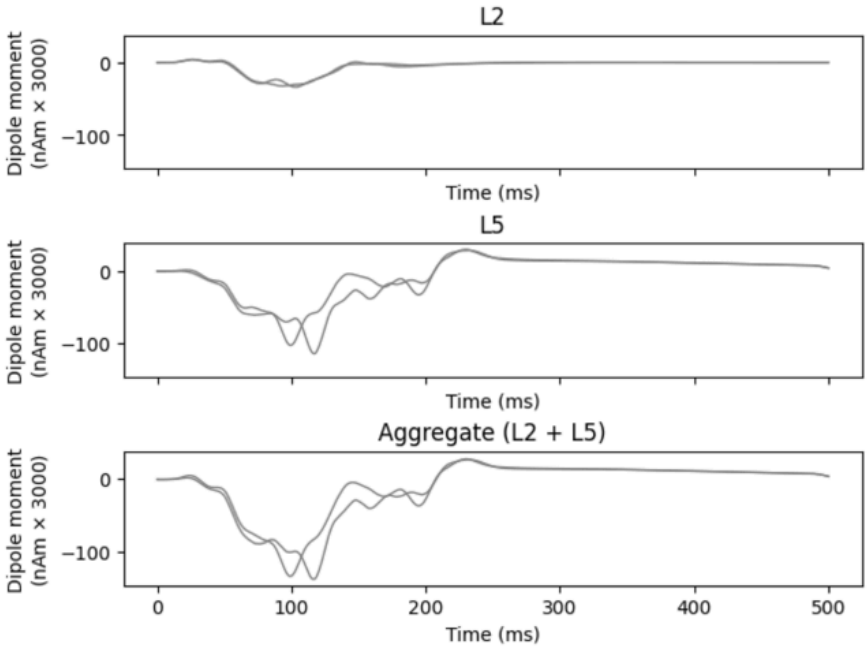


Figure 12 (above): This figure analyzes the individual dipole moments of each layer individually and in the aggregate L2 + L5 time series.

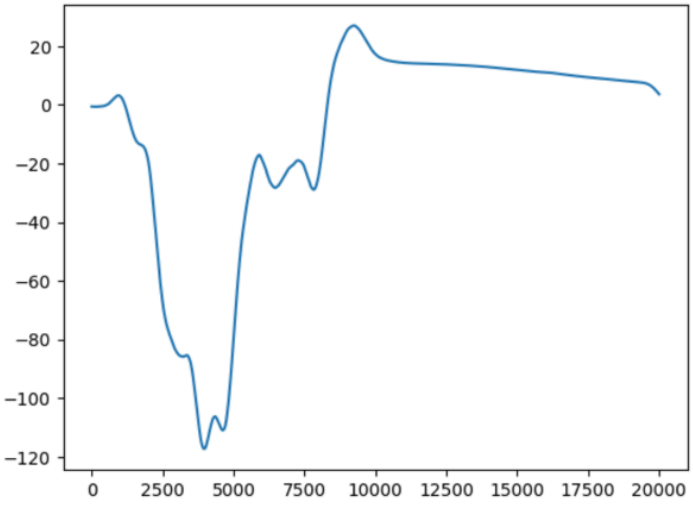


Figure 17 (above): This is the average of both ERP trials shown in figure 16. I averaged the trials of each simulation to stay consistent with the Ethridge 2016 study.

Despite invalidating my hypothesis with only 2 tests, I was curious to see what spiking variability would look like with an increased number of trials. To simulate this, I ran 10 trials

over 2 jobs and generated the following plots. There were still changes in neural spiking present in the L5 layer as shown before, and this was present in the unaveraged time series plot of all 10 trials. However, the averaged time series plot appeared to "smooth over" these differences in spiking activity, which made me wonder if these spiking differences were also present in the 2016 Ethridge data but not considered since they were not present in the final weighted average of all trials.

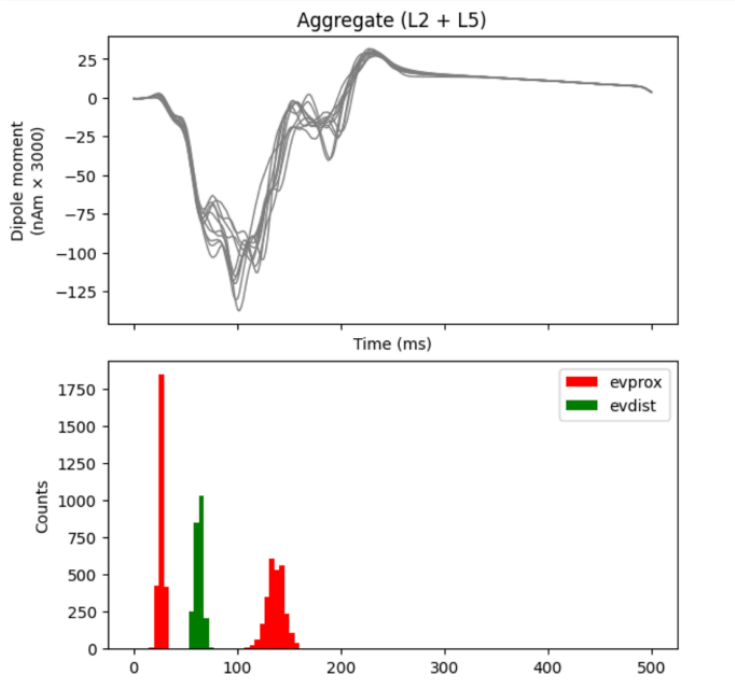


Figure 18 (above): The ERP simulation above shows the results of an increase in the synaptic weights of the distal drive by a factor of 1000 from the original ERP tutorial values, but instead over 10 trials instead of 2. In the AMPA weights of the distal drive, the L2 pyramidal neuron value was increased from .0007 to .007 and the L5 pyramidal neuron value was increased from 14.23 to 142.3. In the NMDA weights of the distal drive, the L2 pyramidal neuron value was increased from 0.4317 to 4.317 and the L5 pyramidal neuron value was increased from 8.0074 to 80.074.

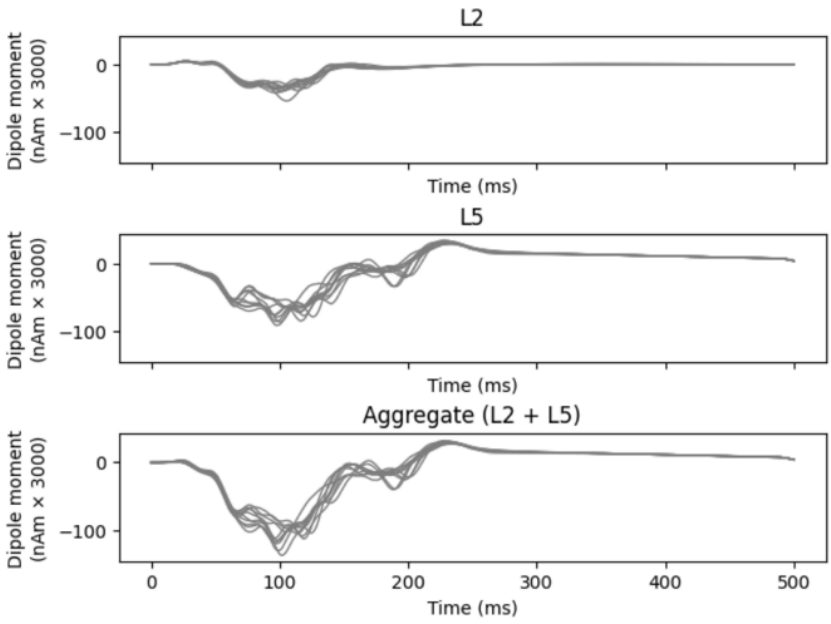


Figure 19 (above): This figure analyzes the individual dipole moments of each layer. Here we can see that the increase in neuronal spiking present in the aggregate L2 + L5 time series is primarily due to spiking changes in the L5 layer.

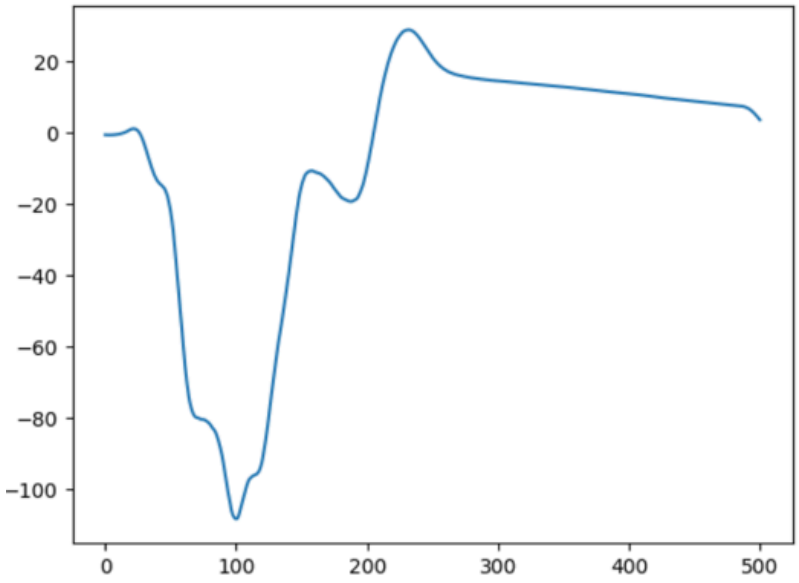


Figure 20 (above): This is the average of both ERP trials shown in figure 14. I averaged the trials of each simulation to stay consistent with the Ethridge 2016 study.

Discussion

Overall, my first hypothesis "differences in extrinsic inputs can explain the differences in habituation responses between Fragile X patients and healthy controls in the ASSR paradigm" was invalidated, since I was unable to successfully model the healthy ASSR paradigm from the 2016 Ethridge paper. While attempting to simulate the ASSR of healthy patients, manipulation of extrinsic inputs unexpectedly increased neuronal spiking, and demonstrated that network connectivity also plays a strong role in the ASSR of healthy patients. The discussion of differences in network connectivity between healthy and autistic has been an ongoing conversation in the scientific community and has been explored in several studies like that of Khan and Kenet 2015. In this study, researchers investigated functional connectivity abnormalities in ASD using MEG by focusing on how these abnormalities manifest during auditory processing tasks, specifically through responses to vibrotactile stimuli. The authors hypothesized that ASD patients would exhibit reduced local functional connectivity, show increased feedforward connectivity between the primary and somatosensory cortex, long-range feedback connectivity would be reduced in individuals with ASD, and the magnitude of functional connectivity abnormalities would correlate with behaviorally measured tactile processing abnormalities and ASD severity. The results found that ASD patients had reduced local connectivity, increased feedforward connectivity, reduced feedback connectivity, and significant correlations between the strength of the mu-b rhythm and behavioral sensory processing scores depending on an individual's ASD severity.

Considering the similarities between ASD and Fragile X disorder, it would be interesting to review if changes in network connectivity have been explored yet in scientific literature.

The results of this initial exploration of the effect of extrinsic inputs on ASSR has shown the need to consider the role of network connectivity in healthy vs. Fragile X patients. My new hypothesis for future work is that changes in functional connectivity, feedforward connectivity, and/or feedback connectivity explain the differences in habituation responses between Fragile X patients and healthy controls in the ASSR paradigm.

Chapter 5: Customer Discovery Report and Competitive Landscape

Unmet Need

According to the World Health Organization (WHO), one billion people are affected by neurological disorders worldwide (Amer, 2023). Of those one billion people, 50 million people suffer from epilepsy and 24 million suffer from dementias and other brain disorders (Amer, 2023). Additionally, 1 in 100 individuals has autism and 1 in 8 individuals live with a mental disorder (Amer, 2023).

Beachhead Market Identification: Preclinical Schizophrenia

Prior to our customer discovery work, our team conducted baseline interviews to gauge interest in the HNN and get a better idea of what the neuropharmaceutical market looked like. Upon interviewing key opinion leaders within the R&D space, our team found that many industry giants such as Abbvie, Daiichi Sankyo, Neurocrine Biosciences, were pursuing therapeutics for schizophrenia in their pipeline. Additionally, many scientists emphasized a need for objective measures to validate preclinical schizophrenia research and to better avoid “the valley of death” (Zurdo, 2013). This prompted our team to select the preclinical schizophrenia market as our beachhead.

Competitive Landscape

Our team has identified the competitive landscape of the HNN to include the intersection of three domains which are (1) Pharmacology and Computational Modeling and Simulations, (2) Pharmacology and Electrophysiology, and (3) EEG Neurotechnology and Computational Modeling and Simulations (Grand View Research, 2023 ; Grand View Research, 2024).

Pharmacology and Computational Modeling and Simulations

Computational Modeling and Simulations in Pharmacology is known as Quantitative Systems Pharmacology (Grand View Research, 2023). Quantitative Systems Pharmacology (QSP) is an expanding field that leverages computational modeling to map out how drugs interact dynamically with biological systems (Grand View Research, 2024). These models aim to capture drug effects from the molecular level up through cellular biochemical pathways to better understand their impact on disease mechanisms. Industry leaders like Certara and Schrödinger are at the forefront, using QSP approaches to simulate drug behavior both at the cellular scale and across the body, which has enabled them to develop predictive models for pharmacokinetics (how a drug moves through the body) and pharmacodynamics (how a drug affects the body). Despite their strengths in modeling drug absorption, distribution, metabolism, and effect, current QSP models fall short when it comes to the brain—they don't incorporate the electrical activity of neurons or the dynamic behavior of neural circuits. As a result, they are not equipped to simulate the complex brain activity underlying neurocognitive disorders or the EEG-based biomarkers that reflect these conditions (Grand View Research, 2023).

Pharmacology and Electrophysiology

At the crossroads of neuropharmacology and electrophysiology, contract research organizations (CROs) like Charles River Laboratories and PsychoGenics are increasingly offering specialized services to collect high-quality in vivo data from rodent models, including EEG recordings and behavioral assessments. These studies represent some of the few available sources of translational brain activity data that can inform and guide drug development.

However, interpreting this complex data remains a significant challenge due to a lack of mechanistic insight into the underlying neural processes. Most electrophysiology-focused CROs also fall short in providing access to raw datasets or in delivering advanced neural biomarkers that could enhance interpretation (Grand View Research, 2024). Additionally, existing approaches do not adequately bridge the gap between rodent and human brain physiology at the circuit level, making it difficult to translate rodent EEG biomarkers into clinically relevant signals. This gap highlights the urgent need for computational modeling and simulation (CM&S) strategies that can better interpret electrophysiological data (Grand View Research, 2023).

EEG Neurotechnology and Computational Modeling and Simulations

A number of academic institutions and a small group of neurotherapeutic companies are working on computational modeling and simulation (CM&S) approaches to analyze EEG signals. However, our Human Neocortical Neurosolver (HNN) and its associated intellectual property stand out by uniquely integrating all three key domains of innovation in neurotherapeutic development. In terms of industry competition, only a few companies are engaged in related efforts. In Silico Biosciences, for example, develops electrophysiological models focused on brain cells and circuits, but their work is restricted to subcortical regions and is not designed for simulating human EEG. Sim4Life offers personalized brain models, yet there is no clear indication that they simulate EEG signals specifically.

We have identified only two companies actively developing whole-brain models intended for EEG simulation: Dassault Systèmes (in partnership with The Virtual Brain) and Neuroelectrics (Sanz, 2013). However, unlike HNN, their approaches rely on neural mass models—simplified mathematical representations of brain activity that lack the detail needed to

explore drug-target interactions at the cellular or circuit level. This makes them less practical for pharmacological applications.

Shifting Landscape

The future of drug development is trending in a direction that aligns strongly with our strengths across several key areas. There's a growing shift within neuropharmaceutical research and development toward the adoption of electrophysiological biomarkers as reliable translational tools that can bridge the gap between preclinical studies and clinical outcomes. At the same time, the FDA is actively promoting the use of innovative tools and alternative methods that improve the efficiency of animal testing and enable deeper, multiscale physiological insights through computational modeling and simulation (CM&S) (Perez, 2023). Positioned at the intersection of neuropharmacology, CM&S, and electrophysiology, our platform is uniquely equipped to capitalize on these evolving industry and regulatory priorities. It directly addresses the increasing demand for predictive, mechanistically grounded technologies to support and accelerate the development of effective neurotherapeutics.

Customer Discovery: Untested Hypotheses

Our team tested a number of hypotheses during customer discovery to help us validate or invalidate our target market. Our ideal customer profile was a small biotech company, our end user persona was a principal scientist, and our decision maker persona was a director of electrophysiology. The five most notable hypotheses tested are listed in this table.

	Hypotheses	True	False
1	Pharmaceutical companies utilize EEG data in preclinical research to validate target engagement.	?	?
2	Most neuropharmaceutical companies have dedicated QSP teams actively involved in evaluating drug efficacy.	?	?
3	R&D teams would use QSP to study EEG and assess drug efficacy.	?	?
4	R&D that uses EEG are looking for new ways to analyze and interpret data.	?	?
5	Scientists show a strong willingness to adopt new technologies that accelerate drug discovery.	?	?

Table 3: Untested Hypotheses for Preclinical Schizophrenia Market Validation and Customer Discovery

Our first hypothesis assessed the use of EEG rodent data in preclinical research at pharmaceutical companies. The second hypothesis assessed the presence of QSP teams at neuropharmaceutical companies. The third hypothesis assessed the use of QSP and EEG at pharmaceutical companies. The fourth hypothesis was to evaluate customer interest, and the fifth hypothesis was to assess their willingness to adopt.

Customer Discovery: Hypotheses Results & Market Validation

I participated in a number of incubators that helped our team to conduct customer discovery research and validate our market including NSF iCorps Spark@Brown, Faculty Founders at MIT, The Engine by Blueprint, and NSF Fusion. I interviewed over 38 individuals to validate these hypotheses which included 27 end users (research scientists, contract research organizations (CROs)), 9 C-Suite officials, directors, or partners, and 2 potential partners. The

table below summarizes our hypotheses results.

	Hypothesis	True	False	Key Findings
1	Pharmaceutical companies utilize EEG data in preclinical research to validate target engagement.	✓		• R&D scientists record and use EEG data in-house for preclinical applications. • CNS drug targets are validated using EEG data.
2	Most neuropharmaceutical companies have dedicated QSP teams actively involved in evaluating drug efficacy.	✓		• Pharma companies use QSP models to reduce costs by identifying promising drug candidates earlier and to minimize trial-and-error.
3	R&D teams would use QSP to study EEG and assess drug efficacy.	✓	✗	• 50/50, no companies currently use QSP for EEG. • When presented with the ability to, some companies indicated pretty strong interest. • Not an existing market, but a new market
4	R&D that uses EEG are looking for new ways to analyze and interpret data.	✓	✗	• 50/50, R&D is looking for new ways to analyze and interpret data with QSP, but the bar for qualifying biomarker use is high.
5	Scientists show a strong willingness to adopt new technologies that accelerate drug discovery.	✓		• Scientists were enthusiastic to use HNN and/or collaborate with our team • Translational neuroscience is challenging, so new technologies are welcomed.

Table 4: Hypotheses Results and Key Findings for Preclinical Schizophrenia Market

We found that our first hypothesis was in fact true, because R&D scientists record and use EEG data in-house for preclinical applications. Our second hypothesis was also true - we found that pharmaceutical companies use QSP models to reduce costs by identifying promising drug candidates earlier and to minimize trial-and-error. However, our 3rd and 4th hypotheses yielded mixed results, and were split 50/50 amongst our interviewees. No companies currently use QSP for EEG, so there isn't an existing market for the intersection of these technologies. However, when presented with the ability to utilize both, some companies indicated pretty strong interest, showing us that there's the potential for a new market in this space for us. Additionally, R&D is looking for new ways to analyze and interpret data with QSP which validates our 4th hypothesis. However, the bar for qualifying biomarker use is high, and there's a lot of debate in pharma on the use and validity of biomarkers. When we think about this hypothesis and whether

they'd be looking for new ways to analyze and interpret data, we need to look at how they're evaluating the standards of this technology. Nevertheless, our last hypothesis affirmed that scientists show a willingness to adopt technologies that accelerate drug discovery.

Overall, the results from our customer discovery interviews invalidated our beachhead market as preclinical schizophrenia. The market for neurotechnology in preclinical schizophrenia research was not as large as anticipated. Only a few pharmaceutical companies from our interviews were interested in buying software to use translational biomarkers in EEG for preclinical discovery. However, we were able to validate our user personas, our approach to our proof-of-concept, and the value of our technology.

Prior to interviewing, we were unsure if pharmaceutical companies outsourced all EEG work, raising the question if a research scientist was the correct end user persona for the HNN. We learned that pharmaceutical companies will only occasionally outsource data collection of EEG data, but never the data interpretation because they are solely responsible for communicating findings for that data to the R&D team and leadership. Because of this, we have insight into the decision making unit, validating that scientists are our champion (Aulet, 2024). The decision making unit consists of three key roles - the champion, primary economic buyer, and influencers (Aulet, 2024). The champion is the person that wants your product and pushes for its adoption, the primary economic buyer is the person who controls the budget and has purchasing authority, and influencers are other stakeholders that impact the decision (Aulet, 2024). Knowing the DMU and each person's motivations lets us design our sales, messaging, and product roadmap more effectively.

Moreover, we were able to validate our approach to our proof-of-concept and the value of our technology through our customer's responses. Throughout all of our interviews, there was a

large amount of interest in the HNN and a need for validated biomarkers. Majority of research scientists were interested in using our technology or collaborating with our team for EEG data analysis. Additionally, many research scientists expressed a need for objective measures to validate CNS drug targets. This demonstrated to our team that schizophrenia may still be the right research area to tap into, but outside the preclinical space.

New Beachhead Market Identification: Clinical Schizophrenia

The key findings and hypotheses results from our customer discovery work have prompted our team to select the clinical schizophrenia market as our new beachhead. We have decided to abandon the preclinical schizophrenia from an initial bottom-up analysis. With roughly 24 small pharmaceutical companies pursuing CNS drug targets, ½ were using EEG and were interested in adopting our technology. Of that ½, if 6 to 12 actually purchased and adopted our technology for $100,000 each, that would amount to roughly $1,000,000 in revenue, which is not enough income to sustain our company. Because the value of our technology remains intact, there is still an opportunity for using the HNN in the schizophrenia market. But instead at the preclinical level, it would be more effective at the clinical level. Therapeutics for schizophrenia patients fail ⅓ of the time they're administered, showing that there are hundreds of thousands of patients that need a new type of therapy intervention and could benefit from optimized treatment decisions. Treatment response prediction is a huge opportunity for patients in the neuropsychiatry space, but given what we've learned about the schizophrenia market, there may be a large opportunity for the HNN to predict treatment response in schizophrenic patients.

Chapter 6: Intellectual Property Considerations for the HNN

Patents

The HNN commercialization team at the Jones lab currently has 2 patents filed:

ID Number (application, serial, or patent)	Title	Assignee	Date Filed	Status	Type (eg. Device and method, composition of matter)
Appl. No.: US2025/020531 Pub. No: N/A Pub. Date: TBD	ESTIMATION OF PARAMETERS IN BIOPHYSICALLY DETAILED NEURAL MODELS WITH SIMULATION BASED INFERENCE	Tolley, Nicholas and Jones, Stephanie	3/19/24	Filed as provisional	System and method
Appl. No: 18/656,157 Pub. No.: US 2024/0366141 A1 Pub. Date: Nov. 7, 2024	SYSTEM AND METHOD FOR DISSECTING BETA WAVEFORMS USING CONVOLUTIONAL DICTIONARY LEARNING	Zhou, David and Jones, Stephanie	5/6/24	Filed as provisional	System and method

Table 2: List of patents filed by the Jones lab for HNN (Jones Lab, 2024).

Patent one is a new method for simulation based inference, and patent two is a method for biomarker identification. Simulated-based inference in EEG enables the reverse-engineering of complex neural mechanisms by matching simulated brain activity to observed EEG data, using statistical inference driven by machine learning and Bayesian approaches. It's especially valuable in computational neuroscience and neurotechnology for extracting biologically meaningful parameters (Bürkner, P.-C., et al., 2022).

IP Strategy

The IP strategy for the HNN software is patenting proprietary methods around an

open-source core. The HNN source code is copyrighted. There is no IP risk for open source access to the HNN because gaining valuable insight requires use of supplemental methods and packages in addition to the HNN core. HNN core on its own cannot complete the proof of concept demonstrated earlier in Chapter 3.

Incorporation, Data, and Development Team

The Jones lab is currently collaborating with the Broad Institute at MIT to analyze real human and rodent data for our schizophrenia proof-of-concept, which is one part of the two-pronged approach for demonstrating the HNN's value. The Broad Institute's human data is being utilized to validate preclinical biomarkers, and the rodent data is being used to validate our model. Our development team includes 3 domain experts with irreplaceable knowledge of HNN and field expertise. We have yet to incorporate, but we have just recently found a law firm to start this process.

Chapter 7: Regulatory Strategy for the HNN

In the realm of preclinical drug development, there is currently no formal regulatory pathway required for the use of computational modeling and simulation (CM&S), meaning there are no regulatory barriers that would prevent commercialization of our technology. However, the FDA has established multiple programs aimed at supporting the integration of CM&S tools into drug and medical device development. These initiatives offer valuable guidance for shaping our commercialization strategy. For instance, the FDA's *Credibility of Computational Models Program* (2023) promotes the adoption of standardized reporting practices and credibility assessments when CM&S tools are used in regulatory submissions. These standards not only enhance the reliability of such tools in the regulatory space but also stimulate broader industry demand. Additionally, the FDA's Center for Drug Evaluation and Research (CDER) operates the Drug Development Tools (DDT) Qualification Programs, which aim to accelerate innovation and expand access to validated tools that support drug development and regulatory decision-making (U.S. Food and Drug Administration, 2024). We plan to engage directly with this program—and potentially lead an industry consortium—to seek qualification for our EEG biomarker simulations as evidence-generating tools for specific use cases, such as neural pathway validation and safety-related dose determination. Achieving qualified status would allow pharmaceutical developers to use data from our simulations in regulatory filings (e.g., INDs and NDAs) without requiring separate model validation for each submission (U.S. Food and Drug Administration, 2024). We view this qualification not as a regulatory hurdle, but as a strategic customer acquisition mechanism. Even in the absence of formal qualification, our CM&S tools would still deliver significant value to the preclinical development process by raising the standard of evidence available beyond current practices.

To further align with FDA expectations and enhance the credibility of our HNN technology, we will implement a comprehensive Verification, Validation, and Uncertainty Quantification (VVUQ) framework. This approach, as outlined in the FDA's guidance document "Assessing the Credibility of Computational Modeling and Simulation in Medical Device Submissions" (FDA, 2023), involves several key steps:

1. **Verification**: Ensuring that the computational model accurately represents the underlying mathematical model and its solution. This includes code verification to identify errors in the numerical algorithms and calculation verification to determine the solution accuracy of a calculation (U.S. Food and Drug Administration, 2024).
2. **Validation**: Determining the degree to which the model or simulation is an accurate representation of the real world for its intended use. This may involve comparing model predictions with experimental data from bench tests, in vivo studies, or other empirical data sources (U.S. Food and Drug Administration, 2024).
3. **Uncertainty Quantification (UQ)**: Quantifying the uncertainty in model inputs and outputs to understand the confidence in simulation results. This step is crucial for assessing the reliability of model predictions in various scenarios (U.S. Food and Drug Administration, 2024).

By systematically applying VVUQ processes, we aim to build trust in the predictive capabilities of our HNN simulations. This rigorous approach will not only support the use of our technology in regulatory submissions but also facilitate its adoption in the pharmaceutical industry by demonstrating its reliability and relevance in drug development processes. Notably, the field of cardiac electrophysiology has already established a precedent for the application of

VVUQ methodologies, with cardiac models undergoing formal verification, validation, and uncertainty quantification to ensure their credibility for clinical and safety-critical applications (Pathmanathan, 2014). As we advance VVUQ studies for HNN, we will draw on the frameworks and best practices developed in cardiac modeling, following a similar pathway to establish robust standards for model evaluation and regulatory acceptance.

Chapter 8: HNN 5-year Business Plan

Below are two commercialization plans that our team has been working on to bring the HNN to market. Prior to the completion of our customer discovery work, our team thought it most feasible to commercialize the HNN as a drug development tool. This aligned with our initial beachhead market of preclinical schizophrenia, assuming that this market would be the best fit for the HNN and our future company. This plan was generated prior to our customer discovery research.

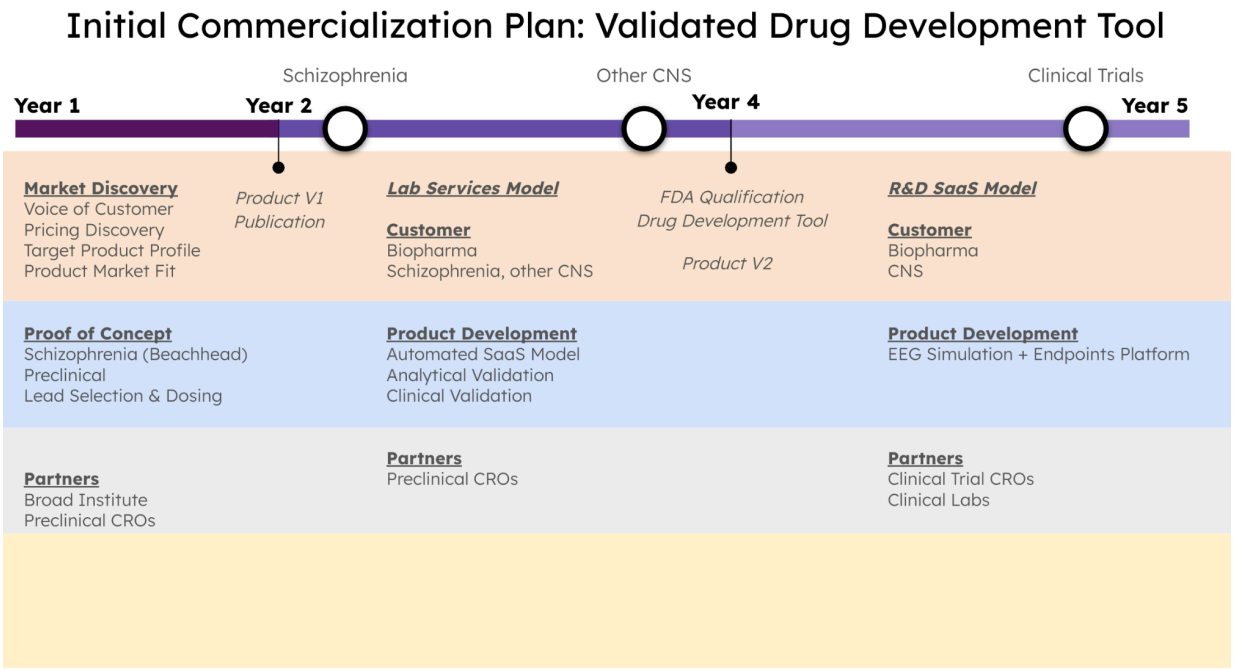


Figure 22: Commercialization Plan for the HNN as a Validated Drug Development Tool

Based on the results of our customer discovery interviews, I've adjusted our commercialization plan for our new beachhead market: the clinical schizophrenia market. Initial changes are shown in red. Because the value of our technology and our proof-of-concept stayed

intact, our team will proceed with preclinical validation, but need to pursue clinical validation afterwards to satisfy our beachhead market.

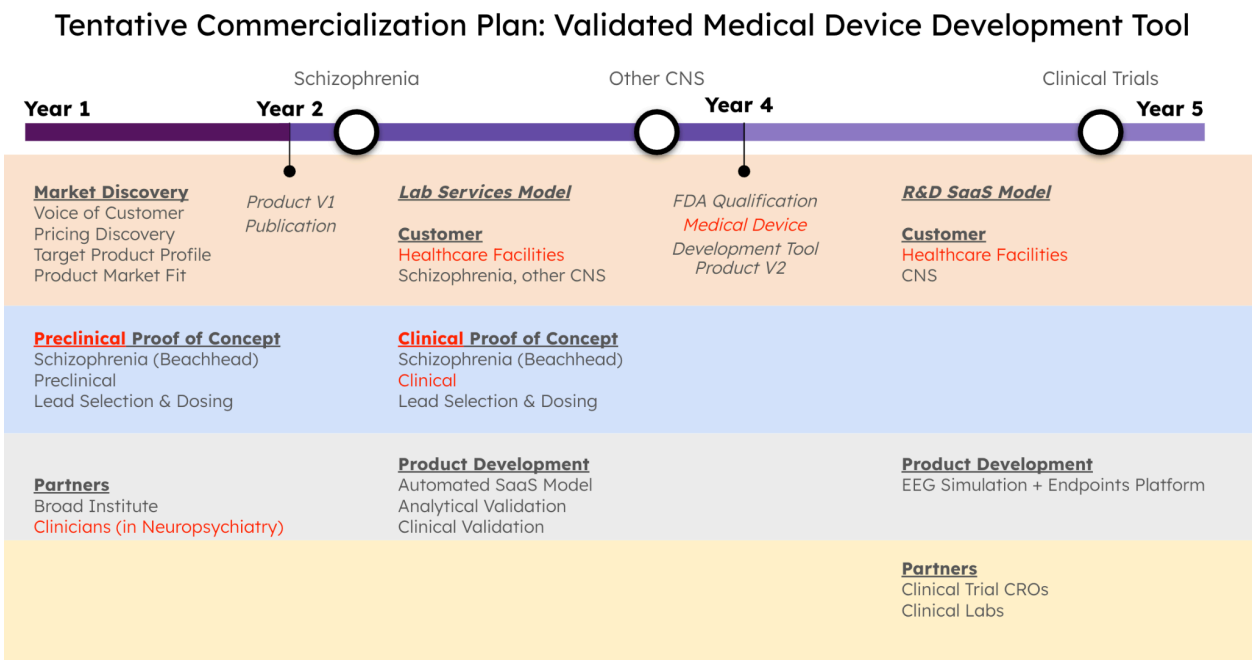


Figure 23: Tentative Commercialization Plan for the HNN as a Validated Medical Device Development Tool

Chapter 9: Final Recommendations for HNN

The HNN platform is uniquely positioned to become a category-defining product at the intersection of electrophysiology, neuropharmacology, and computational modeling. While initial market exploration into preclinical schizophrenia demonstrated technological value, it also revealed commercial limitations: too few companies in this niche expressed sufficient willingness or budget to adopt EEG-based QSP tools at scale. However, the clinical schizophrenia market represents a far more promising and scalable opportunity, both in terms of unmet patient need and economic viability.

With over 24 million people globally suffering from brain disorders, including 50 million with epilepsy and 1 in 8 individuals affected by mental disorders, the demand for better diagnostic and treatment tools is overwhelming (Amer, 2023). In schizophrenia alone, where 1/3 of patients fail to respond to first-line therapies, the clinical imperative for predictive treatment response tools is urgent (Samara, 2019). Here, the HNN's ability to simulate EEG biomarkers from biophysically detailed neural circuit models offers a path to objective, mechanistically grounded tools that can enhance patient stratification, optimize treatment decisions, and reduce the costly trial-and-error of psychiatric drug regimens.

Furthermore, the shifting regulatory landscape plays in the HNN's favor. The FDA has explicitly encouraged the use of advanced computational modeling and simulation (CM&S) techniques to improve translational science and reduce reliance on traditional animal testing. To capitalize on this, our team's strategy to integrate Verification, Validation, and Uncertainty Quantification (VVUQ)—as outlined in FDA guidance (FDA, 2023)—will enhance the HNN's credibility for clinical use and build a foundation for potential qualification through the Drug

Development Tool (DDT) program (FDA, 2023). This regulatory strategy could create first-mover advantage and incentivize adoption by de-risking use of the platform for pharmaceutical partners.

Our customer discovery work validated the core product value: R&D scientists are actively seeking tools to interpret EEG data more effectively, and there's widespread interest in collaborating with HNN for CNS biomarker development. While most companies currently do not combine QSP and EEG, their interest in our integrated approach suggests a category creation opportunity—one that the HNN is uniquely equipped to lead.

Finally, because our end-user (scientists) and decision-making unit (including budget holders and influencers) are well-identified, we are now able to tailor a go-to-market strategy that aligns technical benefits with business outcomes for stakeholders. While pivoting to the clinical market, we can continue validating HNN within preclinical settings to refine its capabilities, collect real-world proof-of-concept data, and maintain relationships with R&D teams. The goal is not to abandon the preclinical market, but to use it as a testing ground and early validation path while pursuing broader applications.

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