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Study Protocol and Statistical Analysis Plan

Official Study Title:

Intracranial Recording and Stimulation of the Human Hippocampal-Amygdala Circuit During Virtual Reality Fear Conditioning

Principal Investigator:

Anthony I. Jang, M.D.

NCT Number:

Not yet assigned

Date:

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PROJECT SUMMARY/ABSTRACT

Anxiety disorders, including generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD), are the most prevalent mental health conditions globally with a high rate of treatment resistance and significant healthcare costs. These disorders are believed to stem from fear responses that are excessive and disproportionate to the situation. Therefore, understanding how the human brain processes fear and how this may go awry in anxiety disorders is crucial for developing effective therapies. Research using rodent models of anxiety suggests that the formation and regulation of fear responses to the environment require a complex interplay between two brain structures, the amygdala and hippocampus. However, it is unknown whether these findings can be translated to the human brain, and whether directly modulating fear-related neural activity can be used as a therapeutic tool to treat anxiety disorders. This project aims to 1) elucidate the neural mechanisms governing the acquisition, extinction, and renewal of fear and anxiety responses within the hippocampal-amygdala circuit in humans, 2) investigate whether targeted stimulation of the amygdala, aimed at dampening its role in maintaining fear responses to innocuous stimuli, can facilitate recovery from maladaptive fear responses, and 3) explore potential differences in outcomes based on individuals' baseline anxiety levels. To address these questions, we will work with participants who have previously undergone surgical implantation of a responsive neurostimulation (RNS) device in the amygdala and hippocampus for the treatment of epilepsy or PTSD. The RNS is a chronically implanted intracranial recording and stimulation device that provides a unique opportunity to directly record from the hippocampal-amygdala circuit and deliver electrical stimulation. To simulate real-world fear responses and assess the impact of environmental context on fear learning, we designed a virtual reality (VR) experiment where participants acquire and subsequently extinguish fear responses to various stimuli within different virtual environments (e.g., library, grocery store), providing insights into how contextual cues influence fear responses.

This award will allow me to complete a multifaceted career development plan in a world-class academic institution under an interdisciplinary mentoring team consisting of experts in basic neuroscience, neurotechnology, virtual reality, neurosurgery, psychiatry, and psychology. Building upon my prior experience with acutely implanted intracranial electrodes, I will broaden my skill set to encompass mobile (chronic) intracranial electrophysiology and targeted electrical stimulation. I will also develop expertise in the implementation of immersive, ambulatory VR experiments in human neuroscience research. Lastly, through seminars, coursework, and conferences, I will develop both as an experimental scientist and scientific communicator. Taken together, this will allow me to grow into an independent physician-scientist who effectively leverages innovative technologies to study the mechanisms of anxiety disorders.

PROJECT NARRATIVE

Anxiety disorders such as generalized anxiety disorder and post-traumatic stress disorder are the most prevalent mental health conditions worldwide and are associated with high rates of treatment resistance, disability, and significant healthcare expenses. Studying the basic neural mechanisms of fear and anxiety in humans is crucial for gaining deeper insights into the development of anxiety disorders and pioneering novel therapeutic interventions. Working with individuals who are already implanted with intracranial electrodes for epilepsy or PTSD, our research will employ virtual reality experiments to study the neural correlates of fear and investigate whether direct stimulation of the fear circuitry can ameliorate anxiety-related behaviors, thereby paving the way for innovative neuromodulatory treatments for anxiety disorders.

FACILITIES & OTHER RESOURCES

Scientific environment: UCLA is a renowned research center with extensive academic resources, including regular scientific seminars, a comprehensive electronic library system, excellent core facilities, and accomplished research collaborators. The David Geffen School of Medicine at UCLA, the Ronald Reagan UCLA Medical Center, and the Resnick Neuropsychiatric Hospital are consistently ranked among the top academic medical institutions in the United States. Dr. Jang has the full commitment of his departmental leadership as described in the Institutional Letter of Support and the Department of Psychiatry offers generous internal grant mechanisms including the Friends of the Semel Institute Research Scholars Program. In addition, the UCLA Clinical and Translational Science Institute (CTSI) provides numerous programs (e.g., K Scholars Society) and support services (e.g., drop-in statistical consulting, grant application reviews, intramural funding opportunities) specifically designed to assist early-career investigators in their career development.

The Suthana laboratory has two locations for mobile ambulatory virtual reality (VR) tasks in humans, one at UCLA (Westwood) and one in Santa Monica (3 miles away). The Westwood laboratory is a part of the UCLA Brain Research Institute which includes about 300 faculty members representing nearly 30 departments from 6 different schools, serving a rich interdisciplinary academic environment. The Westwood location is on the 4th floor of the Semel Institute for Neuroscience and Human Behavior and consists of a computer lab (Room 47-355) and an experimental AR/VR motion capture laboratory (Room 48-136). The computer laboratory (810 sq ft) consists of 16 workstations equipped with numerous Mac/PC desktop/laptop computers and a large cluster server and computing server available to all laboratory members for any research-related activities such as data analysis and manuscript preparation. It also consists of an enclosed office room with a door for behavioral testing for pilot projects. The experimental laboratory is a square-shaped (420 sq ft) open space room equipped with several pieces of equipment (see ***Equipment*** section) including multiple iterations of virtual and augmented reality headsets (e.g., Meta, VIVE), a computer capable of implementing graphics-intensive VR experiments, and wearable sensors that can measure photoplethysmography (i.e., heart rate), electrodermal activity (i.e., skin conductance), electromyography (i.e., startle eye-blink amplitude), pupil diameter, and gaze direction. It is also equipped with full-body wireless motion tracking equipment that includes 23 wall-mounted high-resolution cameras (Optitrack, Natural Point, Inc.,) for sub-millimeter motion capture and body positioning.

The second location is in Santa Monica (total of 4,331 sqft) located at 1618 Stanford St, Suite D, Santa Monica, CA 90404, and consists of 4 office spaces, a conference room/computer lab, a break area, a large experimental VR/AR motion capture laboratory, and a space for sleep/nap studies and behavioral testing. The are a total of 20 workstations and numerous computers and servers that are available for use by laboratory members. The experimental laboratory is a square-shaped (1,947 sq ft) open space room equipped with several pieces of equipment including scalp EEG, VR/AR headsets, wearable technology (e.g., biometrics), eye tracking headsets, and full body wireless motion tracking equipment that includes 41 wall mounted high-resolution cameras (Optitrack, Natural Point, Inc.).

Software available includes Matlab, Python, SPSS, FSL, BrainLab, Vitrea, Freesurfer, ASHS, ANTs, R, Adobe Suite, E-prime, Steam VR, Maya, Blender, Unity, and custom design software.

The Veterans Affairs Greater Los Angeles Health System (VAGLAHS) is one of the leading healthcare systems serving U.S. military veterans in Southern California. It comprises a tertiary care hospital, the West Los Angeles VA Medical Center, alongside several community-based outpatient clinics, fostering a robust environment for collaboration. This proposal features two key collaborators, Dr. Langevin and Dr. Koek, who hold joint VA appointments. They play integral roles as liaisons in participant recruitment efforts, with a particular focus on veterans with severe anxiety- and trauma-related disorders. Neurosurgical patients particularly from the VA have a higher prevalence of anxiety disorders including GAD and PTSD. This collaboration between the Suthana lab and the VAGLAHS has been ongoing successfully for the past several years yielding high-impact publications[1,2].

Institutional Investment in the Investigator: Dr. Jang is currently completing his third year in the Research Track of the UCLA Psychiatry Residency Training Program. Upon graduating from residency, the Department of Psychiatry fully commits to the appointment of Dr. Jang as a full-time faculty member with at least 75% protected time to devote to his research and career development activities, with full departmental salary support in addition to salary supplementation through the Suthana laboratory and external grant mechanisms. Dr. Jang's appointment at UCLA is not contingent upon the receipt of the K08 Award (please see the Institutional Commitment Letter for details).

EQUIPMENT

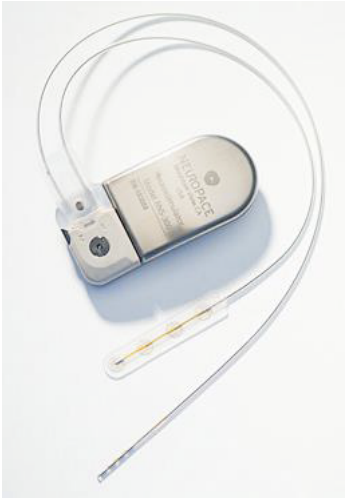


Figure 1. The RNS System

NeuroPace RNS system: The FDA-approved RNS® System (**Figure 1**) will be used to record intracranial electroencephalography (iEEG) activity from the amygdala and hippocampus and deliver targeted electrical stimulation. Each participant in the study will have one or two implanted depth electrode leads 1.27 mm in diameter each with four platinum-iridium electrode contacts, each with a surface area of 7.9 mm^2, 1.5 mm long with an electrode spacing of 3.5mm. The RNS System will continuously monitor iEEG on four bipolar channels at 250 samples/sec with its default closed-loop electrical stimulation turned off during experimental sessions. The RNS System contains a microprocessor that runs software that may be transmitted wirelessly to the device. The RNS System communicates wirelessly with a Programmer and Remote Monitor using secure protocols. Our group developed a specialized research protocol using the RNS System Programmer Accessory which allows for continuous iEEG recording and the delivery of electrical stimulation at specified times during the task. Our laboratory has also built a small oscillating electromagnet that emits a "Marker" signal that is inserted and delivered to the iEEG data in programmable time intervals (i.e. 30-240 sec) to sync the iEEG data with motion capture cameras and other continuous data (e.g., physiological data, scalp EEG) while the participant completes behavioral tasks[3,4]. All research accessories have been tested with the NeuroPace RNS System device with high reliability and repeatability. We have used this setup in more than 20 participants thus far, including data reported in the Preliminary Results of the Research Strategy.

Motion capture: Recording of participant movement is done using the Optitrack system (Natural Point, Inc.) with 41 ceiling-mounted infrared high-resolution cameras that allow for sub-millimeter motion tracking (see Facilities and Resources). The motion tracking cameras detect reflective markers that are manually attached to the virtual reality headset which models the head as a rigid body object and tracks its center of mass (i.e., position) and rotational information (i.e., yaw, pitch, and roll with respect to the experimental room).

Virtual reality setup: The VR fear conditioning paradigms will be implemented in a state-of-the-art Alienware Gaming Computer running the Unity game engine which is wirelessly cast to the Meta Quest Pro VR headset. The paradigm can be flexibly adapted to run in other VR headsets that are also available to lab members, including the Samsung GearVR goggles and the HTC VIVE VR headset. VR applications can also be configured to communicate with the motion capture system via WiFi through custom Unity scripts (Interactive Lab, Russia), allowing for real-time room-scale virtual navigation (i.e., the physical movement of the participant tracked with motion capture cameras can be mapped to their position in the virtual room, allowing for real-time updating of the virtual scene). A wireless joystick can be paired with the headset and used to track button presses during the survey portion of the VR task. The laboratory also has Microsoft Hololens and Magic Leap headsets for augmented reality and simultaneous eye tracking for future adaptations of the VR task.

Wearables for physiological activity: The laboratory is equipped with the wireless BioNomadix Smart Center (BIOPAC® Systems, Inc.) which can record heart rate and skin conductance while the participant undergoes the ambulatory VR task. Data can be streamed in real-time to the computer that is running the Motion Capture system for synchronization of multiple data streams. The BIOPAC system is also capable of recording respiration and eye-blink activity if the decision is made in the future to incorporate those data into the VR paradigm.

Study Title: Intracranial Recording and Stimulation of the Hippocampal-Amygdala Circuit During Fear Conditioning

Conditions or Focus of Study

Intracranial electrophysiology

Intracranial electrical stimulation

Virtual reality

Fear conditioning

Fear renewal

Anxiety

Amygdala

Hippocampus

Humans

Eligibility Criteria

Inclusion Criteria:

- Male

- Female

- Between 18 and 70 years of age

- Adequate visual and auditory acuity to allow neuropsychological testing

- Have undergone surgical placement of NeuroPace RNS System for the treatment of epilepsy or post-traumatic stress disorder

Exclusion Criteria:

- History of traumatic brain injury

Protocol Synopsis

The purpose of this study is to understand the neural mechanisms that underlie fear and anxiety in humans using intracranial neurophysiological recordings and electrical stimulation during laboratory virtual reality-based tasks. We will investigate the physiological (heart rate variability, skin conductance response) and neurophysiological changes that occur during both conditioned and unconditioned fear responses as well as the consequent effects of intracranial electrical stimulation of the relevant neural circuitry.

Outcome Measures

Physiological Change
During trials with and without intracranial electrical stimulation
Physiology (heart rate variability and skin conductance response) will be measured using virtual reality tasks where participants will encounter fear-associated stimuli (e.g., light color change) and fear-inducing stimuli (e.g., spider jump scare).

Neurophysiological activity change

During trials with and without intracranial electrical stimulation
Changes in theta, gamma, and theta-gamma coupling will be measured both in relation to changes in physiology on trials with and without intracranial electrical stimulation as well as in relation to changes in the contextual virtual environment.

INCLUSION OF INDIVIDUALS ACROSS THE LIFESPAN

Both children under 18 and adults over 70 will be unlikely to have intracranial surgery for the treatment of epilepsy or PTSD, and it is unlikely that they will be in the subject pool. Nevertheless, these participants would be excluded from the proposed study because they would introduce considerable heterogeneity in the study results related to age. Children are undergoing brain maturational changes and older adults are more likely to have neurodegenerative changes in the hippocampus and amygdala which would introduce confounding variables for the analysis and interpretation of the electrophysiological data from these age groups. The investigative team has extensive experience in clinical and research work with individuals of the ages included. Dr. Suthana (primary mentor) has conducted numerous research studies, including clinical trials, utilizing deep brain recordings via intracranially implanted depth electrodes in research participants of all ages. Dr. Jang has extensive experience in research with young and older adults within the age range that will be recruited for the proposed research. All studies will be performed in close collaboration with the clinical team including collaborators Dr. Jean-Philippe Langevin (neurosurgeon) and Dr. Ralph Koek (psychiatrist) to ensure a safe experimental environment for all research participants.

INCLUSION OF WOMEN AND MINORITIES

Approximately 12 participants with epilepsy and 2 participants with PTSD with the chronically implanted RNS system will be recruited annually, for a total of 70 participants during the total project period of 5 years. We will not include pregnant women, individuals who are hospitalized due to severe mental illness, incarcerated individuals, individuals with primary psychotic disorders, or anyone who do not have the capacity to adequately provide voluntary informed consent. For participants with epilepsy, we expect a roughly equal number of individuals assigned female versus male at birth, with no exclusion based on ethnicity or race. The estimated enrollment by race/ethnicity and sex/gender is summarized in the Inclusion Enrollment Report. It is based on the percentages of prior and ongoing enrollment of neurosurgical epilepsy patients at UCLA. UCLA serves large urban populations that are racially and culturally diverse, and the full array of racial/ethnic minority populations within the referral base includes Hispanics, Blacks, Asians, Pacific Islanders, and Native Americans. Between the years 2011-2021, the estimated distributions of the UCLA epilepsy neurosurgical study population have been as follows: Caucasian 73%, Hispanic 21%, Asian and Pacific Islander 3.5%, and Black 2%. For participants with PTSD recruited through a separate NIH UH3 clinical trial (PI: Langevin), only individuals of the male sex are eligible for the study with no exclusion based on ethnicity or race, per request from the NIH.

RECRUITMENT AND RETENTION PLAN

Recruitment

Participants with epilepsy: All participants 18 years or older who have undergone implantation of the NeuroPace Responsive Neurostimulation (RNS) device for the treatment of epilepsy with at least one electrode implanted in the hippocampus or amygdala will be invited to participate in this study. UCLA is a worldwide leading center for clinical and research work with RNS patients, and the Suthana lab maintains a database of approximately 250 RNS patients who have expressed interest in participating in research experiments and/or have already participated in the lab's previous studies. Given the Suthana lab's track record, we do not expect any issues with recruiting enough RNS participants for the proposed experiments.

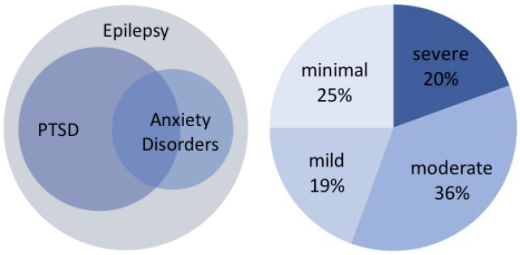


Figure 1. Comorbidity of anxiety disorders and severity of related symptoms in the VAGLAHS epilepsy veteran population in 2020. Left: number of individuals with epilepsy (N=66) and those with a co-morbid diagnosis of PTSD (N=29), other anxiety disorders (N=16), or both (N=8). Note the high prevalence of individuals with a PTSD diagnosis (44%) as well as those with any anxiety-related disorder (56%). Right: percentage of patients with minimal, mild, moderate, and severe symptoms of anxiety in a portion of the epilepsy patients (N=36) who were assessed for symptom severity.

The epilepsy veteran population at the Veterans Affairs Greater Los Angeles Health System (VAGLAHS) will serve as the primary recruitment site for participants with co-morbid anxiety-related disorders including generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD). While the prevalence of anxiety-related disorders in epilepsy patients is around 25-30%[5,6], in the veteran population it is a bit higher, at about 36%[7]. In an internal analysis of the epilepsy population who received care through the VAGLAHS in the year 2020, our group found a high rate of comorbidity (**Figure 1**, left) where patients with a current diagnosis of PTSD or any anxiety disorder comprised of 56% of the total number of patients (37 out of 66). In a portion of the patients who were assessed for anxiety symptom severity using standardized questionnaires such as the Beck Anxiety Inventory[8] or GAD-7[9], we found that there was a balanced distribution across the spectrum of symptom severities from minimal to severe (**Figure 1**, right). Therefore, we anticipate recruiting epilepsy patients with a diverse range of anxiety-related symptom severities in the proposed studies.

Participants with post-traumatic stress disorder (PTSD): We will also explore data from our ongoing parallel NIH UH3 Clinical Trial (PI: Langevin) that includes six PTSD participants implanted with an RNS system in the bilateral basolateral amygdala and ventral (anterior) hippocampus. PTSD participants will be enrolled in the currently proposed study once they have completed their 1-year clinical trial period.

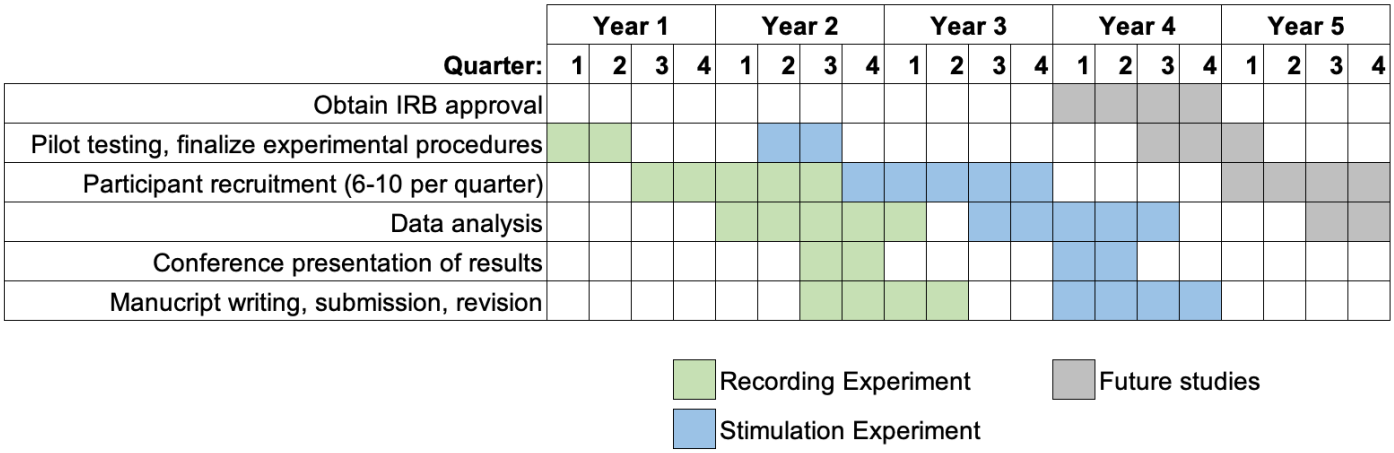
All participants volunteering for the study will be given their own copy of the IRB-approved informed consent document and a Bill of Study Subject Rights. These documents comprehensively outline the experimental tasks, electrode recording procedures, study duration, potential risks, discomforts, and benefits. Dr. Jang and Dr. Suthana will be available to address any questions the participants might have. Informed consent will be formalized through the participant's signature. Duplicate copies will be retained for record-keeping in the research records of both Dr. Jang and Dr. Suthana's offices.

Retention

Our team will maintain regular telephone communication with participants both before and after the protocol to maintain volunteer interest. We have consistently observed a high level of cooperation from participants, even with multiple study visits. Study personnel will remain on call at all times to address any urgent questions or medical concerns participants may have. Dr. Jang and Dr. Suthana will be readily available to address study-related inquiries and offer counseling and general information. To ensure maximum participant retention, clear objectives will be communicated to each participant before enrollment, ensuring they understand the study's commitments before consenting. Participants will also be informed about the importance of minimizing dropout rates in research studies, thereby encouraging enrollment only if they are confident in completing the full study. Recognizing potential transportation and financial limitations, participants will receive $30 per hour during research studies along with transportation expenses. For out-of-town participants, airfare and hotel accommodations will be provided. To maintain participant engagement, open communication channels will be maintained throughout the week of their visit, with readily available contact information for the study team. This approach has proven successful in previous studies with similar time commitments with nearly 100% retention rates and with over 30 RNS participants successfully completing similar experiments to date.

STUDY TIMELINE

For an overview of the proposed study timeline please see the figure below. To acquire sufficient data for both the Recording and Stimulation experiments during the first 3 years, we anticipate enrolling between 10-14 epilepsy participants and 1-2 PTSD participants per year across the two clinical sites (UCLA and VAGLAHS). This will allow each study to recruit at least 20 participants which should be more than sufficient to address the specific aims. At the end of each year, study recruitment methods will be evaluated and modified if needed to meet our recruitment goals. Based on historical recruitment data from the Suthana Lab involving both returning RNS participants and individuals newly implanted with the RNS device for the treatment of epilepsy or PTSD, we do not expect any issues with recruiting these participant numbers locally. As data collection proceeds during years 1-3, we will develop and apply behavioral and neurophysiological analyses on an ongoing basis. Group statistical analyses will be performed at the conclusion of each experiment for manuscript preparation and submission. Years 4-5 of this award will provide time to prepare and conduct follow-up studies based on the findings from the previous years. Concurrently, time will be dedicated to preparing an R-level independence award and a move to my independent laboratory space (either through UCLA or an alternative institution).



PROTECTION OF HUMAN SUBJECTS

1. Risks to Human Subjects
   a. Human Subjects Involvement, Characteristics, and Design
      i. **Study Design**: Participants will perform a virtual reality (VR) task involving ambulation in a virtual environment (e.g., library, grocery store, museum) while intracranial electrophysiological and biometric data are recorded. For a follow-up study (Stimulation Experiment), intracranial recordings will be halted, and participants will receive targeted electrical stimulation of the right amygdala during specific trial periods to test for enhancement of fear extinction learning.
      ii. **Subject Population**: Participants between 18 and 70 years of age who underwent implantation of the NeuroPace responsive neurostimulator (RNS) to the hippocampus and/or amygdala for the treatment of epilepsy or PTSD will be recruited (see Eligibility Criteria). Importantly, our studies will only be performed voluntarily for individuals who already received the RNS implantation for clinical care. There is no instance in which the research will play any role in the clinical decision-making process. In particular, the placement of electrodes will strictly be based on clinical criteria.
   b. Study Procedures, Materials, and Potential Risks
      i. **Study Procedures**: All participants will have already completed a brain MRI, head CT, and neuropsychological evaluation as a part of their standard clinical evaluation. Participants can provide their consent to share these medical records including any other major health problems such as conditions limiting mobility. The discussion regarding the research studies occurs at least 3 months after RNS surgical placement and is conducted by Dr. Jang who has no other involvement with clinical cases and no relationship of authority or unequal power with the participant. Participants will be advised that the decision to participate in the research is entirely voluntary and will not affect their medical care in any way. For participants traveling long distances, the in-person discussion may take place over the phone.

      If a participant agrees to join the study, they will follow the informed consent protocol as it is approved by the UCLA Institutional Review Board (IRB) committee. The RNS system has been approved for use since 2013 by the FDA and recently by the IRB committee at UCLA. Importantly, the stimulation configuration for the RNS device will be turned off during testing sessions. A staff member with seizure first-aid training will be present at all times during experimental sessions. No participant has experienced a seizure thus far during testing in more than 30 participants who completed similar studies in the Suthana Lab. Testing will stop at the participant's request or when any of the staff members including Dr. Jang and Dr. Suthana deem it necessary for the comfort or safety of the participant. There will not be a conflict of interest between the medical needs of the patient and the research procedures.

      During the virtual reality task, participants wear a head-mounted display (i.e., Meta Quest Pro). The VR headset is also equipped with small reflective markers that are detected by the motion capture cameras installed in the laboratory for wireless 3-D motion tracking. The BioNomadix Smart Center (Biopac) will record heart rate and skin conductance. Data can be streamed in real-time to the computer that is running the behavioral task for synchronization of multiple data streams.

      ii. **Materials**: Electrophysiological data are recorded digitally for storage and analysis. Clinical data is extracted from patient records with signed consent from participants. Participants are given number codes for identification to maintain confidentiality, and thus data is anonymized to remove any personally identifiable information. The identifiers are stored on encrypted drives secured with UCLA standards and available only to Dr. Jang, Dr. Suthana, and Staff Research Associate Sonja Hiller. Local backup is also performed on encrypted hard drives. Computers are protected from cyber-attacks thanks to the UCLA firewall.

      Anonymized participant data may be used for teaching purposes or publication in journals, but no identification of patients is made on any of these materials. Data is stored on the secured UCLA Hoffman cluster server, computer hard disks, and external hard drives, which are secured in the Suthana laboratory which is dedicated to this work alone. Only researchers involved in the study have keys to the laboratory and thus access to the data. They do not share this data with any outside researchers. If applicable, we will ask the participant for permission to use photographs or

videos that will be used only for scientific communication (e.g., presentation of results at conferences or as supplementary material for publications). HIPAA regulations will be carefully followed in all cases.

For RNS System data, after it is collected for study participants it is also uploaded to the NeuroPace Patient Data Management System (PDMS). Afterward, the data will be converted to a non-proprietary anonymized format and copied directly from the commercial RNS Programmer or received from NeuroPace through a secure method. From UCLA, RNS System data may be downloaded by authorized individuals. UCLA will provide the following information to NeuroPace: (1) authorization for NeuroPace to manage and transfer RNS System data as described here, (2) the identities of authorized users, including NeuroPace-approved individuals as authorized users, and (3) documentation of informed patient consent to share their RNS System data with authorized users and NeuroPace for research purposes.

iii. **Potential Risks**: The RNS is an adjunctive therapy in reducing the frequency of seizures in individuals 18 years of age or older with partial onset seizures who have undergone diagnostic testing that localized no more than 2 epileptogenic foci, are refractory to two or more antiepileptic medications, and currently have frequent and disabling seizures. The VR study is completed in 30 minutes up to 1.5 hours. During this time seizure detection and responsive stimulation are withheld with the participant's informed consent. It is important to note that this does not deviate significantly from baseline RNS activity – since the RNS device has a prescribed daily limit on the frequency of therapy (i.e., stimulation), many participants already have periods during a typical day when therapy is withheld for an extended period. However, to minimize risks, a staff member with seizure first aid training will be present at all times and the patient's behavior and real-time iEEG will be observed during the experiment. Therefore, the risk of a seizure will be obviated by continuous EEG monitoring for early signs of seizure activity and immediate cessation of the study if needed. Dr. Jang will be alerted if there are any signs of a seizure or psychological distress, at which point the session is discontinued.

Regarding risks related to intracranial electrical stimulation, the patients recruited in this clinical study have already undergone the placement of intracranial electrodes to undergo electrical stimulation for the treatment of epilepsy or PTSD. The risk of an electrical stimulation leading to seizure activity has been assessed by measuring the threshold stimulation parameters that cause synchronous discharges that can be recorded through EEG, also known as the after-discharge threshold. Stimulation in our protocol will be similar to that of prior studies where it was current-regulated, charge-balanced, with biphasic rectangular pulses set below the after-discharge threshold which typically ranges between 1.0 mA–2.0 mA[10]. For each pulse, total stimulation will range between 2.5-10.1 $\mu C/cm^2$ per phase, which is well below the safe maximum used for chronic (30 $\mu C/cm^2$ per phase) and acute (57 $\mu C/cm^2$ per phase) stimulation[11,12]. The absence of any after-discharge threshold at a particular site will be carefully established before each session and stimulation will always be set below the threshold. Previous human studies, using parameters of up to 3.0V, 450μs pulse width, and 130Hz, have shown to be safe and well tolerated in patients with epilepsy with depth electrodes in the temporal lobe[12]. Similar stimulation levels are used in our patients and clinical studies for seizure control[13]. As mentioned above, there will be continuous monitoring of the current charge and after discharges throughout the stimulation session to monitor for any possible seizure activity which will lead to an immediate reduction of the current if needed.

We will also recruit individuals with PTSD from our ongoing UH3 clinical trial, which includes six participants who have RNS electrodes implanted within the bilateral amygdala and hippocampus for the treatment of PTSD. These participants do not have a co-morbid diagnosis of epilepsy. We have now completed 6 years of continuous stimulation in one patient and 2 years in a second patient with PTSD both of whom showed no serious adverse side effects. Further, monthly surveillance EEG showed no seizures or epileptiform activity in these two patients. We have found that transient nausea may be related to the stimulation, however, tends to improve quickly. To mitigate the risks of neurostimulation in patients with PTSD, the stimulation sessions will be performed with continuous scalp EEG monitoring. Psychological side-effects related to neurostimulation can occur after extensive modulation of the network. Acute short-term stimulation is not expected to be associated with significant emotional deterioration. In addition, PTSD patients will have completed the 1-year UH3 RNS clinical trial where during the trial they will have been admitted to the epilepsy

monitoring unit to be observed during neurostimulation sessions. In case a psychological deterioration occurs, the study psychiatrist and team would evaluate the patient to treat the condition at the time during the clinical trial before considering eligibility for the proposed study. PI Langevin will determine the eligibility of the PTSD patient for the proposed study based on these outcomes assessed during the clinical trial.

Regarding potential risks associated with the VR fear-conditioning experiment, participants may find the VR experiments aversive, challenging, boring, or frustrating. Participants have the option to stop should they feel uncomfortable for any reason. There is a small risk of dizziness or nausea associated with wearing a head-mounted display that is mitigated when the participant can move their head freely due to associated vestibular input. Of our participants thus far, no one complained of disorientation during ambulatory VR tasks likely because head movements are allowed during the tasks. Risks associated with photosensitivity in epilepsy, which ranges between 2-14% prevalence[14], will be reduced to negligible by ensuring that characteristics (flash rate, color, intensity, light-dark patterns) of visual stimuli are not in the range to provoke seizures in this population[15].

2. Adequacy of Protection Against Risks

   a. Informed Consent and Assent

   The proposed studies are experimental in nature; none of the interventions involve clinical treatment of participants. However, they will strictly adhere to HIPAA regulations at every stage. Before commencing the study, all participants will provide informed consent following the UCLA IRB-approved protocol (UCLA Human Subjects Protection Committee, IRB#21-000697). To ensure compliance, all study personnel will have completed the HIPAA Privacy Rule Research Education Course and will undergo annual renewal. Additionally, study personnel will have completed Collaborative Institutional Training Initiative (CITI) courses, including those related to biomedical, social, and behavioral research, with renewal every 3 years. Initial phone screening will involve an oral screening script, and eligible participants will be invited to UCLA. Written consent will be obtained before proceeding with study procedures. Informed consent meetings will be conducted in a private research office space with Dr. Jang, where all legal and ethical safeguards for participants will be implemented following standards set by the UCLA Human Subject Protection Committee. During the informed consent process, participants will be presented with detailed information about the potential risks and benefits of the study, procedures involved (including the fear conditioning task, electrode recordings, and stimulation), study duration, location, confidentiality, and their right to withdraw. Dr. Jang will review the UCLA IRB-approved informed consent document in detail, explaining the purpose of the study, potential risks and benefits, and participant rights, and addressing any questions. Participants provide informed consent by signing the consent form; one copy will be securely stored in a locked drawer in Dr. Jang’s office, while the other copy will be provided to the participants for their records. Additionally, participants will receive a copy of the California Subject’s Bill of Rights before giving consent, ensuring transparency and understanding throughout the consent process.

   b. Protections Against Risk

   Neurological, psychological, and psychiatric clinical data are securely stored in files within Dr. Jang's office, accessible only to investigators directly involved in the project within the clinical research program, and in compliance with HIPAA regulations. All investigators have been briefed on the confidential nature of this data and the imperative of maintaining strict confidentiality. In publications, precautions have been taken to remove any personal identifying information. No files have been disposed of as our program emphasizes long-term follow-up. Upon termination of the research project, these files will be transferred to UCLA hospital records for selection of relevant material for medical records, with the remainder disposed of. Referring physicians are informed of all procedures used in our protocol as per program policy. The principal investigator (Dr. Jang) retains the authority to withdraw a subject from the research if circumstances necessitate, such as posing a risk to the participant's safety or health. In the rare event of profound psychological distress or suicidal ideation, Dr. Jang, trained as a psychiatrist, will intervene to de-escalate the situation and ensure the participant receives appropriate emergency medical care. Participants will receive medical treatment at no cost for any injuries resulting directly from the research procedures, adhering to UCLA policy. The VR fear conditioning paradigm typically lasts between 30 minutes to 1.5 hours. For RNS patients, seizure

detection and responsive stimulation are temporarily withheld during this period (see Potential Risks above). Discomfort associated with the research protocol is generally minimal compared to the discomfort of clinically mandated RNS electrode implantation.

c. Vulnerable Subjects, if relevant to your study

The proposed study will recruit and consent participants with possible co-morbid psychiatric disorders and therefore will evaluate each subject's ability to participate in the consent process. The procedure includes the use of the UCLA Decision-making Capacity Assessment Tool, which includes a series of questions that are asked to determine whether the participant understands the research procedures, risks, alternatives to not participating, and other related topics. If it is determined that a participant does not have the capacity to provide consent, surrogate consent may be obtained from one who is a legally authorized representative with reasonable knowledge of the research participant.

3. Potential Benefits of the Proposed Research to Research Participants and Others

There are potential benefits of the research protocol if clinically relevant data is obtained. For example, recordings of a seizure may aid in understanding the neurophysiological changes occurring during epilepsy and seizures. The risks of the research protocols, as described above, are minimal. For the research studies, there are no benefits to the patients, except for incidental findings relating to cognitive abilities that may be useful in their clinical treatment. To achieve an understanding of the neural mechanisms of behaviors that are uniquely human, it is ultimately necessary to carry out observations directly in the human brain. Patients with epilepsy provide important opportunities to carry out studies that have been validated in animals. The modern development of safe, effective pre-operative techniques for evaluating and treating a patient has made it possible to safely carry out intracranial neurophysiology studies that will increase our knowledge of human brain mechanisms and ultimately lead to novel therapeutics.

4. Importance of the Knowledge to be Gained

Understanding human brain function is not only valuable for the pursuit of knowledge but also holds immense potential to unveil new treatment pathways for psychiatric conditions like PTSD and GAD. Given the tremendous impact of anxiety and fear-related states on daily functioning and well-being, the knowledge potentially gained by this project about the neural mechanisms of fear- and anxiety-related states and the potential for treatment of anxiety disorders are extremely important. The large number of people affected by anxiety disorders combined with the potential to benefit their symptoms provides a reasonable justification for exposure to the minimal potential risks.

DATA AND SAFETY MONITORING PLAN

Monitoring Procedures

As detailed in the Protection of Human Subjects, the proposed study carries only minimal risk since it recruits participants who already have the RNS system implanted for the treatment of epilepsy or post-traumatic stress disorder (PTSD). Research considerations never influence the clinical decision-making process. In particular, the placement of electrodes is based solely on the data obtained from previous scalp EEG recordings, imaging, and other clinical tests undergone by the patient. Dr. Jang will closely monitor all study procedures carefully on a per-participant and per-day basis to make sure everything is carried out according to the IRB-approved protocol. Specifically, Dr. Jang will ensure that informed consent is obtained prior to beginning any research procedures and that all participants meet eligibility criteria. Data will be immediately accessible to Dr. Jang for review. Dr. Jang will review any adverse events in real-time as well with Dr. Suthana every quarter. Serious adverse events will be reviewed by Dr. Jang and Dr. Suthana immediately. Dr. Jang will ensure that any adverse effects as well as any protocol deviations are reported to the NIH, FDA, and UCLA IRB in a timely fashion and according to regulatory requirements.

Adverse Events

For this study, the following adverse event definitions are used:

Adverse event: Any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure.

Serious Adverse Event: Any adverse event that results in any of the following outcomes:

- Death
- Life-threatening event
- Event requiring inpatient hospitalization
- Persistent or significant disability/incapacity

ClinicalTrials.gov

The proposed study will be registered with clinicaltrials.gov.

Data Management

The data generated by this proposal will be available to the academic community after initial publication upon request to the PI. We recognize that we are building a unique behavioral and electrophysiological dataset that can be used to probe many more questions than we specifically seek to address. We anticipate a substantial number of data-sharing requests. Below, we outline steps that will be taken to ensure proper HIPAA-compliant data handling and sharing.

Training: Before handling or collecting data, all researchers will be certified by the David Geffen School of Medicine at UCLA with regards to Protecting Human Research Subjects in both Biomedical and Genetics Research, and Social and Behavioral Research with re-certification completed every year. All researchers will also complete the HIPAA Privacy Rule Research Education Course.

Data types, collection, and sharing: Demographic data, medical history, imaging data, neuropsychological data, behavioral task data, and contact information will be collected in addition to the neuroimaging data (iEEG, MRI). File types (format) include neuroimaging (.nii), electrophysiological (.cnt, .edf, .set), neuropsychological (.xls), demographic (.xls), and statistical output files (.mat). Participant contact and personal information will not be shared with researchers or any collaborators; each participant will be coded and only Dr. Jang, Dr. Suthana, and the Staff Research Associate (Sonja Hiller) will have access to the code. Before sharing data amongst researchers and the larger scientific community, Dr. Jang will ensure that data is de-identified in compliance with HIPAA; some information may be recoded or removed to ensure confidentiality as necessary. No private records or identifier information will be made available or transferred. Participant identities are never disclosed by publication or any other means. Informed consent forms are kept in locked files for at least 7 years beyond subject participation as legally required. Information in the computerized database is protected by a firewall and special username/password accounts are given only to authorized personnel. Designated employees maintain the security of computerized databases. Data will be shared between lab members using a secured server with encryption and performed by authorized users using password-protected accounts. We will provide

open access to all tools developed and anonymized data using several data-sharing avenues, including the NIMH Data Archive (NDA), DABI (Data Archive BRAIN Initiative), Blackfynn, and Neurodata Without Borders (NWB). Any hardware or software developed for the platform will be shared via publications and GitHub repositories. A shared goal of our group is to make it possible for as many researchers to begin studies of a similar nature within their laboratories.

Data Standards and Storage: Data analysis will be completed using a centralized neurophysiological and imaging analysis and storage infrastructure (UCLA Hoffman) under the UCLA Institute for Digital Research and Education (IDRE) High-Performance Computing (HPC) and Storage, Operations & Support, which will provide computational power to process large data. Consultation will be provided through IDRE Staff in the following areas: multiprocessor/multicore/GPU programming, efficient algorithms for scientific computing, code optimization for using HPC resources, scaling and analysis of parallel code, parallelization or porting on different platforms, debugging, scientific visualization with large datasets, and grid/cloud computing.

Data Archiving: After publication or the funding period has ended, the UCLA Hoffman data will be compressed and archived using two enterprise-class Synology Diskstation 1815+ network-attached storage devices with dual 1515+ expansion units creating a combined total storage of 200TB. All Diskstation and expansion units are fault tolerant and have enterprise-class uninterruptible power supplies to condition power, protect against surges, and ensure battery backup protection against power problems.

STATISTICAL DESIGN AND POWER

Data Analysis Strategy

Task-induced physiology: To assess the effect of task events on physiology (e.g., skin conductance, heart rate), both within and across participant analyses will be performed. Within-subject analyses will be performed on the mean physiological response across trial types (e.g., conditioned stimulus CS+ versus CS- trials, acquisition versus extinction trials). Detection of task-event-induced physiological changes observed across subjects will be assessed using a linear mixed-effect model. Here, each participant's physiological variable of interest will be reported as a 'response variable' and trial type (CS+, CS-) as 'predictor variables' to predict physiological changes in response to task events. All predictor variables will be specified as fixed effects. Participant number will be used as a random effect variable to control for variation in the magnitude of task event-evoked changes across participants.

Task-induced iEEG power: To assess the effect of task events on intracranial signals, we will calculate changes within specific frequency bands (e.g. theta, gamma) surrounding the presentation of various stimuli during task performance (e.g., conditioned versus unconditioned stimulus). Frequency power will be calculated using the "better oscillation detection" (BOSC) method where power is detected via continuous wavelet transform using a Morlet wavelet[16]. Unlike other time-frequency analyses, the BOSC method calculates a power threshold for each frequency defined as the 95th percentile of the theoretical x2 distribution of wavelet power values centered at a mean derived from an estimate of the local background spectrum using linear regression. By only detecting segments of activity that meet rigorous criteria, BOSC rejects transient, non-oscillatory events that would otherwise be misinterpreted as rhythmic activity. For each event of interest, power will be calculated over an iEEG trace that extends from 2 seconds before stimulus/physiology onset to 1 second after the event ends. Event-related spectral changes will be detected via z-scoring event activity to pre-stimulus baseline activity, defined as 500-2000ms before the CS onset. Comparison of normalized spectral changes between various events will provide insight into the neurophysiological signatures underlying fear memory processing. The significance of within-subject event-related oscillatory power differences will be assessed via non-paired t-tests on mean total frequency band power (e.g., theta, gamma) across various task events. Detection of task-event-induced spectral changes observed across subjects will be assessed using a linear mixed-effect model analogous to the one describe above (see Task-induced physiology). Models will be implemented using each participant's total baseline normalized power in a designated frequency (e.g., theta: 4-8 Hz) within a task-relevant time window as a 'response variable' and event type (e.g., CS+, CS-) as 'predictor variables' to predict oscillatory band power variability in response to task events. Using this strategy, we can examine the effect of event type on oscillatory dynamics across participants despite individual differences.

Physiological data preprocessing: Skin conductance activity, measured in units of μS, will be log-transformed to correct for known skew in distributions[17], then z-transformed using the pre-stimulus baseline activity, defined as 500-2000ms before the CS onset. Using a previously described convex optimization approach[18], skin conductance activity will be decomposed into two components, tonic and phasic, which have different time scales and relationships to fearful stimuli. Although the phasic component is theorized the represent the sudomotor nerve activity corresponding to acute increases in sympathetic tone, both phasic and tonic activity will be evaluated in their relationship to fearful stimuli. Heart rate will be computed by counting the number of beats within a 4s sliding window moving at 4ms (sampling frequency). Beats will be detected from the photoplethysmography (PPG) signal using the Multi-Scale Peak & Trough Detection (MDPTD) algorithm [19,20].

iEEG-physiology correlation analysis: To examine the relationship between iEEG dynamics and physiological parameters such as heart rate, heart rate variability, and skin conductance response, a linear mixed model framework will be used. Here, time-varying iEEG data is modeled as a function of the above-mentioned variables and time delay. The design matrix, which links these variables with the iEEG data, consists of the following basis functions: cubic B-splines, sines and cosines, and polynomials. Model selection will be done using Bayes Information criteria to avoid overfitting. Additionally, to determine variables with larger modulatory effects, we will use the analysis of deviance coupled with the chi-squared test of significance. Furthermore, we will generate surrogate data by circularly shifting the iEEG data with respect to the physiological variables (N = 2000) to calculate a z-score value for the correlation coefficients for a given electrode in each participant. We will use binning methods to compute the theta or gamma power across behavioral tasks. Recording channel numbers will be used as random effect variables ('blocking variables'), to control for the variation coming from different channels and to account for different numbers of recording

channels between participants. All continuous predictor variables (physiological data) will be standardized (z-scored) before model fitting to enable a direct comparison of the impact of each variable on oscillatory band power against each other. Each of the predictor variables' impact on the response variable will be reflected by the variable's beta weight (standardized effect size) and corresponding t-statistic. A similar strategy will be used to examine the relationship between neural and physiological variables with movement variables such as velocity, acceleration, and position.

Inter-regional phase synchrony: The Phase Locking Value (PLV) is a statistic that can be used to investigate task-induced changes in long-range synchrony of neural activity[21,22]. This may be used as a proxy of the relative amount of communication within a network during different periods. The PLV assesses the degree phase (Φ) covariance between two channels (e.g., amygdala and hippocampus) averaged across N trials using the following formula:

$$PLV(t,f) = \frac{1}{N}\left|\sum_{n=1}^{N} \mathrm{e}^{i(\Phi_{n,a}(t,f)-\Phi_{n,b}(t,f))}\right|$$

Where n = trial number, a and b designate electrodes (e.g., hippocampus vs amygdala), f = frequency, t = time point. PLV will be calculated for each electrode pair allowing us to assess the variability of two structures' phase relationship across repeated exposure to different experimental variables. PLVs that approach 1 indicate strong phase synchrony and little variability across trials. To test the statistical significance of PLV, a null distribution will be created by randomly shuffling the signal from each electrode pair and computing the corresponding PLV spectrograms 1000 times. PLVs that exceed the 95th percentile of the surrogate distribution will be deemed significant.

Intra- and Inter-regional Phase-Amplitude Coupling: To compute cross-frequency phase-amplitude coupling, the Modulation Index (MI) is calculated between the phase component of a low-frequency band (e.g., theta; Φ_θ), and the amplitude component of a high-frequency band (e.g., gamma; A_γ) within each time point. To measure the strength of coupling between the theta phase (Φ_θ) and gamma amplitude (A_γ), those two signals are combined into an analytic complex-valued signal via Euler's formula, and then the vector mean is computed, with the PAC quantified as the vector length and the preferred phase measured as the resultant vector's angle:

$$MI = \left|T^{-1}\sum_{t=1}^{T} A_\gamma e^{i\Phi_\theta}\right|$$

Where t = time point and T = total number of time points. For example, to determine MI for N trials of a particular condition (e.g., CS+), a trial-by-time matrix pre-filtered at the theta (4-8 Hz) range, $\{X_\theta[t]\}_{1:N}$, is truncated to time regions $[t_0{:}\,T]$ corresponding to the time window of the trial, reshaped into a 1-by-$(N * T)$ array of concatenated trials, then converted into a complex valued signal with the Hilbert transformation. The phase angle time series of this complex-valued signal results in the instantaneous phase of the low-frequency band, Φ_θ. Similarly, iEEG trials pre-filtered at the gamma range (60-120 Hz) range $\{X_\gamma[t]\}_{1:N}$ are truncated, vectorized, and converted via the Hilbert transform to complex-valued form. The complex modulus (absolute value) is used to compute the instantaneous high-frequency amplitude signal A_γ. To measure strength of coupling between the theta phase (Φ_θ) and gamma amplitude (A_γ), those two signals are combined into an analytic complex-valued signal via Euler's formula, and then the vector mean is computed, with the raw modulation index measured as the resultant vector length, while the preferred phase is measured as the resultant vector's angle.

For surrogate analyses potential autocorrelations in the signal are accounted for by generating 2000 surrogate Modulation Indices, $\{MI_{surr}\}_{1:2000}$, in which the amplitude component is shifted with respect to the phase component by a pseudo-randomly generated lag value of at least 300 msec in duration. A normalized MI index is generated by z-scoring against the normal distribution fitted to $\{MI_{surr}\}_{1:2000}$.

Neural similarity analysis: To assess whether an electrode or a set of electrodes within an anatomical region of interest form distinct neural representations of different environmental contexts, we use a form of representational similarity analysis on the spectral iEEG data[23,24]. Within a given trial, for every 500ms time window spaced every 100ms, we construct a feature vector containing oscillatory z-scored power from five frequency bands (theta, 3.–8 Hz; alpha, 8–12 Hz; beta, 13–25 Hz; low gamma, 30–58 Hz; high gamma, 62–

100 Hz) across all relevant electrodes. For each time window (i) of a given trial, we define feature vectors as follows:

$$\vec{T_i} = [z_{1,1}(i) \dots z_{1,F}(i) \dots z_{L,F}(i)]$$

Where $z_{l,f}(i)$ is the z-transformed power of electrode $l = 1 \dots L$ at frequency band $f = 1 \dots F$ in time window i. For L electrodes and F frequency bands, we thus create a feature vector at each time window that contains a total of $L * F$ features. We quantify the neural similarity between two trials by calculating the cosine similarity between these feature vectors for all possible pairs of time windows, generating a precise temporal map of neural similarity between the two trials. We compute this neural similarity matrix for every pair of trials, then compare the mean similarity matrix between trials within the same environmental context (e.g., library-library) versus trials with mismatched contexts (e.g., library-museum).

Stimulation effects analyses: The effect of intracranial electrical stimulation on physiology will use a between-subjects design. For descriptive analysis, the time series corresponding to a particular physiological variable of interest (e.g., skin conductance) will be averaged and compared between CS++ trials (with electrical stimulation) versus CS+ trials (without stimulation). Time points where there is a significant difference in the variable of interest will be determined using an independent samples t-test and controlled for multiple comparisons using the Bonferroni-corrected p-values. We will also use a linear mixed model similar to the ones described above where time-varying physiological data (e.g., skin conductance) is modeled as a function of binary dummy variables corresponding to whether a trial was CS++ or CS+.

Justification of Sample Size

Previous iEEG studies from Dr. Suthana's group involving RNS participants during memory tasks found statistically significant effects in a sample of 5 participants[25]. Intracranial stimulation studies with 6 and 13 participants yielded an effect size (Cohen's d) of 1.74 and 1.18 respectively when comparing stimulated and non-stimulated trials[10,26]. We anticipate a minimum of 10 participants will be required each for the Recording Experiment and Stimulation Experiment. Since most participants have multiple electrode contacts that fall within both the right and left amygdala, we can test several potential stimulation regions in each participant. We propose to enroll 40 total participants during the proposed project period and will also combine results with our current preliminary findings.

DISSEMINATION PLAN

Dr. Anthony Jang will be responsible, with support from his mentor Dr. Nanthia Suthana, for handling the clinical trial registration and reporting requirements for this project. This includes registration of the clinical trial on “ClinicalTrials.gov” (or an alternative publicly available platform, as discussed below). Once a record is established, Dr. Jang will confirm the accuracy of the record content, resolve problems, and maintain records including content updates and modifications. Registration of the study on the platform will occur no more than 21 days after the first participant is enrolled and required information will be updated annually. Dr. Jang will also be responsible for posting any results including adverse events no later than 12 months after the completion of the trial. UCLA has an internal policy in place that ensures that clinical trials are registered and that the results are reported in compliance with the NIH policies. In coordination with the UCLA Office of Contract and Grant Administration, Dr. Jang and Dr. Suthana will certify policy compliance with each submission of a required report. Dr. Suthana is the principal investigator of separate clinical trials funded by the National Institute of Mental Health (NIMH), the National Institute of Neurological Disorders and Stroke (NINDS), and the National Institute on Aging (NIA), and thus she is well-positioned to support and guide Dr. Jang in the safe and responsible conduct of clinical trials and to help him meet all required registration and reporting timelines. UCLA has further established two institutional accounts in the Protocol Registration and Results System (PRS) for “ClinicalTrials.gov” to support UCLA investigators who serve as the Responsible Parties on a clinical trial. Moreover, the UCLA Office of Contract and Grant Administration as well as the UCLA Clinical and Translational Science Institute (CTSI) have support staff to facilitate the process of registration and results reporting for clinical trials. Dr. Jang will work closely with them to register this trial and to submit summary results in a timely manner.

As per the Funding Opportunity Announcement (FOA PA-20-201), the proposed study falls under the category defined by the NIH as “Basic Experimental Studies with Humans (BESH)”, which meets both the NIH definition of a clinical trial and the definition of basic research. According to the NIH (notice number NOT-OD-22-205), BESH studies are not required to register and report results on ‘ClinicalTrials.gov’ but can choose to register and report results on alternative publicly available platforms. Dr. Jang and Dr. Suthana may thus use an alternative publicly available registration and reporting platform instead of ‘ClinicalTrials.gov’ and adapt the details of the dissemination plan described above to meet the requirements of this alternative platform. However, this will be in close coordination with NIH officials to always ensure compliance with the NIH’s policy requirements.

SPECIFIC AIMS

Fear responses that are excessive and disproportionate to the situation are a hallmark of anxiety disorders such as generalized anxiety disorder and post-traumatic stress disorder (PTSD). Anxiety disorders affect up to 25% of the adult population in developed countries[27] while specific conditions like PTSD can affect up to 11.5% of U.S. military veterans[28]. Despite treatment with medications and psychotherapy, about one-third of affected individuals will experience residual symptoms associated with high morbidity and healthcare costs[29]. Despite advancements in animal models of fear and anxiety, there remains a substantial gap in understanding the fear circuitry in humans and how it may go awry in anxiety disorders. The proposed research aims to address this gap by combining mobile intracranial electroencephalography (iEEG) and virtual reality (VR) experiments to study the neural correlates of fear processing in humans and assess whether targeted stimulation can be employed to enhance fear-related learning, potentially offering a novel therapeutic approach[30,31].

A key component of anxiety disorders is the impairment in fear extinction, namely, the ability to extinguish a previously formed association between benign stimuli and a fearful experience. For example, a veteran who experiences explosions in the presence of helicopters in Afghanistan may panic upon hearing a helicopter in Los Angeles[32,33]. Rodent studies highlight the hippocampus and amygdala as key brain regions in the formation and extinction of fear[34–37]. It is hypothesized that the hippocampus forms a representation of the environmental context while the amygdala ties this context to emotions[38,39]. Consistent with this, when rodents are exposed to a room associated with electric shocks, specific oscillatory patterns (e.g., 3-8 Hz theta activity) are observed in the amygdala[40–45] along with increased communication between the amygdala and hippocampus (e.g., via phase-amplitude coupling)[46,47]. However, human studies have largely relied on imaging methods without direct access to these subcortical structures (e.g., fMRI). Furthermore, traditional experiments delivered on computer screens had constraints in manipulating the contextual environment, limiting our understanding of these neural circuit mechanisms in real-world, ecologically valid settings[48–57].

In collaboration with the Veterans Affairs Greater Los Angeles Healthcare System, we will work with individuals who have already been implanted with a Responsive Neurostimulation (RNS) device for the treatment of epilepsy or PTSD. The RNS uses implanted electrodes to directly measure and modulate iEEG activity within the amygdala and hippocampus[58]. Our approach integrates mobile iEEG, peripheral physiological recordings, and a novel, ambulatory VR fear conditioning paradigm where participants acquire and subsequently extinguish associations between conditioned stimuli (CS; e.g., a change in light color) and a fearful experience (e.g., a large spider)[3,59,60]. The paradigm incorporates changes in the virtual environment to probe the interplay between the hippocampus and amygdala during contextual fear learning. Targeted stimulation of the amygdala will be used to modulate fear extinction[30,31]. Lastly, given the veteran population comprises a wide range of comorbid anxiety and PTSD symptoms, we will measure trait anxiety characteristics (e.g., generalized anxiety, fear sensitivity) and their relationship to neural and physiological activities involved in fear. The proposed research aligns with the applicant's training goals, focusing on mobile iEEG and VR experiments in psychiatry.

Specific Aim 1: Investigate hippocampal-amygdala iEEG dynamics that underlie the acquisition and extinction of contextual fear. Hypotheses 1 & 2: The fear-associated CS will lead to increased theta power in the amygdala (H1) and increased communication (via phase coherence and theta-gamma phase-amplitude coupling) between the amygdala and hippocampus (H2). Hypothesis 3: Hippocampal, but not amygdala activity, will exhibit distinct neural representations of different environmental contexts during fear conditioning.
Specific Aim 2: Test the effects of amygdala stimulation on fear extinction. Hypothesis 4: Amygdala stimulation during the CS will facilitate fear extinction as measured by reduced fear-related physiological responses (heart rate and skin conductance) when compared to a separate, non-stimulated CS.
Specific Aim 3: Characterize the physiological and hippocampal-amygdala iEEG dynamics associated with anxiety. Hypothesis 5: Trait anxiety across a battery of clinical assessments will be associated with increased skin conductance and amygdala theta power and decreased amygdala-hippocampal connectivity.

Implications: The proposed program will characterize intracranial neurophysiological signatures of fear and assess the efficacy of targeted neurostimulation in reducing fear-related behaviors in individuals with varying anxiety levels. By building on decades of findings across species, the program will uncover human-specific hippocampal-amygdala oscillatory mechanisms, shed light on the pathological states associated with anxiety, and make substantial contributions to the comprehensive understanding and treatment of anxiety across a wide spectrum.

RESEARCH STRATEGY

SIGNIFICANCE

Anxiety disorders such as generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD) are among the most prevalent mental health conditions in the world[27]. In the United States, GAD has an estimated lifetime prevalence of 5-12%[61] and is associated with significant utilization of healthcare services[29]. PTSD has a lifetime prevalence of 6-9% in adults and affects up to 11.5% of U.S. military veterans[28,62]. While anxiety disorders can respond to standard treatments with antidepressants and psychotherapy, it is estimated that one-third of affected individuals will experience residual symptoms or remain treatment-refractory[63,64], leaving them with a chronic illness associated with increased rates of occupational disability[65], substance use disorders[66], depression[65,67], and suicide[68]. It is theorized that anxiety disorders result from the development of fear responses that are excessive and disproportionate to the situation. Our limited understanding of the neural mechanisms that underlie such maladaptive fear responses is a major barrier to developing new treatments.

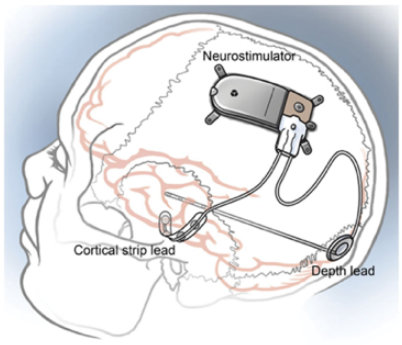


Figure 1: RNS neurostimulator[58].

Recent advancements in implanted neural devices (**Figure 1**) originally used for the treatment of epilepsy provide a unique research opportunity to study these mechanisms through direct recordings and stimulation of brain regions implicated in fear and anxiety. In particular, the ability to record from multiple circuit nodes simultaneously (e.g., the amygdala and hippocampus) allows us to investigate not only distinct brain regions but also the communication between these structures. At the same time, technological advancements allow for the simulation of real-world experiences using virtual reality (VR) and physiological measurements through wearable devices. Our proposed study will leverage these innovations and a unique collaboration with the Veterans Affairs Greater Los Angeles Healthcare System (VAGLAHS) to work with individuals who have anxiety-related disorders and are implanted with a Responsive Neurostimulation (RNS) device for the treatment of epilepsy. To enhance the generalizability of our findings, we will also include participants without epilepsy who have an implanted RNS in the amygdala and hippocampus for PTSD through a separate clinical trial in our research group. Findings will provide a first insight into intracranial neurophysiological signals that underlie functional and dysfunctional emotional responses to threat.

INNOVATION

Rare access to individuals with anxiety-related disorders who have implanted electrodes: Our collaboration with the VAGLAHS provides the ability to recruit veterans who received the RNS for epilepsy. Our preliminary assessment of the VAGLAHS epilepsy population shows a 44% comorbid diagnosis of PTSD and 56% for other anxiety-related disorders, a significantly higher anxiety disorder burden compared to the general epilepsy population[5,69]. This provides a valuable opportunity to engage with hundreds of potential participants with comorbid anxiety disorders with readily accessible intracranial electroencephalography (iEEG).

Research platform combining iEEG recording, intracranial stimulation, and wearable technologies: Our research platform can synchronize iEEG and intracranial stimulation with external wearable sensors that can measure heart rate, skin conductance, gaze direction, eye movements, head rotation, body position, and velocity while the participant ambulates freely. This platform allows for the investigation of relationships between deep brain activity, anxiety-related physiological patterns, and spatial navigation in humans who are not constrained to the hospital bed or neuroimaging suite.

VR to enhance immersion and translational efficacy: Traditional laboratory computer-based tasks are limited in their investigation of fear-related behaviors, especially in studying the impact of environmental context. VR environments presented on a head-mounted display provide a powerful method for removing the participant from the laboratory context and transporting them into an endless number of distinct environments while maintaining full control over the similarities and differences between them. Moreover, VR provides a heightened sense of presence and immersion, increasing the likelihood that our findings will be more applicable and transferable to real-world situations and lead to a more nuanced understanding of fear-related behaviors.

APPROACH

❖ General Approach

Fear conditioning as a model for anxiety disorders**:** Decades of research on fear and anxiety in both humans and nonhuman animals have predominantly used fear conditioning paradigms. In a typical fear conditioning task, a neutral conditioned stimulus (CS+) is repeatedly paired with an aversive unconditioned stimulus (US), leading to the development of fear-related behaviors in response to the CS+ such as increased skin conductance, change in heart rate, freezing, and fear-potentiated startle. Subsequently, the acquired fear response can be extinguished by repeatedly exposing the participant to the CS+ without the US. The process of acquiring and extinguishing fear responses serves as a model for understanding both the development and recovery from various anxiety- and trauma-related disorders[70]. For example, a military veteran with PTSD may exhibit fear reactions to stimuli reminiscent of combat trauma, such as the sight of camouflage clothing, the sound of helicopters, or the smell of smoke. Such PTSD symptoms may improve after repeated exposure to fear-associated stimuli through prolonged exposure therapy (i.e., fear extinction). The fear conditioning paradigm can also be expanded to include a test of fear renewal, a phenomenon seen in clinical settings where individuals may reexperience previously extinguished fear responses upon re-exposure to the CS+. For example, a veteran previously treated for PTSD may have a re-emergence of symptoms after watching the news about the war in Ukraine or the Israeli-Palestinian conflict. Our VR fear conditioning paradigm will address fear acquisition, extinction, and renewal thereby investigating the full spectrum of fear learning.

Virtual reality to study fear and anxiety**:** VR has become an effective method of enhancing the ecological validity of human neuroscience research, thanks to advancements in portable VR headset technology and the capability to simulate life-like, three-dimensional visual environments. VR fear conditioning paradigms demonstrate that virtual CS+ compared to CS- contexts can elicit fear-potentiated startle responses, increased skin conductance, and avoidance[71–78]. Importantly, unlike traditional fear conditioning studies that use aversive unconditioned stimuli (US) such as an electric shock or air blast, VR paradigms can use stimuli that are more realistic and appropriate for the virtual context, such as verbal insults from another person, various insects, or falling from an elevated position[71,76,79–81]. The proposed project will combine the immersive capabilities of VR with simultaneous recordings of physiological and intracranial brain activity. In the VR paradigm, participants acquire and subsequently extinguish associations between neutral stimuli and aversive outcomes over 2 days. Our study comprises two experiments: The first experiment (Recording Experiment; **Figure 2A**) aims to investigate the neural correlates of fear conditioning while the second experiment (Stimulation Experiment; **Figure 2B**) will investigate the impact of amygdala stimulation on fear extinction. This dual-experiment approach allows for a comprehensive exploration of both fear acquisition and extinction processes.

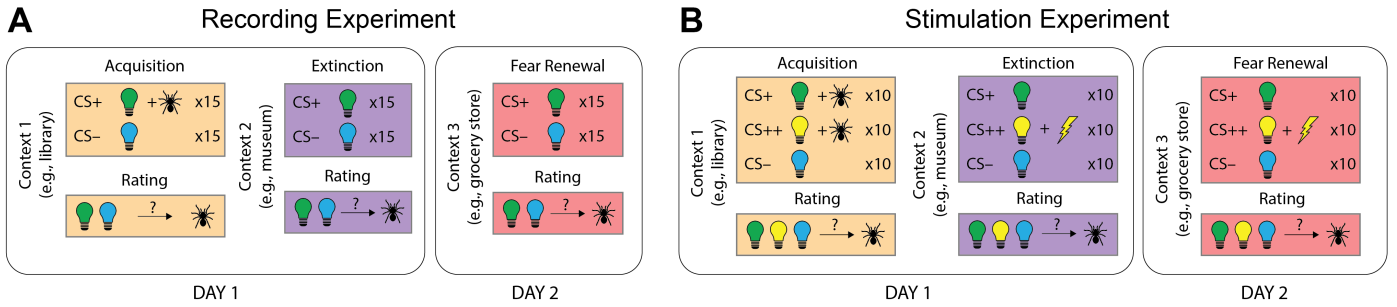


Figure 2: Schematic of the VR fear conditioning paradigm. **A:** Recording Experiment to study hippocampal-amygdala circuit activity with fear acquisition, extinction, and renewal. **B:** Stimulation Experiment to determine the effects of targeted amygdala stimulation during extinction learning. See Experimental Design under Aims 1 and 2 for further details.

Participants**:** Participants are patients with pharmaco-resistant epilepsy who are implanted with the FDA-approved NeuroPace RNS® system. The RNS detects abnormal electrical activity in the brain and responds by delivering imperceptible levels of electrical stimulation to normalize brain activity before an individual experiences a seizure. During our experimental studies, the seizure detection and stimulation system will be temporarily paused and the RNS will be used for continuous iEEG recording and/or targeted electrical stimulation with custom stimulation parameters (see Aim 2 for details). The two program sites, UCLA and VAGLAHS, already have access to a substantial cohort of over 250 participants with RNS implants in the amygdala and/or hippocampus. Additionally, approximately 10 new participants per year are expected to undergo implantation. To assist with the generalization of the data, we will also include 6 participants without

epilepsy who have an already implanted RNS System in the bilateral amygdala and hippocampus for the treatment of PTSD after completing their 1-year NIH clinical trial (UH3; PI: Langevin).

Electrophysiological and biometric data acquisition**:** Each participant has two implanted depth electrode leads, each with four electrode contacts. iEEG activity is continuously recorded at a sampling frequency of 250 Hz. Specially authorized research tools for the RNS system allow for wireless real-time control of the implanted system and synchronization with external physiological measurements via wearable sensors. To enable real-time data storage and synchronization, we will utilize a single-board computer (i.e., Raspberry Pi) which runs a server-like state machine that allows any experimental device or application to connect and control the RNS system over a secured network. The Raspberry Pi will trigger a noise signal into the iEEG data through a telemetry wand (NeuroPace Near Field Telemetry Wand) that is secured over the implant site on the participant's head, and these noise artifacts will be used to synchronize the iEEG data with other task variables (e.g., spatial location, biometrics). All necessary electronics are carried by the participant in a custom-built backpack (**Figure 3**)[3]. Heart rate and skin conductance will be recorded using the wireless wearable Smart Center system (BIOPAC® Systems, Inc.). A pulse transducer is secured to the participant's middle finger which uses wireless photoplethysmography (PPG) to measure heart rate. Two electrodes are placed on the participant's palm to record skin conductance as a measure of eccrine (skin sweating) activity.

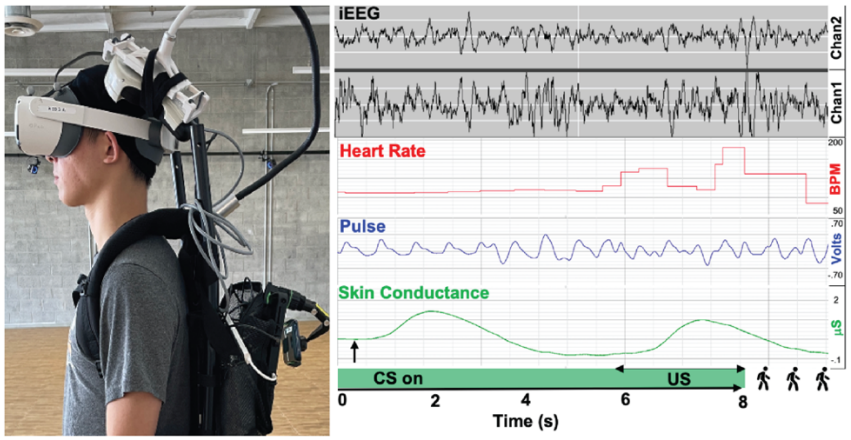


Figure 3. Mobile deep brain recording and stimulation platform. Left: example participant equipped with the VR headset, RNS telemetry wand (secured on the head), and custom backpack that carries equipment. Right: example tracings of two iEEG channels, heart rate (calculated as a sliding window average of pulses), pulse (PPG), and skin conductance.

Electrode localization**:** The precise anatomical location of each electrode contact will be determined by co-registering a high-resolution post-operative head computed tomography (CT) image to a pre-operative high-resolution structural MRI image[82]. The CT and MRI are also registered to a high-resolution T2 image where the amygdala and hippocampus can be automatically segmented based on atlases correlating MRI visible landmarks with underlying cellular histology[83]. Each MRI and CT will also be registered to an MNI standard brain using advanced normalization tools (ANTs)[82] to look for left vs. right difference patterns across the group. The estimated electrode locations of an example participant are shown in **Figure 4**.

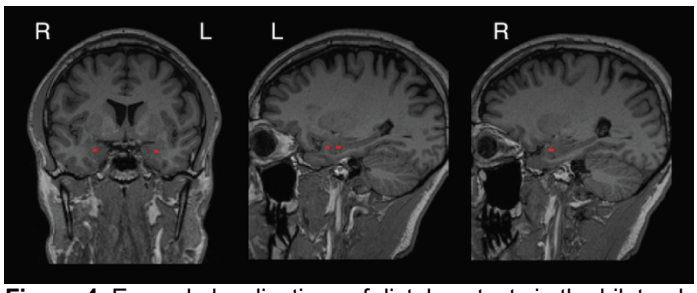


Figure 4. Example localizations of distal contacts in the bilateral basolateral amygdala.

Detection of epileptic events**:** The proposed research uses recordings in participants with epilepsy, which will limit the extent to which results can be generalized to healthy participants. Recent iEEG studies in these patients, however, provide evidence that neural activity is largely unaffected in brain regions outside the epileptic seizure onset area[84–86]. We will further use specialized algorithms to detect and exclude periods of epileptic activity in all recordings. Inter-epileptic discharges (IEDs) are abnormal electrical distortions related to epilepsy that can be observed in iEEG intermittently. IEDs are removed from all iEEG channels before the normalization of power and all additional neurophysiological analyses. For IED detection, we will use a previously described double thresholding approach in which a data point is identified as an IED when either of the following conditions is satisfied: 1) the envelope of the unfiltered signal is 6 standard deviations above the baseline, 2) the envelope of the signal band-pass filtered in the 25-80Hz range is 6 standard deviations above the baseline. Removed IED samples are replaced using cubic spline interpolation. Using this method, we anticipate that between 2–10% of samples will be removed per channel[1,2,59].

❖ **Aim 1: Investigate hippocampal-amygdala iEEG dynamics that underlie the acquisition and extinction of contextual fear**

Background: Rodent fear conditioning studies highlight the basolateral amygdala and ventral hippocampus as key brain regions in the formation, expression, extinction, and generalization of fear processes[34–37]. It is hypothesized that the amygdala forms an association between stimuli and emotions while the hippocampus encodes the broader multisensory context such as visual, spatial, and auditory cues. Thus, the interplay between these two structures leads to the formation of contextual fear memories[87–89]. Rodent electrophysiological studies, investigating network oscillations through local field potential (LFP) recordings, consistently reveal oscillatory patterns in the amygdala within the theta (3-8 Hz) and gamma (60-120 Hz) frequency ranges[40]. These oscillations are thought to represent the synchronous synaptic activity among large neuronal populations[90]. The hippocampus encodes contextual information such as spatial location independent of the emotional valence[38,39,91]. Lastly, successful contextual fear learning in rodents involves communication between the amygdala and hippocampus through mechanisms like theta-gamma phase-amplitude coupling, phase coherence, and single-unit synchrony between the two regions[43,92]. Despite these advancements in rodent models of fear, there remains a significant gap in our understanding of these oscillatory dynamics in humans during the acquisition, extinction, and renewal of fear responses and whether they covary with clinically relevant symptoms observed in psychiatric disorders like GAD or PTSD.

Experimental Design: Participants will undergo the 2-day Recording Experiment shown in **Figure 2A**. On day 1, the task consists of two phases: acquisition and extinction. During the acquisition phase (**Figure 5A**), the participant is instructed to freely navigate a virtual environment (e.g., a library) and informed that the color of the room will temporarily change to one of two colors. When this occurs, they are instructed to stop walking and remain in place until the room returns to natural lighting. One of the colors (conditioned stimulus, CS+; e.g., green lighting) is followed by an aversive, audiovisual jump scare (unconditioned stimulus, US; e.g., giant spider). The other color (CS-; e.g., blue lighting) will not lead to the US. The CS+ and CS- are encountered 15 times each. To assess whether the participant properly learned the CS-US associations, following the acquisition phase, they will be asked to rate their level of fear of each light (1-10) and state whether they believed each colored light led to an aversive outcome (yes/no) and their confidence in this belief (1-10). Afterward, participants rest for 3 minutes before entering a second environment (e.g., a museum) for the extinction phase (**Figure 5B**). During extinction, the participants receive the same instructions as acquisition but now neither of the colored lights leads to the jump scare. The CS+ and CS- are encountered 15 times each, followed by the same rating task they completed during acquisition. Day 1 of the task will take approximately 40 minutes excluding equipment setup. On day 2, participants will undergo another extinction phase that occurs in a third environment (e.g., a grocery store) to test for fear renewal, in which re-exposure to the CS+ in a new environment leads to renewal of a previously extinguished fear response. Other than this environment change, the task will be identical to the extinction phase from day 1. The color of the CS+/CS- lights and the order of the three environments will be randomized for each participant.

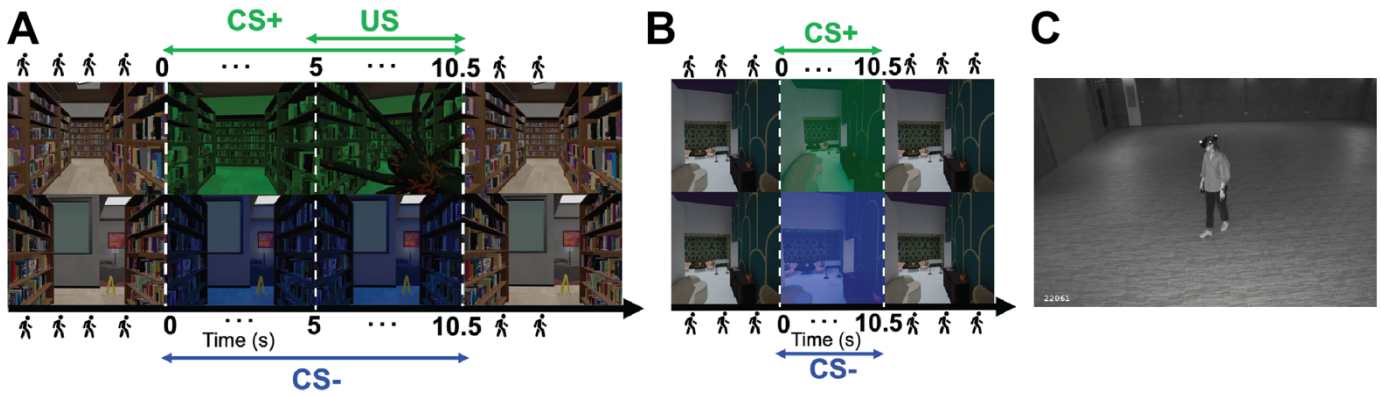


Figure 5. VR fear conditioning. **A**: Acquisition phase in the library environment. The top row shows an example CS+ trial in which a US (i.e., a jump-scare of a spider leaping on the headset) occurs 5 seconds after the onset of the green light (CS+). The bottom row shows an example CS- trial in which no US follows the onset of the blue light (CS-). **B**: Extinction phase in the museum environment. Neither the green nor the blue light is paired with the US. **C**: Snapshot of video footage of a participant during the task.

Analysis and Interpretation**:** The raw iEEG trace will undergo processing to obtain frequency-specific information. To compute the magnitude and instantaneous phase information in the frequency domain, the

iEEG signals will be convolved with 6th-order complex-valued Morlet wavelets. The result of the convolutions is complexed-value signals from which we can extract phase, amplitude, and power to calculate time-frequency spectrograms[2,21,93]. All time-frequency points will be z-score normalized to a baseline period (500-2000ms before the onset of the CS). We will calculate trial-averaged normalized power for different trial conditions (e.g., CS+ vs CS-) in each experimental session. To assess differences in spectral power between conditions across recording sessions, we will use a non-parametric permutation procedure that compares the real difference to an empirical distribution created by shuffling the trial labels and generating the shuffled mean difference 2000 times[94]. Phase-amplitude coupling (PAC) will be used to quantify the relationship between the phase component of a low-frequency (e.g., theta) band, and the amplitude component of a high-frequency (e.g., gamma) band, either within a single anatomical region or between two regions (e.g., amygdala-hippocampus). To measure the strength of coupling between the theta phase (Φ_θ) and gamma amplitude (A_γ), the two signals are combined into an analytic complex-valued signal via Euler's formula, and then the vector mean is computed. PAC is quantified as the length of the resultant vector: $PAC = \left| n^{-1} \sum_t^n A_\gamma e^{i\Phi_\theta} \right|$. To assess whether the hippocampus and/or amygdala form distinct neural representations of different environmental contexts during fear conditioning, we use a form of representational similarity analysis on the spectral iEEG data[23,24]. Within a given trial, for every 500ms time window spaced every 100ms, we construct a feature vector containing oscillatory power information from all frequency bands across all relevant electrodes (e.g., all electrodes within the hippocampus). We quantify the neural similarity between two trials by calculating the cosine similarity between these feature vectors for all possible pairs of time windows, generating a precise temporal map of neural similarity between the two trials. We compute this neural similarity matrix for every pair of trials, then compare the mean similarity matrix between trials within the same environmental context (e.g., library-library) versus trials with mismatched contexts (e.g., library-museum).

Preliminary Results: We recorded iEEG activity in six participants with implanted electrodes in the hippocampus and amygdala. On average, across all electrodes and participants, we found a reliable increase in theta power for CS+ trials compared to CS- trials in the acquisition phase during the onset of both the CS+ and US (**Figure 6A**). We also observed an increase in skin conductance and heart rate during CS+ trials compared to CS- trials (**Figure 6B, C**), consistent with the hypothesis that both the fear-associated stimulus (CS+) and the fearful experience itself (US) lead to an increase in amygdala theta power and sympathetic nervous system activity. In contrast, trials during the extinction phase (where neither green nor blue light led to the jump scare) did not show this difference between CS+ and CS-, consistent with the hypothesis that the CS-US associations are extinguished (**Figure 6D-F**).

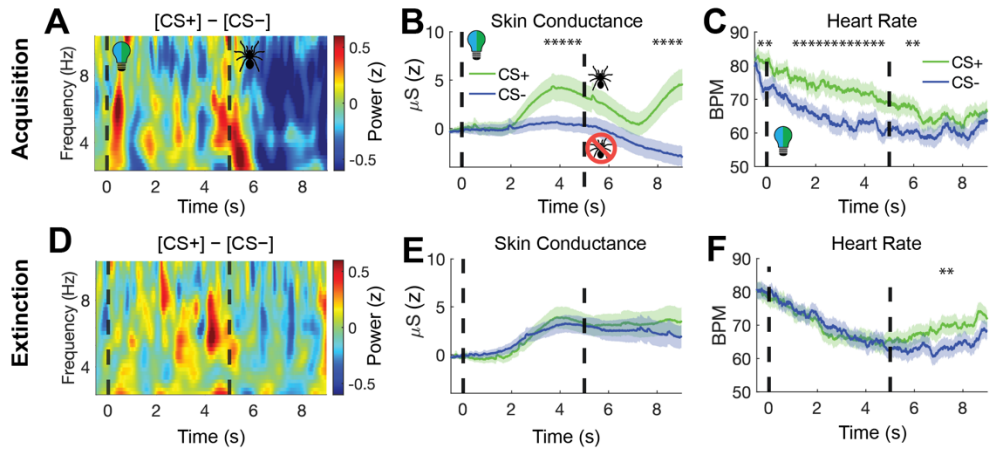


Figure 6. Mean iEEG and physiological activity during the fear conditioning task. First row (**A-C**): acquisition phase, second row (**D-F**): extinction phase. **A**: Mean difference in the time-frequency spectrogram of CS+ and CS- trials, pooled across participants (N = 6) and electrodes (4 per participant). Y-axis shows frequency in the log scale. Color represents z-scored power. **B-C**: Mean skin conductance response (**B**) and heart rate (**C**) across all CS+ (green) and CS- (blue) trials. Asterisks represent points with an uncorrected p < 0.05. **D-F**: Analogous time-frequency, skin conductance, and heart rate plots for the extinction phase. For all plots, the x-axis indicates time within a trial where vertical dotted lines denote the CS onset (first line; either green or blue light) and US onset (second line; spider).

We also hypothesize that due to the hippocampus's involvement in encoding context, hippocampal activity patterns will be discernible between different virtual environments. Using the representational similarity analysis described above, we quantified the neural similarity between every pair of trials in an example participant who completed the 2-day task spanning three different environments (library, museum, grocery store). In the hippocampus, we observed significantly greater neural similarity between trials in the same environment when compared to trials in different environments (**Figure 7A and B, left**). This difference was

not seen in the amygdala (**Figure 7B, right**), consistent with the idea that the amygdala forms associations between conditioned stimuli and fear while the hippocampus encodes the broader multisensory context.

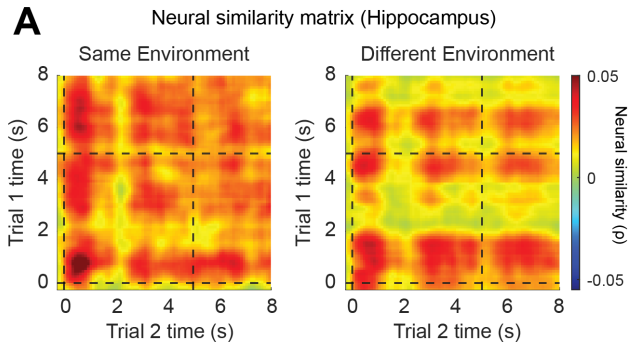


Figure 7. Neural similarity between trials of the same (e.g., library-library) versus different (e.g., library-museum) environment in an example participant. **A**: Mean neural similarity matrix across all pairwise trials with the same (left) versus different (right) environment, using electrodes in the hippocampus (N = 3 electrodes). The Y and X axes represent time within the two trials being compared. Color represents the mean cosine similarity of neural feature vectors between pairs of time points from the two trials. **B**: Mean neural similarity within the 0-8s time window from the neural similarity matrix. The left plot shows data from the hippocampus (i.e., taking the average of the 0-8s windows of matrices in plot **A**), and the right plot shows data from the amygdala (N = 1 electrode). The asterisk indicates $p < 0.05$ using an independent samples t-test.

Potential Challenges and Limitations**:** We anticipate challenges involving the optimization of the VR fear conditioning task. We have found that, out of sixteen total participants who completed the task (7 RNS, 9 non-RNS), only eleven (3 RNS, 8 non-RNS) reliably learned the CS-US associations as measured by the rating task at the end of the acquisition phase. Since individuals with RNS implants are on average older with more psychiatric and neurological co-morbidities, they likely had more difficulty with learning the associations compared to the non-RNS participants consisting of college psychology majors. In consultation with my co-mentorship team, we are currently conducting additional pilot experiments to enhance the salience of both the CS and the US by introducing multimodal sensory experiences such as different tones associated with the CS light colors and vibratory cues paired with the US. Although traditional fear conditioning studies have utilized painful tactile or auditory stimuli, we hope to demonstrate that context-appropriate stimuli in virtual reality can reliably induce fearful responses. Developing a nuanced understanding of the strengths and limitations of VR technology in neuroscience research is aligned with the training goals of the proposal.

❖ **Aim 2: Test the effects of amygdala stimulation on fear extinction.**

Background: Intracranial electrical stimulation has emerged as a potent tool to causally probe neural circuits. In humans, amygdala stimulation has been safely and effectively used to treat focal epilepsy for many years[95–97]. Several lines of evidence converge to suggest that amygdala stimulation will enhance fear extinction. In animal studies, perturbation of amygdala activity and its connectivity to the hippocampus through optogenetic inhibition[98] or electrical stimulation[99] leads to a reduction in anxiety and enhancement in fear extinction. In humans, amygdala stimulation appears to have two main effects: 1) elicits autonomic activity consistent with a response to a threat (increased skin conductance, heart rate deceleration)[30] and 2) enhances declarative memory formation independent of its emotional content[31,100]. Taken together, we hypothesize that amygdala stimulation will enhance fear extinction similar to how prolonged exposure therapy is used to treat PTSD. During prolonged exposure, individuals deliberately confront fear-inducing stimuli in a controlled environment, allowing them to gradually learn that once-feared stimuli no longer pose a significant threat. Thus, the mechanism behind fear extinction is analogous to the development of a separate competing memory, with the new association being strengthened by repeated exposures. Importantly, this re-learning process is more effective when patients experience the physiological arousal associated with the traumatic memory[101]. Therefore, targeted stimulation of the amygdala during the presentation of fear-conditioned stimuli (CS+) during extinction will 1) amplify the physiological arousal to threat, and 2) enhance re-learning that the CS+ is no longer associated with the US. Encouragingly, our group has recently shown that stimulating the amygdala both continuously and in response to increased theta activity leads to PTSD symptom reductions[2,102]. This promising evidence underscores the potential therapeutic value of amygdala stimulation in modulating emotional responses and promoting recovery from treatment-resistant anxiety disorders.

Experimental Design: Participants will undergo a 2-day Stimulation Experiment (**Figure 2B**), which has a similar structure to the Recording Experiment with the addition of an extra conditioned stimulus (CS++). Therefore, participants are informed that the color of the room will temporarily change to one of three colors.

Two of the colors (CS+ & CS++; e.g., green and yellow lighting) are followed by the US (e.g., giant spider) while the other color (CS-; e.g., blue lighting) is not. The CS+, CS++, and CS- are encountered 10 times each. Participants complete the ratings and rest for 3 minutes before entering the extinction phase. During extinction, none of the three colored lights will lead to the US. However, during the presentation of CS++, stimulation will be delivered to the right amygdala. Analogous to the Recording Experiment, an extinction phase will be completed for Day 2 in a third environment to test the effect of stimulation on fear renewal.

The primary site for stimulation will be the right basolateral amygdala, given previous studies in humans that suggest the right amygdala is preferentially active during negative emotions[103], states of high arousal[104], and fear[105]. Further evidence comes from case reports where a lesion to the right amygdala led to reduced fear-potentiated startle response[106] and a reduction of PTSD symptoms[107]. If a participant only has left amygdala contacts, we will stimulate this site and take this into account in our analysis. Stimulation parameters will be derived from previous studies completed by our group using bipolar stimulation that is current-regulated, charge-balanced, with biphasic rectangular pulses set below the after-discharge threshold[2,30,31]. The absence of an after-discharge at a particular site will be carefully established before each session. We will use (putatively inhibitory) gamma frequency (130 Hz) stimulation with a 90-μsec pulse length, following previous studies and our protocol for deep brain stimulation (DBS) in PTSD[2,4,30,31]. For each pulse, total stimulation will range between 2.5-10.1 $\mu C/cm^2$ per phase, well below the safe maximum used for chronic (30 $\mu C/cm^2$ per phase) and acute (57 $\mu C/cm^2$ per phase) stimulation.

Preliminary Results: We hypothesize that amygdala stimulation during the CS++ will facilitate fear extinction as measured by reduced fear-related physiological responses (increase in heart rate and skin conductance) when compared to a separate CS+ (see **Figure 2B**). Individuals from our team have published several studies using medial temporal stimulation including the amygdala specifically[2,30,31,102]. **Figure 8** shows an example raw iEEG trace from a medial temporal lobe electrode adjacent to one that delivered a high-frequency burst of stimulation repeated at a lower frequency for several seconds. We have recently published the preliminary results from our study utilizing electrical stimulation of the amygdala where two combat veterans with treatment-refractory PTSD experienced a significant decrease in symptoms, including one participant who has achieved and maintained complete remission from the disorder[2].

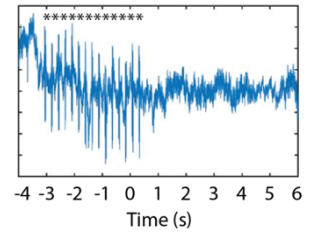


Figure 8. Raw iEEG trace during a 100 Hz intracranial pulse (asterisks) to an adjacent electrode contact.

Potential Challenges and Limitations**:** We foresee potential challenges in quantifying extinction learning during the Stimulation Experiment. Since iEEG recording cannot co-occur with stimulation, we will rely on physiological measurements to measure the acquisition and extinction of fear responses. Upon completion of the Recording Experiment, if the skin conductance and heart rate measures appear to have inadequate resolution to distinguish between responses to the CS+ and CS++, we may introduce additional measures such as pupil dilation and startle eye blinks, both of which have been previously implemented in the lab. Although we will initially use stimulation parameters consistent with previously published research, we anticipate the parameters will require gradual titration to maximize its effect without inducing a subjective emotional experience[30]. The RNS system can deliver stimulation with configurable amplitude (0-12 mA, 0.1 mA steps), pulse width (40-1000μs, 40μs steps), frequency (1-333 Hz), and burst duration (10-5000 ms). In line with the training goals of gaining expertise in intracranial stimulation, I will work closely with our neurosurgeon collaborator Dr. Langevin throughout this process.

- **Aim 3: Characterize the physiological activity and hippocampal-amygdala iEEG dynamics associated with anxiety.**

Background: Classification of psychiatric illness is moving away from a categorical approach and embracing a more transdiagnostic, dimensional perspective. This involves considering diseases along a continuum of biological, behavioral, and cognitive factors rather than relying on a concrete list of symptoms. For instance, although GAD and PTSD are listed under separate diagnostic categories in the DSM-5[108], we understand them as occupying a similar dimensional space characterized by symptoms such as excessive fear, avoidance, hypervigilance, heightened physiological arousal, and sleep disturbances. Consistent with this, fear conditioning has served as an effective model for both disorders. Given the U.S. veteran population comprises a wide range of comorbid anxiety and PTSD symptoms, we have a unique opportunity to investigate how iEEG activity from the fear circuitry relates to anxiety symptoms across a wide spectrum of illness severity.

Given the role of the hippocampal-amygdala circuit in fear learning, there has been a concerted effort to investigate how the structural and functional characteristics of this circuit relate to an individual's baseline (trait) anxiety. Imaging studies have shown that trait anxiety is associated with the amygdala's surface morphology[109], structural connectivity to higher-order brain areas (e.g., dorsal anterior cingulate cortex)[110,111], and resting state functional connectivity to other brain regions[112]. FMRI studies have demonstrated that individual differences in trait anxiety predict activity in the basolateral amygdala in response to fearful stimuli[113,114]. Similar associations are found between hippocampal activity[115] and functional connectivity[116] with trait anxiety. We will build upon these findings by investigating whether fear-related iEEG oscillatory activity and connectivity measures in this circuit exhibit variations among individuals with different levels of anxiety. This effort ultimately aims to identify potential biomarkers associated with anxiety disorders.

Analysis and Interpretation: All participants enrolled in the study will complete clinical assessments including the Beck Anxiety Inventory, State-Trait Anxiety Inventory, Intolerance of Uncertainty Scale, Fear Survey Schedule, Anxiety Sensitivity Index, Patient Health Questionnaire (PHQ-9), and the WHO Disability Assessment Schedule. Participants enrolled in the PTSD clinical trial will also complete the Clinician-Administered PTSD Scale (CAPS). Across participants, the composite score of each clinical questionnaire will be used to predict the degree of change in a physiological or electrophysiological variable of interest using a multiple linear regression model. Of note, we expect the total scores of many of the questionnaires to be correlated, leading to a multicollinearity issue. This will be addressed by either converting highly correlated metrics into a composite score or using statistical regularization techniques such as ridge or lasso regression. The choice of the time window to average data will be determined a priori using the same time window where increased activity is observed across all participants (e.g., 3-9 seconds after CS presentation for skin conductance activity seen in **Figure 6**).

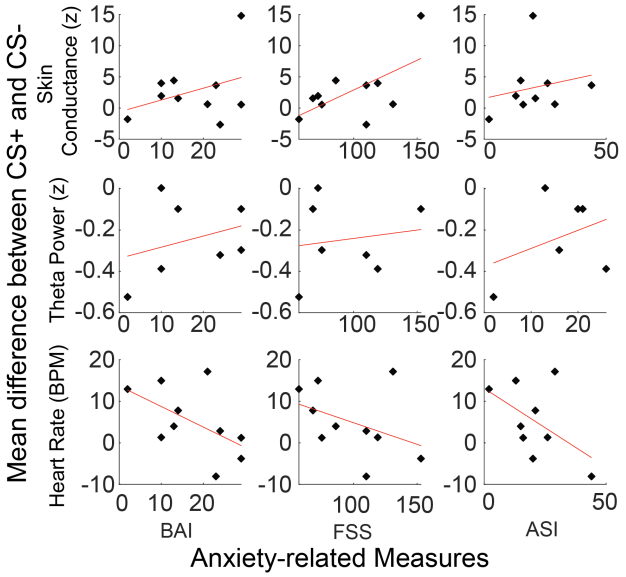


Figure 9. Scatterplots showing the relationship between a measured variable (skin conductance, iEEG theta power, heart rate) and clinical assessment scores. Red lines indicate lines of best fit and each point represents a participant. The y-axis is the mean difference between all CS+ and CS- trials for a given variable, averaged between 3-9s relative to CS onset. The x-axis is the composite score for a given clinical questionnaire. BAI = Beck Anxiety Inventory, FSS = Fear Survey Schedule, ASI = Anxiety Sensitivity Index.

Preliminary Results: We analyzed data from RNS epilepsy participants (N = 7) and pilot participants without RNS (N = 3). Consistent with our hypothesis, we see trends toward a positive association ($p > 0.05$ across all regression models) between skin conductance and theta power and various anxiety metrics (**Figure 9**, first and second rows). Interestingly, we see an opposite pattern with heart rate, whereby a decrease in heart rate is associated with anxiety (**Figure 9**, third row). This is consistent with the hypothesis that animals experience an initial deceleration in heart rate when faced with threatening stimuli to transiently enhance perceptual processing[30,117]. Therefore, in combination with our preliminary finding that heart rate is overall higher during CS+ compared to CS- trials (**Figure 6C**), our data suggest that participants engage in coactivation of both sympathetic and parasympathetic responses to VR-based threat and that these responses are correlated with the individual's baseline level of anxiety across multiple symptom dimensions.

Conclusions and Implications: Currently, a substantial gap exists between rodent fear conditioning studies and human laboratory-based studies of fear and anxiety. Our experimental platform bridges this gap by allowing for direct intracranial recording and stimulation of the hippocampus and amygdala – brain regions heavily implicated in contextual fear learning and anxiety disorders – while participants undergo naturalistic behavior using VR and wearable technologies. Moreover, the inclusion of military veterans, who exhibit higher rates of comorbidity with various anxiety-related disorders, enhances the translational relevance of our research. Taken together, our proposed project aims to elucidate the basic neural mechanisms underlying human fear processing, pave the way for the development of innovative therapeutic interventions for treatment-resistant anxiety disorders, and discover biomarkers across various anxiety- and fear-related traits, thereby contributing to the ongoing mission to adopt a dimensional approach to psychiatric illness.

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

UCLA offers opportunities each semester for thorough training of students, postdocs, and faculty in the responsible conduct of research (RCR). This includes formal courses in RCR that fulfill all requirements and address all components as specified by the NIH. The UCLA David Geffen School of Medicine offers two face-to-face courses on RCR annually: “Responsible Conduct of Research Involving Humans”, a ten-week course in the Fall semester (2 hours of lectures and 2 hours of discussion per week for a total of 40 hours) that combines lectures and scenario-based interactive discussions; and “Ethics and Accountability in Biomedical Research”, a ten-week course in the Spring semester (2-hour discussions per week for a total of 20 hours) based on case-study discussions. I will take one of the above courses during my first year during the K08 award period (the academic year 2024-2025), then again during my 5th year to fulfill the NIH requirements. Both courses cover a broad array of topics including mentoring responsibilities, safe laboratory practices, data ethics, intellectual property, conflicts of interest, misconduct, authorship, collaborative research, responsible publication, peer review, human/animal subjects, Institutional Review Board (IRB), and broad ethical issues. Rotating clinical and research faculty also participate in weekly discussion sessions in these courses.

During my first weeks as a resident physician at UCLA (June 2021), I completed an online training program through the Collaborative Institutional Training Initiative (CITI) offered by UCLA’s Office of the Human Research Protection Program, including courses and tests on Good Clinical Practice, History and Ethics of Human Subjects Research, Conflicts of Interest in Human Subjects Research, IRB Regulations, Clinical Trials, HIPAA, and other topics. I have also completed analogous courses on the responsible conduct of research (RCR) through the VA system (Talent Management System VA Online University). These courses are required to be renewed annually to maintain my status as a resident physician in the UCLA and VA systems.

Lastly, my primary mentor Dr. Nanthia Suthana often circulates articles to facilitate discussion of research ethics during lab meetings, one-on-one meetings, and while training me in collecting data from human participants. Dr. Suthana and my co-mentors (Dr. Craske and Dr. Fanselow) emphasize promoting the highest level of ethical research conduct, especially in the context of working with patient populations.

DESCRIPTION OF INSTITUTIONAL ENVIRONMENT

The University of California, Los Angeles (UCLA) ranks among the top research universities in the United States and has an established record of excellence in health science research, graduate education, and patient care. The university offers unique facilities that collectively create a robust network of research, educational, and collaborative opportunities. This environment is conducive to both my career development and the successful execution of the proposed research plan. At the core of the proposed research plan are electrophysiological recordings from patients with epilepsy who have been surgically implanted with responsive neurostimulation (RNS) devices. UCLA stands as a global leader in conducting clinical procedures and research focused on these patient groups. Multiple UCLA neurosurgeons carry out this invasive surgery on numerous epilepsy patients monthly. My proposed research will be conducted in direct partnership with the UCLA Department of Neurosurgery, with Dr. Jean-Philippe Langevin (collaborator) playing a pivotal role in the recruitment and retention of study participants from both UCLA and the VA systems.

The Suthana lab at UCLA, led by my primary mentor Dr. Nanthia Suthana, has an excellent track record in utilizing both RNS and virtual reality technologies to study the human brain. The Suthana lab has both the expertise and the technical equipment (as outlined in the Facilities and Equipment sections) for this line of research and maintains a large database of more than 250 RNS participants who have expressed their interest in participating in the lab's research studies. Additional support for task design and data analysis regarding fear conditioning will come from my co-mentors Dr. Craske and Dr. Fanselow, both of whom hold the title of Distinguished Professor in the Department of Psychology for their contributions to fear conditioning research in humans and rodents, respectively. The Suthana lab and I have been successfully working on several research projects with all these collaborators and institutes over the past 2.5 years, which indicates that this will continue to be a fruitful collaboration with minimal unexpected administrative hurdles for the proposed experiments.

UCLA also has several institutes to facilitate the career development and research success of early-stage investigators: The Clinical and Translational Science Institute (CTSI) supports and supervises human studies and clinical trials in all therapeutic areas and offers a large variety of training opportunities such as consultation services and formal courses on human subjects protection, IRB-related topics, the administration and coordination of clinical trials, study management, data management, as well as manuscript and grant preparation. The Institute for Digital Research and Education (IDRE) at UCLA offers courses, resources, and support to UCLA researchers in all areas of computational science, data science, and information science. This includes free-of-charge support and consulting services from the IDRE Statistical Consulting Group which assists in applied statistics and data analysis related to grants and publications. Several UCLA-based entities, such as the Brain Research Institute (BRI), the Integrative Center for Learning and Memory (ICLM), and the Training in Neurotechnology Translation (TNT) Program host neuroscience journal clubs and research seminars each week. These events often cover topics that are highly relevant to the proposed research and allow students and postdocs to practice formal presentations and receive feedback from the local neuroscience community. Moreover, the BRI hosts weekly 'UCLA Joint Seminars in Neuroscience' which invites world-renowned neuroscientists and provides networking opportunities with the speakers.

In summary, I am confident that UCLA's extensive network of resources and opportunities will create an excellent and collaborative environment for accomplishing the aims of my research plan and for my training to become a successful independent investigator.

DATA MANAGEMENT AND SHARING PLAN

If any of the proposed research in the application involves the generation of scientific data, this application is subject to the NIH Policy for Data Management and Sharing and requires submission of a Data Management and Sharing Plan. If the proposed research in the application will generate large-scale genomic data, the Genomic Data Sharing Policy also applies and should be addressed in this Plan. Refer to the detailed instructions in the application guide for developing this plan as well as to additional guidance on sharing.nih.gov. The Plan is recommended not to exceed two pages. Text in italics should be deleted. There is no "form page" for the Data Management and Sharing Plan. The DMS Plan may be provided in the *format* shown below.

Element 1: Data Type

A. Types and amount of scientific data expected to be generated in the project:

Summarize the types and estimated amount of scientific data expected to be generated in the project,

Demographic, neuropsychological, behavioral, neuroimaging (iEEG, MRI), and physiological data (heart rate, skin conductance) will be collected from a total of 70 participants with RNS electrodes during the 5-year award period. All data will be de-identified before receipt by the repository, but the information needed to generate a global unique identifier for the NIMH Data Archive (NDA) will be collected for each subject.

B. Scientific data that will be preserved and shared, and the rationale for doing so:

Describe which scientific data from the project will be preserved and shared and provide the rationale for this decision.

Sufficient data from this project will be preserved to enable sharing via NDA data of sufficient quality to validate and replicate research findings described in the Aims.

C. Metadata, other relevant data, and associated documentation:

Briefly list the metadata, other relevant data, and any associated documentation (e.g., study protocols and data collection instruments) that will be made accessible to facilitate interpretation of the scientific data.

Study protocols including participant instructions for the virtual reality paradigm and setup instructions for physiological data collection will be made accessible to facilitate replication and extension of our study from other groups.

Element 2: Related Tools, Software and/or Code:

State whether specialized tools, software, and/or code are needed to access or manipulate shared scientific data, and if so, provide the name(s) of the needed tool(s) and software and specify how they can be accessed.

No specialized tools or software are needed to access or manipulate the shared scientific data. While we use custom code written in MATLAB to analyze our data, the data itself is organized into number matrices that can be accessed by any other analysis software. Most universities have site licenses available to MATLAB, and it is also possible to use an open-source alternative to MATLAB such as Octave. The code used for analysis will be made available to the public via GitHub and can be located by searching for "labname".

Element 3: Standards:

State what common data standards will be applied to the scientific data and associated metadata to enable interoperability of datasets and resources, and provide the name(s) of the data standards that will be applied and describe how these data standards will be applied to the scientific data generated by the research proposed in this project. If applicable, indicate that no consensus standards exist.

No consensus standards exist for our demographic, behavioral, and neuroimaging data. However, within the Suthana laboratory group, it is strongly encouraged to keep digitized data sources in formats that facilitate

consistent collection and interoperability across different systems, sources, and users. The clinical assessments we plan to collect for this study include the Beck Anxiety Inventory (BAI), State-Trait Anxiety Inventory (STAI), Intolerance of Uncertainty Scale (IUS), Fear Survey Schedule (FSS), Anxiety Sensitivity Index (ASI), Patient Health Questionnaire (PHQ-9), WHO Disability Assessment Schedule (WHODAS), and the Clinician-Administered PTSD Scale (CAPS).

Element 4: Data Preservation, Access, and Associated Timelines

A. Repository where scientific data and metadata will be archived:
Provide the name of the repository(ies) where scientific data and metadata arising from the project will be archived; see Selecting a Data Repository).

We will provide open access to all tools developed and anonymized data using the NIMH Data Archive (NDA) and will also consider data-sharing through DABI (Data Archive BRAIN Initiative), Blackfynn, and Neurodata Without Borders (NWB). Any hardware or software developed for the platform will be shared via publications and GitHub repositories.

B. How scientific data will be findable and identifiable:
Describe how the scientific data will be findable and identifiable, i.e., via a persistent unique identifier or other standard indexing tools.

Data will be findable for the research community through the NDA Collection that will be established when this application is funded. For all publications, an NDA study will be created. Each of those studies is assigned a digital object identifier (DOI). This data DOI will be referenced in the publication to allow the research community easy access to the exact data used in the publication.

C. When and how long the scientific data will be made available:
Describe when the scientific data will be made available to other users (i.e., no later than time of an associated publication or end of the performance period, whichever comes first) and for how long data will be available.

The research community will have access to data when the award ends. As required by NDA, studies will also be created that contain the data used for every publication. Those studies will be shared when the pre-print is available. NDA studies have digital object identifiers (DOI) to aid in findability. We will include that DOI in relevant publications. NDA will make decisions about how long to preserve the data, but that data archive has not deleted any deposited data up to now.

Element 5: Access, Distribution, or Reuse Considerations

A. Factors affecting subsequent access, distribution, or reuse of scientific data:
NIH expects that in drafting Plans, researchers maximize the appropriate sharing of scientific data. Describe and justify any applicable factors or data use limitations affecting subsequent access, distribution, or reuse of scientific data related to informed consent, privacy and confidentiality protections, and any other considerations that may limit the extent of data sharing. See Frequently Asked Questions for examples of justifiable reasons for limiting sharing of data.

Any data shared with the public will be completely de-identified with only basic demographic information included such as age, sex/gender, and race/ethnicity. Therefore, we do not anticipate any significant limitations affecting the access, distribution, or reuse of our scientific data. All research participants will be consented for broad data sharing.

B. Whether access to scientific data will be controlled:
State whether access to the scientific data will be controlled (i.e., made available by a data repository only after approval).)

To request access to the data, researchers will use the standard processes at NDA, and the NDA Data Access Committee will decide which requests to grant. The standard NDA data access process allows access for one year and is renewable.

C. Protections for privacy, rights, and confidentiality of human research participants:
If generating scientific data derived from humans, describe how the privacy, rights, and confidentiality of human research participants will be protected (e.g., through de-identification, Certificates of Confidentiality, and other protective measures).

Participant contact and personal information will not be shared with researchers or any collaborators; each participant will be coded and only Dr. Jang, Dr. Suthana, and the Staff Research Associate (Sonja Hiller) will have access to the code. Before sharing data amongst researchers and the larger scientific community, Dr. Jang will ensure that data is de-identified in compliance with HIPAA; some information may be recoded or removed to ensure confidentiality as necessary. No private records or identifier information will be made available or transferred. Participant identities are never disclosed by publication or any other means. Informed consent forms are kept in locked files for at least 7 years beyond subject participation as legally required. Information in the computerized database is protected by a firewall and special username/password accounts are given only to authorized personnel. Data will be shared between lab members using a secured server with encryption and performed by authorized users using password-protected accounts.

Element 6: Oversight of Data Management and Sharing:
Describe how compliance with this Plan will be monitored and managed, frequency of oversight, and by whom at your institution (e.g., titles, roles).

Monitoring and compliance with this Data Management and Sharing Plan will be the responsibility of the project's Principal Investigator. The plan will be implemented and managed by professional staff working under the direction of the PI. The final project report will summarize adherence to this data management and sharing plan.

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