Neurophysiological and Behavioral Effects of Sensory Flicker and Electrical Flicker Stimulation

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ABSTRACT:
Clinical trials have explored the modulation of brain circuits to treat several brain disorders, including Parkinson's Disease, Alzheimer's Disease (AD), depression, and Obsessive-Compulsive Disorder (OCD). However, current means to modulate brain activity are limited. Non-invasive methods, such as transcranial direct current stimulation and transcranial magnetic stimulation, can usually only affect superficial brain areas. Invasive methods, such as deep brain stimulation, can more precisely modulate brain targets and networks, but involve risks that are inherent to brain surgery. *The overall goal of this study is to develop safe, effective, and non-invasive means of modulating the activity of deep brain circuits in a behaviorally relevant manner, potentially opening new treatment avenues for various brain disorders.*

Repeated light flashes and sounds, or visual and auditory flickers, have been shown to induce neural entrainment, called steady-state evoked potentials (SSEPs)[1], in visual and auditory sensory areas, respectively. Furthermore, recent research from our collaborators shows that combined audiovisual flicker at specific frequencies can modulate activity in the mouse hippocampus and prefrontal cortex[2], regions with great relevance to human disorders of cognition and mood. *Here, we propose to study whether sensory flicker can modulate neural activity of deep brain regions in humans, and whether it can have relevant effects on behavior. Moreover, we propose to compare those effects to the gold-standard method of modulating brain circuits, direct electrical stimulation of the brain (the same mechanism as deep brain stimulation), using a powerful within-subjects design.*

Objectives of interest in the current proposal will include: to analyze entrainment of brain activity (recorded directly from local field potentials, LFPs) to sensory flicker in sensory areas; to study the effects of sensory flicker on interictal epileptiform discharges (IEDs); to test whether sensory flicker can entrain LFPs and modulate single neuron activity in higher cognitive areas, such as the hippocampus and prefrontal cortex; to study the optimal frequency at which various brain areas are entrained to sensory flicker; to study the effects of sensory flicker on specific behavioral tasks, to compare the effects of sensory flicker stimulation to direct electrical stimulation at corresponding frequencies in given brain regions. Specific primary, secondary and exploratory outcome measures, as listed in the associated registered clinical trial (NCT04188834), are specified below under "Outcome measures". To accomplish these objectives, we propose to expose subjects to either sensory flicker or direct electrical stimulation of specific brain regions, at given frequencies, while subjects perform behavioral tasks and we record their intracranial neural activity.

All experiments will be performed in epilepsy patients who have already undergone placement of intracranial electrodes and been admitted to the epilepsy monitoring unit of the hospital for identification of their seizure onset zone, so there will be no additional risk from surgery beyond what would otherwise be required for clinical treatment. Such patients already undergo direct electrical brain stimulation as a routine part of their clinical evaluation of seizure onset and mapping of the location of specific brain functions. If successful, results from this project may open new non-invasive therapeutic avenues to modulate circuitry in several brain disorders, such as AD, depression, OCD and epilepsy.

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1. PROJECT OVERVIEW

1.1. Purpose

Many approaches to treat various brain disorders employ pharmaceutical and/or behavioral therapies. When such treatments fail, invasive procedures such as deep brain stimulation (DBS) can precisely target and modulate affected circuits. For example, DBS of the subthalamic nucleus and globus pallidus have been successfully used in late stages of Parkinson's disease (PD)[3] for decades. Clinical trials of DBS of other specific brain circuits to treat other disorders such as Alzheimer's Disease (AD), obsessive-compulsive disorder (OCD) and depression[4] are well underway. Recently, non-invasive means of modulating brain circuits, such as transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) are being developed to try to treat similar disorders[5]. Although these non-invasive methods are promising, they are very limited in their ability to reach deep brain regions such as the basal ganglia, the hippocampus, and the amygdala, which are often involved in key brain disorders. *Our primary goal is to test the effects of a novel non-invasive means of modulating the activity of such deep brain circuits through exposure to a sensory flickering stimulus. Our secondary goal is to compare the effects of temporarily modulating specific cognitive-memory circuits by either sensory flicker or gold-standard direct electrical stimulation within individual subjects.*

1.2. Background

1.2.1. Evidence- sensory flicker modulates brain circuits in mice

Our collaborators recently showed[6] that exposure of mice to 40Hz visual flicker induces local field potential (LFP) entrainment and firing rate modulation in the primary visual cortex (Figure 1), and induces physiologically relevant changes in the primary visual area of an animal model of Alzheimer's disease (AD). Indeed, following 40Hz visual flicker, the primary visual cortex of the 5XFAD mouse model showed reduced amyloid beta load, as well as morphological and gene expression changes of microglia suggesting a transition to a phagocytic state. Moreover, the study showed colocalization of amyloid beta with microglia following 40Hz visual flicker. These beneficial effects suggested that 40Hz sensory flicker may potentially be used as a new therapeutic approach for AD.

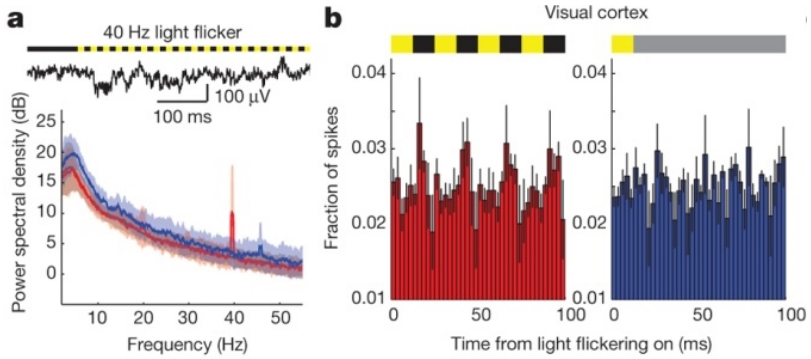

Figure 1 (from Iaccarino et al. 2016): Electrophysiological effects of 40Hz visual flicker in the mouse primary visual cortex. a: LFP trace in primary visual cortex before and during 40Hz light flicker (above). Power spectral density mean and s.d. (below); red represents 40Hz light flicker condition, blue represents random light flicker condition. b: Fraction of spikes in primary visual cortex over 4 cycles of 40Hz flicker (left) or the equivalent time for random flicker (right,

mean +/- s.e.m. across animals). For random stimulation, spiking was aligned to light turning on; grey indicates additional light-on flickers occurring randomly.

Following these findings, our collaborators showed[2] that 40Hz sensory flicker may gently entrain LFPs in the hippocampus and modulate firing rate in both the hippocampus and the prefrontal cortex (Figure 2). Moreover, they showed that in the 5XFAD AD mouse model, repeated 1-h daily exposure to 40Hz sensory flicker reduced amyloid beta load in the hippocampus, and improved spatial memory, thereby suggesting that such neurophysiological modulation may be regionally, physiologically and behaviorally relevant to AD.

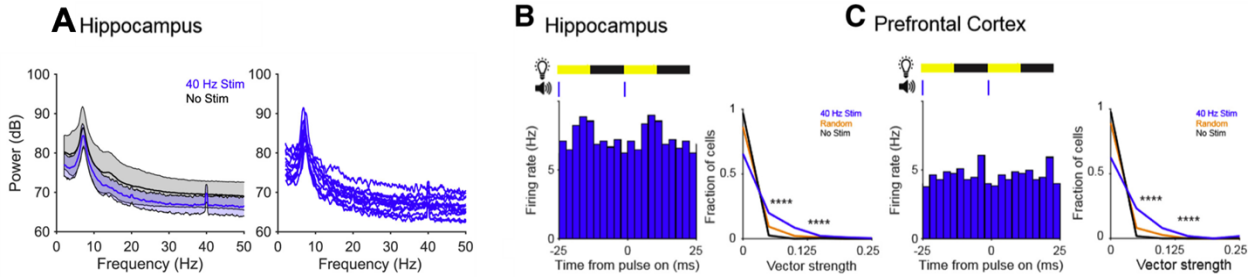

*Figure 2 (from Martorell, Paulson et al., 2019): LFP and single unit response to audiovisual flicker in the hippocampus and prefrontal cortex in mice. A: Power spectral density (PSD) response to 40 Hz audio-visual flicker stimuli and no stimulation periods, with mean and standard deviation across recording days (left), power spectrum LFP response to audio-visual flicker stimulation of all recording days in CA1 (recording site with largest 40 Hz peak during 40 Hz audio-visual flicker per recording depth is shown) (right). B: In CA1, firing rate modulation of a single unit during 40 Hz audio-visual (AV) stimulation (left). Vector strength of responses to 40 Hz AV stimulation, random AV stimulation, and no stimulation periods (right, ****P<0.00005 40 Hz vs. no stim, 40 Hz vs. random; Kolmogorov-Smirnov test; 8 units and 3 units had VS values >0.25 for 40 Hz or random stim, respectively). In all statistical tests for panels B-C, results are significant after controlling for multiple comparisons using the Bonferroni correction unless otherwise stated. C: Same as in B for mPFC (right, ****P<0.00005 40 Hz vs. no stim, 40 Hz vs. random; Kolmogorov-Smirnov test; 5 units had 40 Hz stim VS values > 0.25). For B and C, vector strength is a measure of how much the firing of a given cell is phase-locked to the stimulus.*

In this project, we aim to explore whether we can replicate these neural modulation and behavioral effects of flicker in humans. We ask 2 questions: 1) Can sensory flicker modulate the activity of the hippocampus, and potentially other high-order areas such as the prefrontal cortex, in humans? 2) Would such modulation be correlated with changes in cognition that are relevant to regions affected, such as memory in the case of the hippocampus? To explore these questions, we propose to study intracranial neural activity in treatment-resistant epileptic patients, at the level of populations of neurons (LFPs) and, when microelectrode recordings are present, at the level of individual neurons (single units), as a function of sensory flicker.

1.2.2. Evidence- sensory flicker modulates sensory circuits in humans

Steady-state evoked potentials, which are neurophysiological effects that can be induced by visual and/or auditory flicker in sensory brain regions, have been extensively explored in humans (Table 1)[1]. However, the majority of studies present limitations in their temporal and spatial resolution, as well as the extent of brain areas that they explore. Many studies use scalp electroencephalography, which offers good temporal resolution to characterize neural entrainment in superficial areas of the brain, yet poor spatial resolution as it does not record

directly from the brain. To our knowledge, only three studies looked at the electrophysiological effects of repeated sensory stimuli through intracranial recordings. Two studies focused on the effects of visual flicker in regions involved in visual processing[7,8], while the other studied the effects of repeated auditory beats in the temporal region[9]. Other studies, employing imaging such as functional MRI and/or PET, may allow measures of activity throughout the brain, but provide only results at a very low temporal resolution (on the order of tens of seconds instead of sub-milliseconds).

Table 1
Source location of SSVEPs in recent publications.

Authors	Stimulus	Frequencies	Method	Location(s)
Krolak-Salmon et al. (2003)	Chkb	1.1 Hz, 75 Hz	Implanted electrodes (human subjects)	LGN, V1, V2
Pastor et al. (2003)	Strobe	5–60 Hz (EEG) 5–40 Hz (PET)	EEG + PET	V1[a], cerebellum
Fawcett et al. (2004)	Chkb	1–21 Hz (square) 1–17 Hz (sine)	MEG	V1, V5, MT
Sammer et al. (2005)	Comp	4.7 and 18.8 Hz	Simultaneous EEG + fMRI	Occipital
Zhang et al. (2006)	Comp[b]	26–33 Hz	EEG	V1
Srinivasan et al. (2006)	Dots	3–30 Hz	QEEG + Laplacian	Occipital, parietal, and frontal[c]
Srinivasan et al. (2007)	Chkb	3–14 Hz	fMRI	V1, V2, BA
Di Russo et al. (2007)	Gabor gratings	12 Hz	EEG + fMRI	V1, V5, +(possibly)V3A, V4/V8
Pastor et al. (2007)	Strobe	5–40 Hz	EEG + PET	Frontal eye-fields, Parieto-occipital[a]

Chkb, Strobe, Comp, and Dots = flickering checkerboard, strobe lamp, flickering whole computer screen, and flickering random dot pattern, respectively.
[a] The authors suggest that V1 is not the only area involved in SSVEP generation. In Pastor et al. (2007), the SSVEP harmonics, studied separately, correspond to different sources.
[b] This experiment used the binocular rivalry paradigm.
[c] Occipital and parietal sources are local, whereas frontal sources are either deep or broadly distributed for most frequencies.

Table 1 (from Vialatte et al. 2010): Summary of studies exploring the neurophysiology of steady-state visually-evoked potentials (SSVEPs).

Our study is novel in that it aims to explore the neurophysiological response to sensory flicker in humans 1) in various brain regions- both superficial and deep, both early sensory and higher cognitive regions, 2) at high temporal and spatial resolution through localized intracranial LFP, and 3) at the single-unit level.

1.2.3. Rationale for studying the potential behavioral effects of sensory flicker in humans

Our ultimate goal is to use sensory flicker as a means to modulate pathological circuits in a behaviorally relevant way, potentially opening new avenues to develop non-invasive treatments for various brain diseases. Two studies suggested that modulating hippocampal circuits in humans through direct electrical stimulation may either increase[10] or decrease[11] performance for given memory types. Our team recently showed[12] that stimulation of the amygdala by theta-modulated gamma bursts increases long-term memory for images without causing any changes in emotion or provoking epileptic activity. *Here, we propose that, similar to these studies, modulating neural activity through sensory flicker may affect cognitive processes such as memory.*

This has been explored in a recent study[13], where it was shown that exposing human subjects to 5.5Hz (theta-like) audiovisual flicker during the consolidation phase of memory for words may improve source memory performance. Here, we are particularly interested in 40Hz (gamma-like) audiovisual flicker. Gamma oscillations are thought to be implicated in a range of cognitive processes, including cortical computations, attention[14] and memory[15], and have been shown to be affected in some disorders such as AD and Fragile X Syndrome[16]. In particular, the 5XFAD

mouse model of AD shows decreased gamma power during sharp-wave ripples[6], thought to play an important role in memory. Moreover, recent results from our collaborators[2] showed that 1h daily exposure to 40Hz sensory flicker improved recognition and spatial memory in the same AD mouse model. *Here, we will test the acute effects of sensory flicker in humans on memory, either positive or negative, as well as their neurophysiological correlates. We are especially interested in contrasting the effects of theta-like vs gamma-like sensory flicker. Moreover, we will directly compare those effects to the ones obtained by direct electrical stimulation at corresponding frequencies in target brain regions.*

1.3. Outcome measures

This protocol encompasses the following outcome measures tied to the associated registered clinical trial (NCT04188834):

1.3.1. Primary Outcome Measures

The primary outcome measure is the effect of sensory **flicker** exposure on local field potential (LFP): comparing mean power spectral density at the frequency of flicker being presented between flicker and baseline periods. [Time Frame: Up to 6 weeks]

Power spectral density of the LFP will be measured across stimulus frequencies and modalities of sensory flicker stimuli in visual areas, auditory areas, hippocampus and prefrontal cortex. The mean power spectral density measured in dB at the frequency of flicker being presented will be compared between flicker stimulation and no-stimulation baseline periods.

1.3.2 Secondary Outcome Measures:

The secondary outcome measure is the effect of sensory flicker on interictal epileptiform discharges (IEDs), which represent pathological activity often observed in epilepsy [Time Frame: Up to 6 weeks]

The effect of the sensory flicker will be evaluated by the comparison of the whole-brain rate of IEDs between sensory flicker stimulation and baseline (no stimulation).

1.3.3 Exploratory Outcome Measures:

1) To study whether sensory flicker exposure can entrain LFPs and modulate single neuron activity in higher cognitive areas such as the hippocampus and prefrontal cortex.
2) To identify the modality and frequency of flicker to which given brain regions are most efficiently modulated at the LFP and the single unit levels.
3) To identify potential effects of audiovisual flicker on various cognitive processes through a behavioral task.
    a. If there are effects, to identify their electrophysiological correlates (such as changes in sharp-wave ripples' rate).
4) To compare the effects of sensory flicker modulation to direct electrical modulation of various neural circuits within individual subjects.

2. STUDY DESIGN

2.1. Equipment

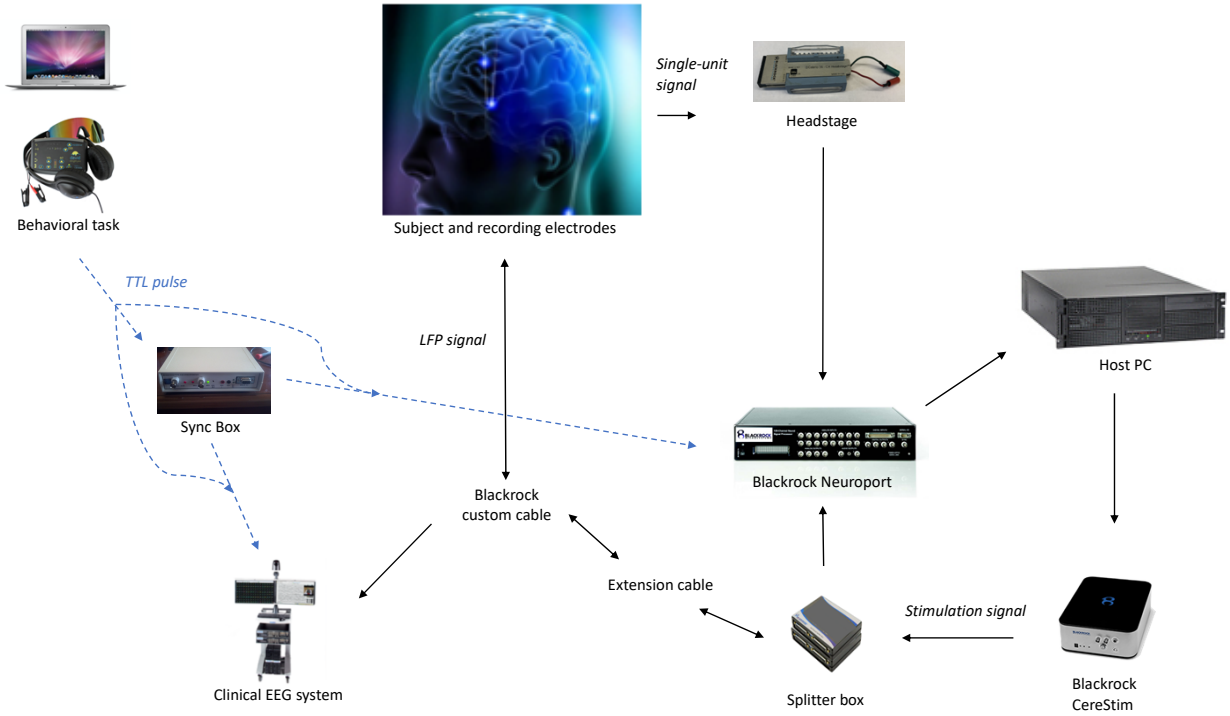

Figure 3: Summary schematic of the data acquisition and stimulation systems.
To synchronize the behavioral task with neurophysiological recordings, a TTL pulse will be sent to the clinical EEG system and the Blackrock Neuroport system. The TTL pulse may be sent to those systems through one of 2 ways: either using the Sync Box, which allows to send a 5V TTL pulse to both systems in dedicated TTL pulse channels, as well as a ~1mV pulse into an empty channel of the clinical system; or directly sending a 5V TTL pulse into dedicated channels of the clinical and Blackrock Neuroport systems (i.e. not using the Sync Box). Moreover, LFP signals will be split using a custom cable and splitter box to be sent to both the clinical EEG system and directly to the Blackrock Neuroport system. Single unit activity will be conditioned via a headstage before being sent to the Blackrock Neuroport system. Data recorded using the Blackrock Neuroport system will be stored on a host PC. Finally, brain stimulation will be applied using the Blackrock CereStim system, controlled by the host PC.

Our project may use several or all of the following items:

Electrodes All intracranial electrodes are FDA-approved equipment for recording intracranial EEG during presurgical evaluation. We will collect data from macroelectrodes and from microelectrodes. Either AdTech Medical Instrument Corporation (Racine, WI), PMT Corporation (Chanhassen, MN), or DIXI Medical (Besançon, France) manufactures and ships the intracranial EEG electrodes after sterilization. Such electrodes are currently in use at Emory University Hospital specifically for collection of intracranial EEGs, single-unit activity, and for intracranial stimulation in epilepsy patients.
Clinical EEG system

Testing Computer Memory tasks are administered using a standard laptop or computer running custom software. Moreover, a game controlled may be connected to such a computer, for the patient to use, for example in spatial navigation tasks.	
Customized DAVID device (Mind Alive Inc., https://mindalive.com/) A customized device will be used to expose patients to sensory (visual and/or auditory) flicker. This device consists of opaque glasses containing LEDs to present flickering light, as well as earbuds or headphones to present flickering sound. The glasses may or may not have see-through holes depending on the experiment.	
Sync Box (previously approved in IRB00076437) The Sync Box sends small electrical pulses to an unused channel of the clinical EEG system, or a 5V TTL pulse to a dedicated channel of the clinical system, in order to synchronize the EEG signals with task events.	
Blackrock CereStim (previously approved in IRB00076437) The Blackrock CereStim is a fully programmable neurostimulator that has been designed to provide guided, intermittent electrical stimulation of the brain for brain mapping procedures for patients with seizure disorder.	
Blackrock Neuroport System (previously approved in IRB00076437) The Blackrock Neuroport System records and analyzes human brain activity. The NeuroPort has been 510(k)-approved by the FDA for recording electrocorticogram signals.	

Cabrio 16 – CH Headstage by Blackrock Microsystems (previously approved in IRB00057509) This device allows to condition small single-unit signals to be sent to amplifiers and the Blackrock Neuroport System.	
Blackrock custom cable (previously approved in IRB00057509) The custom cable splits the signal between the Clinical EEG system and the Blackrock components.	
Blackrock extension cable (previously approved in IRB00067252) The extension cable carries signal (neurophysiological recording or stimulation) between the custom cable and the splitter box.	
Blackrock splitter box (previously approved in IRB00076437) The splitter box can receive LFP data to be sent to the Blackrock system, or transmit a stimulation signal from the Blackrock CereStim to the brain.	
Host PC The Host PC stores the neural recordings from the subject, and controls the Blackrock CereStim.	

All study devices will be used during experiments by experimenters listed on the protocol, and the devices will be stored safely either on the EMU floor in a locked room or in the laboratory. Only the study team can operate the equipment with patients for the purposes of this project.

2.2. Protocols

All research experiments will be carried out in the Emory EMU.

2.2.1. Electrode placement

Electrodes are implanted stereotactically to help localize the seizure focus. As a standard means to increase accuracy, the surgery is guided by contrasted MR images, with post-operative reimaging for confirmation. The electrodes (Adtech, PMT or DIXI) have contacts for electrophysiological (LFP) recording and for stimulation. An electrode may include macroelectrode contacts only or macro- and micro electrode contacts. All electrodes are FDA-approved equipment for recording intracranial EEG during presurgical evaluation. Number and

locations of electrodes will be placed on purely clinical grounds, but may be used thereafter for research purposes in consenting subjects under the supervision of the director of the epilepsy monitoring unit (a neurologist) and the supervising neurosurgeon.

2.2.2. Monitoring participants

The epilepsy monitoring unit (EMU) at Emory University Hospital is a 10-room inpatient ward that is designed for inpatient video-EEG monitoring and is staffed by a multidisciplinary team of specialists, including attending physicians, fellows, psychiatrists, EEG technicians, nurses, and information-technology technicians from the Emory Healthcare Information Services (EHc-IS). The EMU contains 10 computers with monitors, one station per room, to visualize the real-time recording of EEG and video data. The data is automatically stored on a server in the EMU for secure storage and access. The EMU continuously monitors the daily progress and health of each patient server for secure storage and access. The EMU rotates personnel to monitor and care for each patient daily (24 hours per day) during the patient's stay (on average 2-3 weeks) in the EMU. A patient under presurgical evaluation is discharged from the EMU at the discretion of the treating physicians. Patients are discharged generally after 5 weeks with no seizures or if the patient has had at least 3-5 reliable seizures with ostensibly focal epileptic activity according to the EEG.

2.2.3. Intracranial electroencephalographic (iEEG) activity

This study analyzes routine diagnostic EEG data that is generated using intracranial electrodes. The number, type, orientation, and size of the iEEG electrodes depend on the anatomy and clinical considerations per patient. Typically, there will be tens to hundreds of electrode contacts arranged as a depth, strip, and/or grid. An electrode may be relatively large (macroelectrode) or very small (microelectrode). Macroelectrode local field potentials (LFPs) consist of 0.1-1000 Hz signals obtained from iEEG contacts placed either within the brain (i.e. recorded from depth electrodes) or on the surface of the brain (i.e. recorded from subdural strip or grid electrodes). These signals will be acquired at 1000-2000 samples per second and 12-bit precision for electronic storage. Both the standard and investigational macroelectrode iEEGs are automatically stored to a 2-Petabyte RAID server that is securely managed by Emory Healthcare Information Services (EHc-IS). The stored data is directly accessed via a password-protected Epilepsy Monitoring Unit EMU data system that is securely managed by EHc-IS. Microelectrodes record signals at a sampling rate of up to 30,000 samples per second and 16-bit precision for electronic storage. These signals are then processed by applying a high-pass filter between 200 and 750 Hz, in order to extract the extracellular action potential waveforms (single unit activity) from neurons located in the vicinity of the recording contact.

2.2.4. Exposure to sensory pulses and flicker.

To expose the patient to sensory flicker and individual pulses sensory stimulation, we will use modified versions of the DAVID device (Mind Alive Inc., https://mindalive.com/), a commercially available device, and headphones or earbuds. Versions of the DAVID device were used in a recent study described above[13] (Figure 4) and in many other human studies[17–24]. Our device consists of opaque glasses containing LEDs to present light flashes, with or without see-through holes, as well as earbuds or headphones to present sound bursts. Patients will be exposed, for about 10

to 60 minutes at a time, to a sequence of sensory flicker trials each lasting a few seconds to 5 minutes, while their eyes are open or closed. Each trial may include the following modalities and frequencies of flicker:

- Modalities: auditory only, visual only, or audiovisual combined.
- Frequencies: random, or anywhere from 3Hz to 200Hz.

Additionally, subjects may be exposed to individual pulses of light and/or sound, i.e. around or less than 1 pulse /second, for up to 20 minutes at a time.

For experiments characterizing neural response to sensory flicker, patients may be exposed for about 10 minutes to a single modality and frequency combination, such as 40Hz (gamma-like) audiovisual flicker, 5.5Hz (theta-like) audiovisual flicker, or random audiovisual flicker.

For one of the experiments testing the effects of sensory flicker on memory, patients may be exposed to audiovisual flicker for up to about 36 minutes at a time.

For another experiment, patients may be exposed to audiovisual flicker of a given frequency or random frequency, at modalities of visual only, auditory only, or audiovisual combined, for up to 1h at a time.

For another experiment testing the effects of sensory flicker on memory, patients may be exposed, eyes open, to visual flicker via a strip of LEDs secured to the perimeter of a monitor screen that presents images or words, or via wearing a customized DAVID device with glasses that have view holes; sound stimulus will be again provided via earbuds or headphones. In this experiment, sensory flicker would last for a few seconds at a time.

For other experiments testing the effects of sensory flicker on memory or interictal epileptiform discharges, patients may be exposed, eyes open, to visual and/or auditory flicker, anywhere from 10s to 60 minutes at a time, while performing on a spatial navigation task (see more details below). Visual flicker will be administed via customized DAVID device with glasses that have view holes, and auditory flicker will be administered via earbuds or headphones.

For most to all experiments involving exposure to visual and/or auditory stimuli, there will be an occluded condition. In this occluded condition, the subject will wear a sleeping mask or towel on their eyes (under the LED glasses mentioned above), and commercially available earplugs; in this condition, they will be exposed to similar visual and/or auditory stimuli as in the non-occluded condition. Subjects will be instructed on how to properly wear earplugs by specifying instructions similar to the ones available here: https://www.cdc.gov/niosh/mining/content/earplug.html.

Figure 4 (adapted from Roberts et al. 2018): A representation of the experimental setup to expose the patient to audiovisual flicker. For most experiments, the patient will be wearing a customized DAVID device (Mind Alive Inc.).

2.2.5. Spatial Navigation Experiments

We have preliminary data showing that sensory flicker may decrease the rate of interictal epileptiform discharges (IEDs), which constitute abnormal brain activity often detected in epilepsy patients. In the following experiments, we will further test the effects of sensory flicker on the rate of interictal epileptiform discharges, while the patient performs a spatial navigation task described below. The simultaneous running of the spatial navigation task will be used to control for subject's attention and arousal state, which has been shown to influence the rate of IEDs.

Spatial navigation task:

In this task, subjects learn the layout of a computer-generated virtual city environment and the locations of landmarks by navigating from a first-person perspective using a game controller or keyboard[43]. Each session (virtual day) of the task consists of 12 virtual city environments where subjects navigate for 9 minutes. There are hidden shortcuts to goal landmarks where subjects learn to find as they are instructed to navigate to goal landmark as quickly as possible. Behavioral records from the memory testing (e.g. keystrokes, in the form of game controller or keyboard inputs) will be collected.

During these experiments, the patient will do the spatial navigation task above while being exposed to sensory flicker via glasses lined with LEDs with see-through holes, and earbuds or headphones. Subjects may be exposed, up to 60 minutes at a time, to visual and/or auditory flicker at frequencies anywhere between 3-200Hz.

2.2.6. Electrical brain stimulation

To contrast the effects of sensory versus direct electrical flicker stimulation on neurophysiology and cognition (such as memory), we will stimulate various brain regions at various frequencies ranging from 5-200Hz, for up to 10 seconds at a time. We will initially be testing specifically frequencies of 5.5Hz and 40Hz.

During brain stimulation sessions, bipolar electrical stimulation will be applied to one or more areas of the brain at a time either with or without associated memory task. Stimulation in the

absence of any memory task will be applied in order to assess the subject's neurophysiological response to stimulation and to identify the optimal stimulation parameters for use during memory task. Stimulation during memory task will be applied in an attempt to affect the subject's memory.

The proposed intracranial stimulation parameters are both historically common and proven safe in epileptic populations. Stimulation will be current-regulated and charge-balanced, with biphasic rectangular pulses. The anticipated electrode impedance is 1-4 kΩ. Stimulation will be bipolar, involving adjacent electrodes. Prior to testing, current amplitude will be increased in a stepwise fashion in order to identify the threshold for afterdischarge (anticipated range from 0.2mA to less than 8mA), and then decreased by 25-30% for the duration of testing. If no after-discharges are observed, the maximum amplitude of current will be kept under 8mA.

These parameters are considered safe and well tolerated in patients with epilepsy who have depth electrodes implanted in the amygdala and hippocampus for both clinical and research endeavors[10,25,34,26–33]. Furthermore, these parameters are similar to intracranial stimulation parameters used in other ongoing translational research protocols at Emory University Hospital[35,36].

2.2.7. Memory encoding experiment

To determine whether audiovisual flicker impacts recognition memory, subjects may be asked to perform a memory task in which they view a series of images or words from a standard set. Memory for these items will be assessed at the end of the initial session or 1 day later. The general paradigm is based on memory enhancement experiments from our team using amygdala electrical stimulation[12], that have previously been approved by the Emory IRB (IRB00067252).

The subject will be presented with a series of images or words referring to objects. Each item will be presented only briefly (e.g., 3 sec). During presentation of items in the study phase, the subject will be asked to state an intrinsic characteristic of the object (e.g., whether the object is used indoors or outdoors). Presentation of some of these items will be paired with brief (e.g. 1-10 seconds) audiovisual flicker. During presentation of items in the test phases, the subject will be asked to determine whether he or she has previously seen the exact object (e.g., Yes / No) and to gauge his or her confidence in this response (e.g., Low / High).
Trials will be divided into blocks, with extended delay periods (e.g., 10-20 sec) allowing the subject to rest briefly at several points throughout the study phase; the test phases will be self-paced. The anticipated duration of the experiment is about 55-75 min on day 1 and 15-20 min on day 2.

2.2.8. Memory consolidation experiment

To determine whether audiovisual flicker impacts memory consolidation, subjects may go through the following experimental paradigm or a similar one, based on Roberts et al.'s (2018)[13] experiment (Figure 5).

Patients will view 4 series of words or images. In 2 of these series, they will be asked for each word or image whether it refers to a living thing, while in the other 2 series, they will be asked whether each word or image refers to something that is man-made. This will be followed by a 36-minutes period of exposure to audiovisual flicker (either random or 5.5Hz or 40Hz, via the customized DAVID device). Finally, they will undergo an unexpected test of memory for the words or images they've been exposed to (item recognition), as well as the associated contextual sorting they had to perform (alive vs man-made, i.e. source recognition).
For this experiment, depending on his or her choice, a patient may go through the task more than once on different days, being exposed to a different condition each time (random, 5.5Hz, or 40Hz audiovisual flicker). This would allow us to compare the effects of specific frequencies of audiovisual flicker on memory, in the same patients.

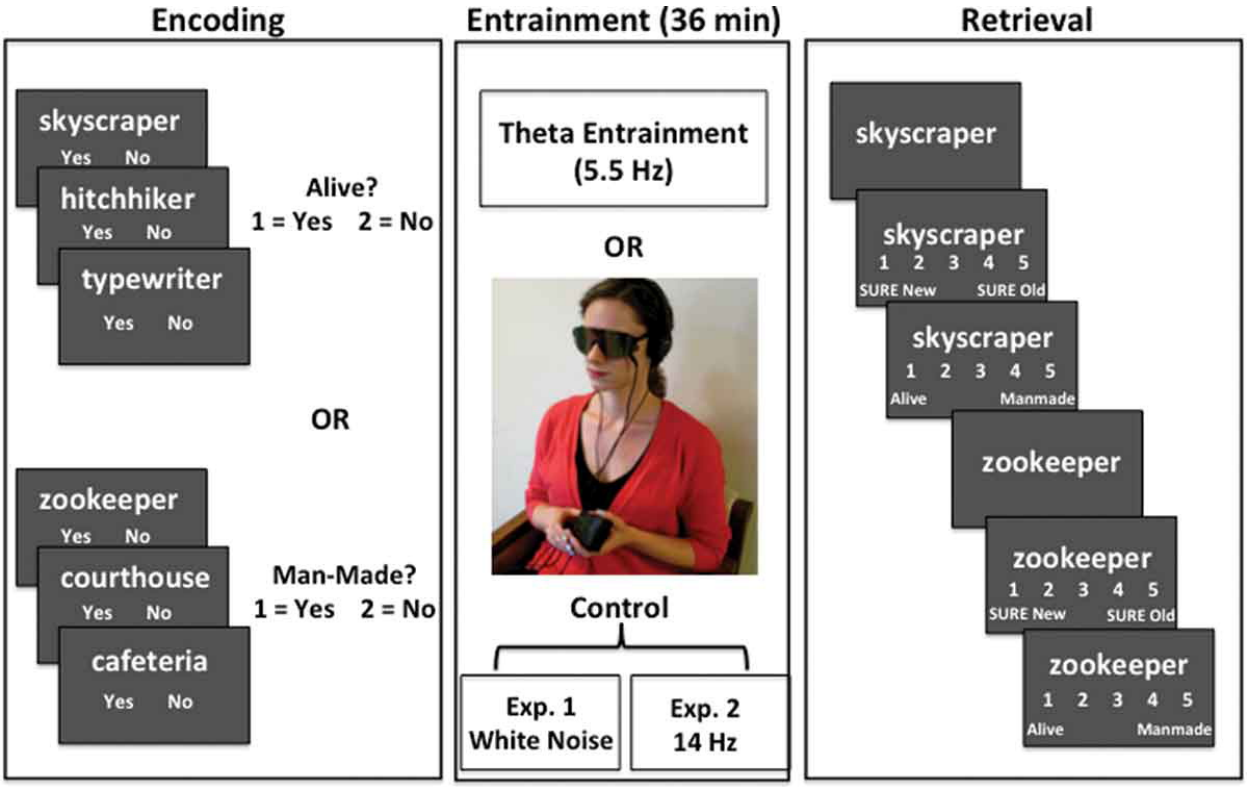

Figure 5: experimental paradigm from Roberts et al. (2018). In our experiment, we may use images instead of words, and patients will be tested for effects of either random, 5.5Hz or 40Hz audiovisual flicker on memory. In case a patient were to be tested for the effects of more than one type of sensory flicker, he or she would be tested for each type of sensory flicker on different days.

The anticipated duration of this experiment is about 120min for each day.

2.2.9. COVID-19 related procedure

Our study subjects are inpatients undergoing intracranial seizure monitoring as part of an evaluation for epilepsy surgery. As part of their clinical care, they are continuously video-monitored for seizure semiology, including facial movements that could be critical to understanding their seizure network; furthermore, sometimes they only have very few seizures

while admitted and getting maximal information from each seizure is critical in determining the surgical approach. Thus, having them wear a mask during a research study session, or during the consent procedure if performed while they are already inpatients, would constitute a substantial departure from their clinical care. As such, we ask for an exemption for our study subjects to wear a mask during interactions with researchers, while they are inpatients and being video-monitored for seizure semiology.Researchers will still wear a mask during any interaction with the patient.

Note: all of our participants are tested for SARS-CoV-2 virus before admission.

2.2.10. Research data storage

Data containing protected health information (PHI), such as link between subjectID and patient name, date of birth and other PHI, will be saved on a secure platform, such as Emory OneDrive.

Data that has been deidentified may be stored indefinitely on various platforms, such as Emory REDCap and the lab server. For example, de-identified metadata, such as patient neuropsychological testing results, MRI reports, electrode implant details, and experiment notes, may be stored on REDCap; other de-identified data, such as imaging, neurophysiological recordings and analysis data, may be stored on the lab server.

2.2.11. Source records and data collection

Data will be collected from either the subjects' electronic medical records (EMR), the EMU medical Natus neurophysiological recording system, or via devices and custom softwares mentioned above (2.1). Data collected in the subjects' EMRs will include metadata pertinent to this study, including age, determination of the seizure onset zone location and seizure medications taken on the day of the experiment.

2.2.12. Long term follow up

Once all research-related procedures are complete, missing data pertinent to the research study may be collected, either in the subjects' EMRs or on the EMU Natus clinical EEG server.

3. RISK ASSESSMENT

There are no direct benefits to participants in this study. However, this study will provide a better understanding of how sensory flicker affects activity in various brain areas relative to direct electrical stimulation, and whether it can impact memory performance or interictal epileptiform discharges. The gains from this study are anticipated to help develop new non-invasive treatments for several brain diseases such as epilepsy, Alzheimer's Disease, depression and obsessive-compulsive disorder.

All subjects will have already undergone an operation to implant macroelectrodes as is necessary for clinical evaluation and treatment. Additional microelectrode contacts are built into the clinically necessary macroelectrodes and are placed in the same surgery. Thus, the current proposal does <u>not</u> present additional risk from additional surgeries.

Every precaution is taken to minimize risks.

3.1. Electrical brain stimulation

Direct electrical brain stimulation is a routine procedure in the Epilepsy Monitoring Unit for localization of brain function and confirming regions of seizure onset in patients with intracranial electrodes. Safety limits have been established for decades[37,38]. Stimulus trains will utilize current regulated, charge-balanced, biphasic rectangular pulses set below the afterdischarge threshold. Stimulation trains will be brief (less than or equal to 10 seconds) and stimulation levels will be kept below the safety limits identified by histological analysis for chronic (30 $\mu C/cm^2$ per ph) and acute (57 $\mu C/cm^2$ per ph) stimulation protocols[37,38].

Brain stimulation can, particularly when applied to a seizure onset zone, cause afterdischarges (brief, localized, epileptiform activity), which are often asymptomatic. Afterdischarges are generally self-limiting but can elicit an actual seizure if repeated or prolonged. Brain stimulation can also elicit clinical seizures, which usually resemble the subject's habitual seizures. The risk of seizure is small and well known to clinicians performing stimulation for functional localization. Moreover, in a recent research study using electrical brain stimulation in patients (770 stimulation sessions across 188 patients), seizure occurred during or within 30 minutes of the stimulation session for less than 2% of sessions (Goldstein et al., In Prep). We have proposed to use stimulation parameters that are less likely to induce afterdischarges, and therefore less likely to induce seizures[29,31,33,34].

We will take the following steps to prevent risk of inducing a seizure:

- Under the supervision of a qualified medical personnel (neurologist, neurosurgeon, or neurology fellow familiar with brain stimulation for epilepsy) we will test different stimulation amplitudes to determine a threshold at which afterdischarges are observed. Subsequent stimulation currents used during stimulation sessions will be kept 25-30% below the afterdischarge threshold to mitigate risk of seizure, and these stimulation current levels will have to be approved by qualified medical personnel.
- During all stimulation sessions, the subject's clinical EEG will be monitored in real-time for afterdischarges by qualified medical personnel or intracranial monitoring reader. If any afterdischarges are observed, the protocol will be immediately halted and the patient assessed. Once afterdischarges have stopped, stimulation current will be decreased by a further 25-30% and testing will resume. If afterdischarges occur again, the protocol will be halted and stimulation sessions will be cancelled for the patient.
- No stimulation will be attempted within 2 hours of a spontaneous seizure.

If at any time clinical seizures are elicited during stimulation, the protocol will be halted and stimulation sessions will be cancelled for that patient. Seizure monitoring units have protocols in place to treat seizures. Clinical seizures that occur during the time of the stimulation sessions will be reported to the medical monitor. The patient will be informed about the risk of seizure as done in routine clinical functional mapping.

Brain stimulation may also temporarily induce unusual sensations such as tingling, movement, interruption of speech and change in mood. If this occurs and makes the patient uncomfortable, we will decrease the stimulation amplitude by 25-30% and reassess.

3.2. Exposure to light flashes

In theory, visual flicker may induce epileptiform EEG activity and seizures. Indeed, flashing lights of certain frequencies can be used to intentionally elicit seizures in certain susceptible patients.

- According to a review for the Epilepsy Foundation of America Working Group published in 2005[39]:
    - The estimated prevalence of seizures from light stimuli is about 1 per 10,000, or 1 per 4,000 individuals age 5-24 years.
    - People with epilepsy have a 2-14% chance of having seizures precipitated by light or pattern.
    - Intensities of 0.2-1.5 million candlepower are in the range to trigger seizures; frequencies 15-25Hz are most provocative, but the range is 1-65Hz; red color is a factor.
- Precautions taken in this study to reduce the risk of inducing seizures:
    - Several studies using visual flicker in humans have already been published[1,13,17–24]. Our project will expose human patients to similar stimuli and is not expected to present a risk that would be higher than in those studies, for the general population.
    - In their study, Wolf and Goosses[40] report 103 out of 1062 patients with epilepsy in their cohort to be photosensitive (i.e. having seizure or abnormal activity induced by photostimulation). Among patients with localization-related (focal) epilepsies in their cohort, only 2.7% showed photosensitivity. Based on this study and expert opinion, we expect that when photic stimulation triggers a seizure in epileptic patients, it typically occurs in patients that have a generalized type of epilepsy disorder. Most of our patients are highly selected to have focal epilepsy characterized by seizures starting in specific brain locations. Thus, we estimate that the risk of photic-induced seizures is very low in our patient population compared to the overall epilepsy patient population, except for: 1) rare patients whose epilepsies start in the occipital region or 2) rare cases where it is suspected that the patient may have a generalized type of epilepsy disorder. For such patients (1 and 2 above), we will first check that they have not been shown to be photosensitive; we will then coordinate with the clinical team to determine, on a patient-by-patient basis, whether exposure to visual flicker or individual light flashes is contraindicated with respect to clinical goals. Causing a photic-induced seizure in such patients, while not necessarily desirable for research, may provide additional information useful to the clinical team.
    - Most of our patients have already been tested for photosensitivity and photic-induced seizures during their phase I (scalp EEG) long term video seizure monitoring, at photic stimulation frequencies of 1-21Hz (for 10s trains). We will check each patient's health records to see whether they have been tested.

Patients that have been identified as photosensitive or susceptible to photic-induced seizures will not be enrolled in this study.

- Before running an experiment involving exposure to light flashes (individual flashes at around 1Hz or less, or higher frequency flicker), we will consult with the clinical team to confirm that the patient is not suspected to be sensitive to photic-induced seizures.
- For patients selected for the study, the researcher will be in the patient room anytime exposure to light flashes is administered, and available to alert the clinical team in case of a clinical seizure. Moreover, the clinical staff (such as ICM technologists) will be monitoring the patient for signs of clinical seizure, as per usual. If a clinical seizure occurs at any point during the experiment, the experiment will be stopped and the patient will be treated by the clinical staff as per usual.
- For any experiment involving exposure to light flashes, we will first choose an intensity of light that is comfortable to the subject, then test the subject for any evidence of seizure in response to the range of visual flicker or individual light flashes' parameters to which we would expose the subject.

Flicker (including in given cases invisible flicker) may trigger a range of temporary symptoms that do not represent an immediate health risk to the patient but may induce pain or discomfort, such as:

- Psychogenic, nonepileptic seizures (PNES). If a patient has a pre-existing diagnosis of PNES, we will check whether they have received photic stimulation in the clinic, and whether such stimulation triggered an episode of PNES; we will also consult the clinical team's opinion on whether sensory stimulation constitutes a risk to trigger PNES in that patient. Patients considered at risk of PNES triggered from such sensory stimulation will be excluded from the study.
- "migraine or severe paroxysmal headache often associated with nausea and visual disturbances"[41].
- "malaise, headache, and impaired visual performance"[41].
- "increased repetitive behavior among persons with autism"[41]. Patients with a pre-existing diagnosis of autism will be excluded from this study.
- "asthenopia, including eyestrain, fatigue, blurred vision, conventional headache, and decreased performance on sight-related tasks"[41].
- "panic attack, anxiety, and vertigo"[41].
- "decreased performance on certain tasks"[41].
- "increased heart rate in agoraphobic individuals"[41].
- Discomfort.

A published human study[13] involved audiovisual stimulation for 36 minutes at 5.5Hz, 14Hz, or exposure to white noise, using a device similar to one of the devices (customized DAVID devices) we will be using. Thus, we expect to find similar minimal levels of discomfort as were found in this study. At any point in the experiment, if any of the issues mentioned above manifest, or if the patient complains of discomfort, we will modify parameters of the light flicker or individual

light flashes (such as flicker frequencies tested, light intensity). If issues persist, we will discontinue exposure to light flashes for this subject.

3.3. Exposure to auditory stimuli

Seizures induced by auditory stimuli are extremely rare and usually well-known to such a patient and their clinical team[42]. As in the case of exposure to light flashes (cf above), the subject will be monitored by both researcher and clinical staff for signs of a clinical seizure. If such signs are noted, the experiment will be stopped and patient treated as per usual.

Exposure to auditory stimuli might theoretically induce discomfort. We will first find a volume of sound that is comfortable to the patient. During an experiment, if the patient complains of discomfort, we will modify parameters of the auditory stimuli (such as flicker frequencies tested, sound volume). If discomfort persists, we will discontinue exposure to auditory stimuli for this patient.

3.4. Memory experiments

The subject could experience eye strain from looking at words or images on a computer screen for extended periods of time, and could experience anxiety from memory testing. In our previous studies, such complaints are extremely rare and minimal.

3.5. Spatial navigation task

The subject may here also experience eye strain or nausea from navigating in a virtual environment for extended periods of time, and could experience anxiety from memory testing. In our previous studies, such complaints were extremely rare and minimal. They may also experience frustration or fatigue from memory testing. Moreover, it is possible that navigating in the virtual environment, while being exposed to visual and/or auditory flicker simultaneously, might induce similar symptoms or discomfort. The patient will be monitored at all times, and the stimulation parameters (brightness, volume) will be modified if the patient complains of any symptoms or discomfort, or the experiment will be stopped altogether if those changes do not resolve the issue. Moreover, special care will be given to the wellbeing of the subject and testing will be stopped at the subject's request.

3.6. Custom cable and splitter box

Custom cables and splitter boxes have been developed with Blackrock Microsystems to split the signal between the clinical EEG system and the Blackrock neural recording and stimulation devices. These components have been extensively tested to ensure that they provide uninterrupted physiological monitoring capabilities. However, as with any component, there is a very small risk of failure.

Cable failure does not put the subject at risk of injury. However, it does introduce the risk of data loss. If the cable fails during the time when the subject is not having a seizure, then there is no risk to the subject. However, if the cable fails while the subject is having a clinically necessary seizure then this may extend the subject's length of stay in the Epilepsy Monitoring Unit (EMU) until the subject has another seizure that can be localized. The risk of a cable failure that extends

the subject's stay in the EMU is anticipated to be very small. In addition, there is no reason to expect that the risk of the Blackrock cable failure is any greater than the risk of the standard clinical cable failing.

3.7. Breach of confidentiality

The link between subject identifying information, such as subject's name, and assigned study subject identifier, will be saved on a PHI-compliant server that only research personnel may access. Furthermore, data will be stripped of PHI as much as possible and as soon as possible. Data with limited subject PHI will be stored on PHI-compliant servers that only research personnel may access. No identifying information will be used in any publication that results from this research. Behavioral data are only coded with an indirect identifier and date of research testing, and stored on a secure, password protected laptop or computer and stored later on HIPAA compliant servers that only research personnel may access. Should at any time a breach of confidentiality be detected, the patient(s) will be notified directly, and we will undertake a thorough reevaluation of methods to protect patient confidentiality.

De-identified data from this study (data that has been stripped of all information that can identify the subjects) may be placed into public databases where, in addition to having no direct identifiers, researchers will need to sign data use agreements before accessing the data. We will remove or code any personal information that could identify the subject before information is shared. This will ensure that, by current scientific standards and known methods, it is extremely unlikely that anyone would be able to identify the subject from the information we share.

Data from this study may be useful for other research being done by investigators at Emory or elsewhere. To help further science, we may provide subject deidentified data to other researchers. If we do, we will not include any information that could identify the subject. If data is labeled with a subject's study ID, we will not allow the other investigators to link that ID to the subject's identifiable information.

3.8. Sharing of results with participants

In general, we will not give the subject any individual results from the study of the data that is produced from their participation. If we find something of urgent medical importance to them, we will inform them, although we expect that this will be a very rare occurrence.

3.9. Potential benefit to participants

There is no direct benefit to participants from this study, except possibly entertaining them during their hospital stay. Subjects will not be compensated for participating in this study.

3.10. Cost to participants

There will be no costs to the subject for participating in this study. They will not be charged for any of the research activities.

4. PARTICIPANT RECRUITMENT

We aim to include, across all experiments, about 100 adult patients (most patients will only participate in one or a few of the experiments) with pharmaco-resistant epilepsy in whom intracranial depth or grid/strip electrodes are implanted in order to determine the area of seizure onset for possible surgical resection. We anticipate having access to an adequate sample size to attain significant preliminary data in one year. Between two neurosurgeons, our center implants >50 epilepsy patients per year for intracranial monitoring. Approximately 90% of these patients will meet all inclusion criteria.

4.1. Inclusion criteria

- Adult (>18 years, regardless of gender, race or ethnicity).
- To be implanted with intracranial depth or grid/strip electrodes for surgical evaluation.
- Patient was not shown, during phase I seizure monitoring, to exhibit abnormal EEG activity in response to photic stimulation, and is not clinically suspected to be susceptible to photic-induced seizures.
- Patient has no pre-existing diagnosis of autism.
- Patient is not considered at risk for psychogenic nonepileptic seizures (PNES) triggered by sensory stimulation.
- Fluent in English.
- Able to understand an informed consent (comprehend potential risks and benefits).
- Give written and verbal informed consent to all experiments patient would participate in.

4.2. Exclusion criteria

Failure to meet any one inclusion criteria.

4.3. Informed consent

To avoid any sense of coercion of the potential participant by the patient's physician, another investigator or the study coordinator will be the recruiter for the study. The physician will refer a patient who satisfies the inclusion criteria to the recruiter, but he will not be the recruiter for the study. All recruited patients engage in a detailed informed consent before the study, prior to becoming an official participant. The below actions surmise the detailed informed consent by the recruiter:

- The recruiter fully explains the purpose, procedures, risks, and benefits of participation, efforts to safeguard all confidential data, their freedom to opt out of the study at any time without affecting any current or future care that they may receive, and other terms, all of which are on a form (see eIRB attachment).
- After the recruiter concludes the explanation, a patient who is interested in becoming a participant in the study signs an informed consent form (see eIRB attachment) to acknowledge their participation and understanding of terms for the study.
- A copy of the consent form is given to the patient and the original document is placed in their medical record.
- A person who declines to participate in the study will sign nothing, but be reminded that their decision will not affect their clinical care.

The consent process may be done in person at Emory University Hospital or remotely. In the case of remote consent:

- The subject will be contacted (by an investigator other than patient's physician, or a study coordinator) via phone or other form of electronic communication such as Zoom, to gage interest in the study. If the subject is interested in participating, a copy of the consent form will be provided to the patient so he/she can read over it, either via encrypted email or an intermediary person, such as the patient's physician or another member of the study team.
- The subject will then be contacted again via phone or other form of electronic communication such as Zoom, to walk the patient through the consent form, answer any questions, and further gage interest in the study. If needed, the patient will be provided further time to read over the consent form.
- If the subject agrees to participate, he/she will sign the consent form, and either:
    - Email (via encrypted email) or fax a scanned copy, or email a picture, of the signed page of the consent form.
    - Give the signed consent form to a study member.
- The investigator (not the patient's physician) or study coordinator who carried out the consent discussion will then sign the consent form, to complete the consent process.

4.4. Study timelines

We anticipate that each recruited subject will participate in one or more experimental sessions (if and when the patient is agreeable to do so) during the 2-4 weeks while they are in the hospital being investigated by the clinical team for intracranial seizure localization.

4.5. Withdrawal from study

Any includable patient who declines to enroll in the study or included patient who wishes to withdraw from the study after consenting to participate is allowed to withdraw from the study with no effect on the clinical care for the patient. Our previous experience with over 100 patients who undergo presurgical evaluation with implanted electrodes demonstrates that the vast majority of patients accept participation in research studies while they are being monitored for seizures.

The researchers will stop a subject's participation in the study without their consent for any reason, especially if they believe it is in their best interest or if they were to object to any future changes that may be made in the study plan.

5. STATISTICAL ANALYSIS

For objectives 1 and 2, we will perform analyses similar to the ones used in Martorell et al.'s study[2]. We will perform a power spectral density analysis of LFP signal in function of frequency and modality (visual, auditory or audiovisual) of the sensory flicker stimulus, using multi-taper methods. Comparisons will be drawn between the mean power spectral density under a given condition and control (random sensory flicker or no flicker) condition, using the Wilcoxon rank-sum test with Bonferonni correction for multiple comparisons. To analyze firing rate modulation of single units to sensory flicker, we will produce a histogram of each single unit's firing rate

aligned to the onset of light and/or sound stimulus. Moreover, we will analyze how strongly the firing rate of single units is phase-locked to the stimulus being played, by calculating and comparing the vector strength of their firing rate modulation under different conditions, using the Kolmogorov-Smirnov test.

For objective 3, we will use similar methods as mentioned above, and compare the effect size of LFP entrainment and firing rate modulation in different brain regions, in function of various sensory flicker parameters (i.e. frequency and modality).

For objective 4, we will do within-subject comparison of the rate of interictal epileptiform discharges during various stimulation and/or baseline conditions. If normally distributed we will use t-test to compare the conditions, otherwise we will use repeated-measures ANOVA.

For objective 5, memory performance will be compared with a repeated-measures ANOVA across the different conditions (e.g. for example, 5.5Hz or 40Hz audiovisual flicker vs. random audiovisual flicker). For one of the memory experiments, performance in the case of random flicker will serve as the baseline from which we will infer any improvement or impairment in memory performance.

All neural data analyses will be performed in Python and/or MATLAB.

6. SAFETY AND MONITORING

6.1. Plans to Monitor the Data to Ensure the Safety of Participants and Integrity of the Data

The study has a plan to monitor data for safety of the recruited patients. The real-time review of participant's data during data collection will be done by a co-investigator on the protocol and everyone on the study team are responsible for primary data collection. The review of regulatory files will be done by one of the study coordinators at least once annually. The review of the consent forms will also be done by one of the study coordinators during regulatory monitoring visits, at least once a year. The review of all adverse events will be done by the study's safety monitor every time an adverse event occurs. The monitoring of critical data points will be done by the PI on the study every time a participant is recruited by Dr. [redacted] or Dr. [redacted]. A review of the subjects' record will be done by one of the study coordinators at least annually.

DSMP Requirement	How this Requirement is Met	Frequency	Responsible Party(ies)
Site Monitoring at pre-determined intervals: The Principal Investigator has a responsibility to ensure that the study is following all aspects of the protocol.	*There should be a standard operating procedure to review data (whether a sample or 100%) at pre-determined intervals to ensure that there is adequate documentation of critical elements such as eligibility criteria. Monitoring is required at the following timepoints (but may be done more frequently):*	*At a minimum, a review is required annually when no one has been enrolled or the study is in long term follow up. Additional interim monitoring at least once every 12-24 weeks based on*	*Delegate a responsible party for each requirement below*. Self-assessment is NOT acceptable. An experienced, knowledgeable person **who is independent of the study team** should serve as monitor. A*

	• *study initiation* • *at least every six months while participants are receiving intervention and* • *annually while participants are in follow-up*	*the site activity, and more as needed, to include the possibility of remote monitoring.*	*Contract Research Organization (CRO) may be used. Consult the IRB Office regarding acceptable qualifications for the independent monitor, if not using an outside expert such as a CRO.*
Real-time review of participant data during initial data collection.	The review will be done by a co-investigator on the protocol.	*Expectation is that this happens every time you obtain information.*	*Everyone on the study team responsible for primary data collection.*
100% review of regulatory files	The study coordinator will review the regulatory files.	*Reviewed at a minimum of first and close-out visits*	Dr. [redacted] or the study coordinator.
100% review of consent forms	The study coordinator will review the consent forms.	*Reviewed for every participant as they are recruited*	Dr. [redacted] or the study coordinator.
Review of credentials, training records, the delegation of responsibility logs (if applicable)	This review will be part of an annual regulatory monitoring	Once a year	Dr. [redacted] or the study coordinator.
Comparison of case report forms (CRF) to source documentation for accuracy and completion	This review will be part of an annual regulatory monitoring. Sample (10%) will be monitored, with additional CRFs monitored if significant discrepancies are found during the initial 10% sampling.	N/A	N/A
Review of documentation of all adverse events	The Safety Montor will review them.	Every time they occur.	Dr. [redacted]
Monitoring of critical data points (eligibility, study endpoints, etc.)	The PI on the study.	Every patient recruited.	Dr. [redacted] or Dr. [redacted]
Laboratory review of processing and storage of specimens	N/A	*N/A*	N/A
Assessment of laboratory specimens stored locally	N/A	*N/A*	N/A
Test article accountability review	N/A	*N/A*	N/A
Accountability logs, dispensing records, and other participant records	N/A.	*N/A*	N/A

For FDA regulated studies, the following requirements apply:	How this Requirement is Met	Timing, frequency, and intensity of monitoring	Responsible Party(ies)
Monitoring methods (may include centralized, on-site, and self-monitoring)	On-site	At minimum annually	Dr. [redacted] or an independent monitor
*For international studies, you are required to engage a CRO that is working in the site country and/or to consult with Emory's legal counsel regarding compliance with the country's clinical research regulations.			

6.2. Study monitoring and adverse event reporting

No data and safety monitoring board (DSMB) is used in this study. However, the study monitors the progress of the research and the welfare of the participants.

Dr. [redacted] will be the Data and Safety Monitor. The study team will keep track of any adverse event occurring for any research session, looking for any of the expected adverse events that are listed in Section 3 (Study Risks) and will follow and document the preventative and mitigation steps related to risk management as described. The study team will review the patient clinical progress notes for the days of the research testing to assess for potential adverse events beyond the mere testing window.

Potential adverse events will be tracked in a log using with the following information:

- Description of the adverse event.
- Start and end time of adverse event.
- Flicker sensory modality (neurostimulation, aural or photic)
- Grade: mild, moderate, minor, severe.
- Whether the adverse event was anticipated or not.
- Research related: not related, possibly related, definitely related.
- Status: recovering, recovered or recovered with sequelae.
- Mitigation steps taken to address the adverse event.

Additionally, any adverse event that involved a seizure or was not anticipated in the protocol, will be promptly reported to the Data and Safety monitor, who will review the information above using source data (including patient's stereo-EEG and video recording and electronic medical record). Any unanticipated AE would also be reported to the IRB.

The study data source will also document that no adverse event occurred.

The Safety Monitor will also review the adverse event log and related source documentation at least once annually.

Adverse events with the following features will be promptly reported to the IRB:

- Not anticipated by the protocol.
- Frequency or intensity exceeds what is expected.

- Changes the risk to the subject.
- Necessitates changes to the protocol or consent.
- Affects the willingness to participate in the study.
- Affects the rights of a subject.

Finally, the Data and Safety Monitor will review periodically the AEs (or absence of AEs) against the source data (including stereo-EEG recording, and patient electronic medical record) as well as their timely review and if proper mitigation steps were taken. The study monitor findings (or absence of any findings) are reported periodically to the IRB (at a minimum annually), along with a recommendation about continuation of study with or without changes in the protocol and testing procedures.

The medical doctor for the patient ([redacted]) holds primary responsibility for monitoring the daily progress and safety of each participant. Because the investigational procedures of this study occur while each participant is hospitalized as a patient in the Emory EMU, the EMU staff (not members of this study), the technical team in the EHc-IS (also not members of this study), and the co-investigator performing the experiments will ensure safe and secure daily monitoring and data-collection for each participant (see '2.2.2. Monitoring participants'). The data that is under constant monitoring until the end of this study includes but is not limited to the following information:

- Administrative information
    - proper attainment of informed consent
    - signed forms from informed consent
    - adverse events
    - withdrawals from the study
    - other documentation
- Demographical information
    - demographical data via survey
    - names
    - geographic data
    - dates (directly related to an individual)
    - telephone numbers
    - email addresses
    - medical record numbers
- Experimental data
    - raw and analyzed neurophysiological recordings
    - raw and analyzed video
    - memory performance scores
    - behavioral records from memory testing (e.g. keystrokes, verbal reports in the form of auditory recordings, game controller or keyboard inputs)
- Medical information (epilepsy patients)
    - neurophysiology or neuroimaging of the brain
    - video recordings in the EMU

- clinical notes or reports before, during, or after epilepsy-monitoring
- clinical notes or reports of psychological exams and visits (mental health)
- pre-surgical and post-surgical evaluations (if applicable)
- other clinical data (e.g., charts, tests, reports, labs) that are relevant to this study

Moreover, the EMU staff ultimately reports all medical information to a neurologist and to a neurosurgeon (R.G.) and all technical information to the director of clinical neurophysiology. The principal investigator uses all information to assess the status of each patient. Any adverse event or complication due to the study will be carefully documented, followed, and submitted to the Emory IRB via the "Reportable Event Form."

All data collection and monitoring will be in compliance with the Emory University *Clinical Trials Guidebook*. To adhere to HIPAA guidelines about confidentiality in collecting and disseminating human data, all data will be stripped of identifiable information as soon as possible and as much as possible. Data containing minimal PHI (such as brain imaging, or auditory recordings of the patent's verbal recall for some of this study's tasks) will be stored on HIPAA-compliant servers only accessible by the research team. The confidential data in this study will include medical records, neurological or psychological exams, medical imaging of the brain, EEG, EMG, video, surgical outcomes at typical time points (e.g., 6 weeks, 6 months, 1 year), and the study's behavioral data (such as verbal reports in the form of auditory recordings). Data will be de-identified as much as possible by assigning each participant an alphanumeric ID (study number) as a reference. The relationship between the encoded ID and all identifiable information about the participant (e.g., name, date of birth) will be known and accessible by only our research team. Any publication or presentation that uses the data from patients in this study is reported using the study numbers for the patients. All members of this study have completed the Collaborative IRB Training Initiative (CITI) tutorial on the responsible conduct of human research.

If a patient joins our study, they will be donating data that is produced from their participation in the study, and their study information. These data and study information may be used for research purposes related to this study or for research purposes unrelated to this study. If a patient withdraws from the study, data and study information that were already collected may still be used for this study or other research purposes that may be unrelated to this study.

The subject stopping rules are voluntary withdrawal by the participant, withdrawal due to an adverse event by the safety monitor or attending neurologist. The study stopping rules is when the PI decides to stop recruiting patients for the study and sufficient data for primary and secondary objectives have been collected.

This study includes persons from Emory University (EU), and only EU members recruit potential subjects (EU patients) and collect any de-identified or personally identifiable data before it is de-identified for other researchers. Since the main research, such as recruitment and data-collection, for this study occurs at EU and since the only review and approval of this protocol is by the Emory Institutional Review Board (IRB), all EU members, especially the PI (R.G.), accept

full responsibility for the safety and monitoring of each participant and their data as well as all aspects of this protocol. Please see the protocol for the list of the above personnel.

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