Title: Electrophysiological biomarkers during invasive monitoring of mesial temporal lobe epilepsy patients

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Emory IRB Biomedical Protocol Outline
For Investigator-Initiated Studies

Title Page

Electrophysiological biomarkers during invasive monitoring of mesial temporal lobe epilepsy patients

Short study title: **Electrophysiologic biomarkers in MTLE patients**

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- No external collaborators.

Protocol version number and version date

Version 2.0 , September 9, 2021

Revision #	Version Date	Summary of Changes
2.0	September 9, 2021	Changes to the Intracranial Stimulation Protocol section (Study Design and Methods): (i) addition of 2 new stimulation frequency pulse-width combinations for safety threshold testing; (ii) Addition of stimulation-trains of up to 15 minutes for use in revised memory testing protocol. Change to ADMES effects on memory (Study Design and Methods): (iii) Revisions to the memory testing procedure to include object recognition tasks after initial encoding and 15-minute distraction period along with continuous 15-minute neurostimulation-trains and ("Electric WADA").

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Study Summary

Study Title	Electrophysiological biomarkers during invasive monitoring of mesial temporal lobe epilepsy patients
Study Design	Nonrandomized interventional
Primary Objective	Identify biomarkers related to the precictal state; determine stimulation patterns that modify biomarkers
Secondary Objective	Assess free recall during stimulation
Research Intervention	Brain stimulation via clinically implanted electrodes
Study Population	Subjects with medial temporal lobe epilepsy undergoing phase II surgical work up with clinically implanted intracranial electrodes
Sample Size	18 Subjects
Study Duration	2 years
Study Duration for Individual Patients	3 recording sessions over 1 week
Study Specific Abbreviations	ADMES- asynchronous distributed multi-electrode stimulation CT- computerized tomography EEG- electroencephalography EMU- epilepsy monitoring unit IV – intravenous LFP- local field potential MRI- magnetic resonance imaging MTLE- mesial temporal lobe epilepsy NHP- nonhuman primate RNS- responsive neurostimulation SEEG- stereo electroencephalography
Funding Sources	None

Objectives- The Primary Aim and Outcome Measures

The objective of this study is to identify biomarkers related to the brain state prior to medial temporal lobe seizures and to use brain stimulation effectively change these biomarkers.

Specific Aim 1. Identify biomarkers related to the pre-ictal state

Specific Aim 2: Perform an acute parameter search to determine the stimulation pattern that most effectively modifies these biomarkers.

Specific Aim 3: Identify changes in memory (free recall) during asynchronous distributed multi-electrode stimulation (ADMES)

Background

While resective surgery for medically intractable epilepsy can be curative, only a small proportion of the 1 million people in the US with this condition can under go surgery due to risk of neurological deficits[1]. For example, some patients with mesial temporal lobe epilepsy (MTLE), the target population for this study, can be at risk for significant memory decline and associated disability[2]. The only option for these patients at present is electrical neuromodulation which, although effective at reducing the number of seizures, only achieves seizure freedom in ~10% of patients ([3, 4]). As seizure freedom is the clear goal of therapy, there is a large unmet need for novel approaches.

Currently responsive neurostimulation (RNS, Neuropace, Inc) is the only FDA approved implantable neurostimulation device approved for neuromodulation directed at the hippocampus[3]. Recommended stimulation protocols include the use of high frequency (130-200 Hz) stimulation synchronized across implanted electrodes in response to detected seizures[5, 6]. In contrast, we have found that delivering *asynchronous* pulses distributed across a multi-electrode array of 16 micro-electrodes and stimulating at low (theta) frequencies is more effective than macrostimulation in controlling seizures in a rodent model of MTLE[7]. Asynchronous distributed multi-electrode stimulation (ADMES) can be delivered in a continuous fashion, but has the potential to be used in a responsive or optimized manner as well. These stimulation modes require the ability to identify biomarkers, or neurophysiologic features identifiable on recording on neural activity, indicative of impending seizures to optimize stimulation. The goal of the present study is to explore potential biomarkers that could distinguish between the inter-ictal (between seizures), pre-ictal (before seizures) and ictal states (during seizures) to be used later for optimization of stimulation parameters in human subjects with implantable neurostimulation device.

As a high volume epilepsy surgery center, we have a unique opportunity to study electrophysiology directly from the brain in human subjects with epilepsy using electrodes already in place for clinical purposes. For many patients being considered for epilepsy surgery, invasive monitoring with stereo-EEG (SEEG) electrodes is needed to localize seizure onset zone. As part of routine clinical care, SEEG electrodes are used to monitor and record brain activity and for brain stimulation for the purpose of mapping brain function and eliciting seizures. During SEEG in patients with mesial temporal lobe epilepsy, depth electrode arrays are routinely placed in the hippocampus. We can utilize these implanted electrodes to both stimulate in an asynchronous manner and also simultaneously record local field potentials (LFPs) for analysis of biomarkers.

Informed by our non-human primate (NHP) data, we will identify electrophysiological biomarkers related to the pre-ictal state and determine the influence of specific patterns of stimulation on these biomarkers. The results obtained here will be used to inform an early feasibility study of ADMES neuromodulation in patients using an implantable neurostimulation device (for which a separate IRB proposal will be submitted).

Study Endpoints

Each biomarker (or log biomarker) determined in the parameter sweep experiment will be estimated and compared by epileptic state using repeated measures analyses implemented via the SAS Procedure MIXED. Free recall accuracy will be statistically compared using a paired t-test between the ADMES and non-stimulation lists to determine whether there is any effect of ADMES on long-term memory.

Study Design and Methods

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 (Dixi Medical or Adtech) have platinum contacts for electrophysiological (electroencephalogram [EEG] and local field potential [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.

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.

Data recording and processing

Equipment: All electrophysiological data will be collected through the EMU's Xltek system or other devices that have been approved by the FDA to be sold for studies in human subjects or are equivalent to previously approved devices and have already been approved for use by the Emory IRB (IRB00076437-DARPA; e.g. Blackrock Microsystems Neuroport Data Acquisition System, Blackrock Cerestim R96 neurostimulator, and Medtronic handheld stimulators commonly used for clinical mapping). A summary of device testing and equivalence is attached for the Blackrock CereStim M96. In addition, a letter of non-significant risk determination is attached to the IRB amendment. Intracranial electrical stimulation will be delivered through these devices as well. 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) or Dixi Medical USA (Chaudefontaine, FR) 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. Microelectrode iEEG signals will be acquired using a separate acquisition system from Blackrock Microsystems (Salt Lake City, UT). In addition, the Blackrock custom cable & splitter box splits the signal between the Clinical EEG system and the Blackrock components. This custom EEG cable and splitter is introduced to allow the signal path from the subject's electrodes to be split between the Blackrock recording and stimulation devices and the clinical EEG system. In this configuration, the ECoG signal processed and recorded by the Neuroport and analyzed by the Host PC can be used to control the timing of stimulation via the CereStim. The two different arrangements are depicted in Figures 1 and 2.

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 256 or 512 samples per second and 12-bit precision for electronic storage. Both the standard and investigational macroelectrode iEEGs are automatically stored to a 2-Tetabyte RAID server that is securely managed by Emory Healthcare Information Services (EHc-IS). The stored data is directly accessed via only one password-protected Epilepsy Monitoring Unit EMU computer 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 100 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.

Figure 1: Schematic for study device in System 1.0 configuration. Here, the testing computer interfaces with the existing clinical EEG system via the Sync Box and stimulates via the CereStim.

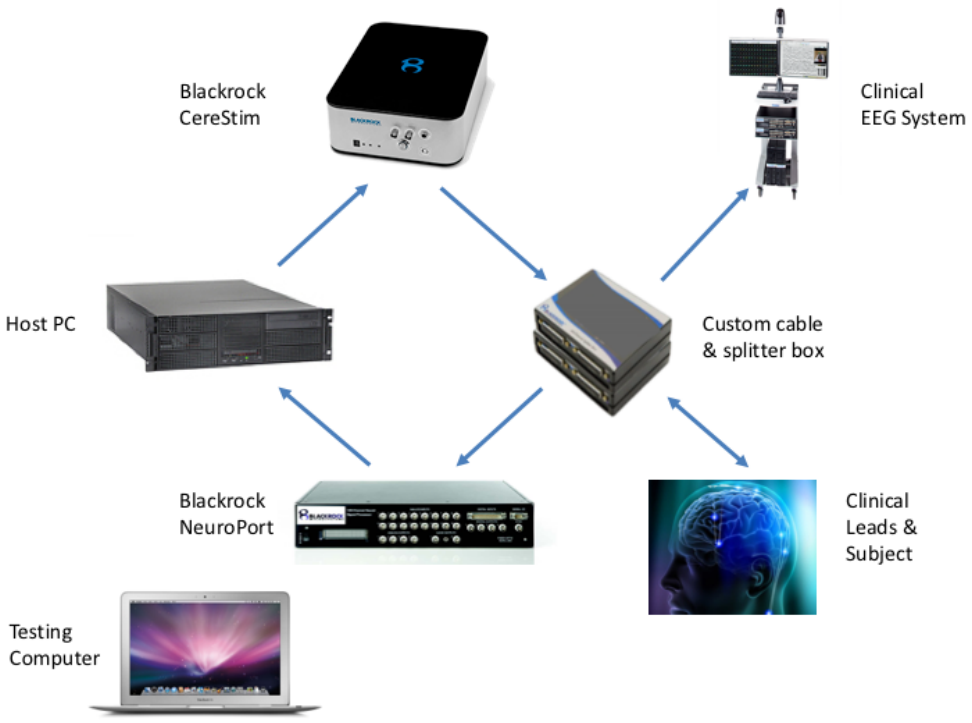

Figure 2: Schematic for the study device in System 2.0 configuration. Here, a custom cable and splitter box connects the Blackrock NeuroPort and CereStim to the subject electrodes for data recording and stimulation.

Intracranial stimulation protocol

LFPs will be continuously collected from intracranial depth electrodes before, during, and after stimulation using our data acquisition and stimulation system (Blackrock Microsystems, Salt Lake City, UT). The goal will be to measure the effect of different combinations of stimulation parameters (amplitude, frequency, and pulse width) on electrophysiological neural features (spectral power, synchrony) shown to correlate with the pre-ictal state. Multiple stimulation parameter sweeps will then be performed to identify parameters that modify these biomarkers most effectively. These parameter sweeps will initially focus on the range of stimulation parameters that will be feasible in a planned subsequent study using the RC+S device (Medtronic, MN) under a subsequent IRB to be submitted. However, given the greater flexibility of the externalized Blackrock CereStim device, additional stimulation patterns may be evaluated if supported by the initial results.

Connection of the stimulation and recording hardware (recording hardware takes a 'split' signal and also does not interrupt the clinical study) will be supervised and/or performed by a neurologist trained in electrophysiology, amplifiers and stimulators. Charge density is calculated based on the surface area of the electrode contacts, and will not exceed the 30 $\mu C/cm^2$/phase safety limit within the parameter space[8]. All stimulations will be composed of a biphasic charge-balanced square wave without an inter-phase delay. In all cases, iEEG will be continuously monitored by a neurologist during the session for the presence of after-discharges and seizures. Patients are admitted to the intracranial monitoring unit to have seizures, so this can coincidentally occur during the stimulation session.

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 impedence is 1-4 kΩ. Stimulation will be bipolar, with the electrodes placed 1.5 mm apart (surface area, 0.059 cm). Prior to testing, current amplitude will be increased in a stepwise fashion in order to identify the threshold for afterdischarge (anticipated range 0.2-2 mA), and then decreased by 25% for the maximum amplitude for the duration of testing. The subjects will be blinded to stimulation condition. These parameters are similar to intracranial stimulation parameters used in other ongoing translational research protocols at Emory University Hospital over many years[44,45].

<u>Pre-stimulation threshold testing:</u>
Before the session, the epilepsy monitoring staff and patient will be consulted to determine if the patient has had any clinical seizures in the previous 2 hours. If the patient has had a clinical seizure in the previous 2 hours, then the experiment will be delayed until a 2 hour,

seizure-free period has been met. For each session, we will ask the patient for verbal consent to testing. During the entirety of all sessions, a study neurologist, clinical neurophysiologist, or neurosurgeon will monitor the subject and view the real-time EEG for afterdischarges. 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 25-30% and testing will resume. If afterdischarges occur again, all testing will be aborted on the patient. If at any time clinical seizures are elicited during stimulation, all testing of that patient will be aborted.

The goal of the first session will be to establish the range of safe stimulation parameters that can be tested in subsequent parameter sweeps. The procedure for safety threshold testing is as follows:

- Seven stimulation frequency pulse-width combinations will be tested:
    - 2 Hz, 450 μs
    - 7 Hz, 450 μs
    - 50 Hz, 450 μs
    - 90 Hz, 100 μs
    - 90 Hz, 450 us
    - 125 Hz, 100 μs
    - 125 Hz, 450 us
- Each frequency/pulse width combination will be tested at 0.25 mA, and incremented by 0.25 mA until the patient reports adverse side-effects, undesired changes are observed in the real-time LFP, or 3.0 mA is reached. This limits the number of tests to 5 frequency/pulse width combinations at 12 current amplitudes, for a maximum total of 60 stimulation patterns to be evaluated for safety.
- The test of each stimulation pattern will consist of 5 seconds of stimulation, followed by 15 seconds of washout (20s total), repeated 3 times, for a total of 60 seconds per stimulation pattern. For lower frequencies, a longer stimulation train may be administered to increase likelihood of observing effects (e.g. 10 – 15 sec). During memory tasks, stimulation trains up to 15 minutes will be used to provide sufficient time to observe any temporary effects of stimulation on memory tasks as has been previously reported.
- The total test time will be approximately 60 minutes.
- For the following parameter search, the maximum current threshold for each frequency/pulse width combination will be set to 80% of the current that induced patient-reported side-effects or undesired changes to the LFP.
- The clinician conducting the threshold testing is at liberty to test additional safe parameters, under the current safety limit, at their discretion.
- Lower current limits may be determined based on the neurologist's discretion given what is known about the patient's epilepsy.

<u>Parameter search:</u>

During subsequent sessions, the focus will be on collecting the data necessary to evaluate the effect of different stimulation patterns (combinations of stimulation parameters) on the neural signal. The default approach will be to conduct a randomized sweep of stimulation patterns. Randomized sweeps are typically superior to the more traditional grid search

approach, as a more diverse array of stimulation patterns can be tested. While a grid search lends itself to more classical statistical analysis (e.g. ANOVA), we've shown that the data collected using a random search can be analyzed using a non-parametric Gaussian process (GP) model. The stimulation parameter space will be discretized as:

- Current – 0.1-3.5* mA with 0.1 mA increments (35 values)
- Frequency – 2-125 Hz with 1 Hz increments (124 values)
- Pulse width – 100-450† μs with 10 μs increments (35 values)
- * The maximum current will be derived based on the results of the initial threshold testing
- † The maximum pulse width is limited depending on the frequency

These three discretized parameters will be combined to produce input space consisting of a maximum 156,240 potential stimulation patterns, minus those that identified as unsafe by the initial threshold testing, and prohibited by the limitations of the hardware.

The random sweep will be conducted using 180 non-repeated stimulations consisting of 5 second of stimulation, followed by an average of 15 seconds of washout. For each stimulation, the duration of the washout will be selected from a uniform distribution on the interval [10 20] seconds. Data will be analyzed by measuring power spectral and time-series features, and using computational tools to estimate biomarkers written in Matlab. This will be done offline following the session.

An alternative approach will utilize a data-driven optimization algorithm. Data-driven optimization algorithms, particularly Bayesian optimization, can be used to efficiently search high-dimensional stimulation parameter spaces where the measured output is noisy, as is the case for neural modulation. However, successfully applying data-driven optimization is contingent on 1) having a desired electrophysiological objective to be optimized, and 2) properly selecting and configuring the algorithm. The first point, the objective function, would be a specific electrophysiological neural feature or biomarker that we want to modulate. Identifying such an objective will be dependent on both the preclinical findings of ADMES in independent non-human primate studies, as well as the results from the earlier randomized parameter sweeps (above). The second point, properly configuring the algorithm, is also critical. While a well-configured data-driven optimization algorithm can-out perform a random sweep combined with a GP model, a poorly-configured algorithm can perform much worse. As such, the data from the randomized parameter sweep will be used to construct simulation models. These will then be used to design a well-configured optimization algorithm to efficiently search for specific electrophysiological objectives. If there is sufficient evidence to use a data-driven optimization approach, it will be designed so that 1) it cannot use stimulation parameters outside safety thresholds identified during the initial testing, 2) can be terminated at any time at the discretion of the supervising clinician, and 3) if necessary, can require the approval of each stimulation pattern prior to delivering the stimulation. These experiments will use the stimulation/recording configuration depicted in Figure 2.

<u>ADMES:</u>

The effects of ADMES (asynchronous distributed multi-electrode stimulation) on neurophysiological biomarkers will be tested. Stimulation parameters will be based on our extensive non-human primate studies, in which stimulation was cycled through 4 sets of contacts within one stimulation cycle (Figure 3). Additionally, we will test whether ADMES influences the encoding and retrieval of long-term memories. This will be accomplished by using a well-established free recall task [9].

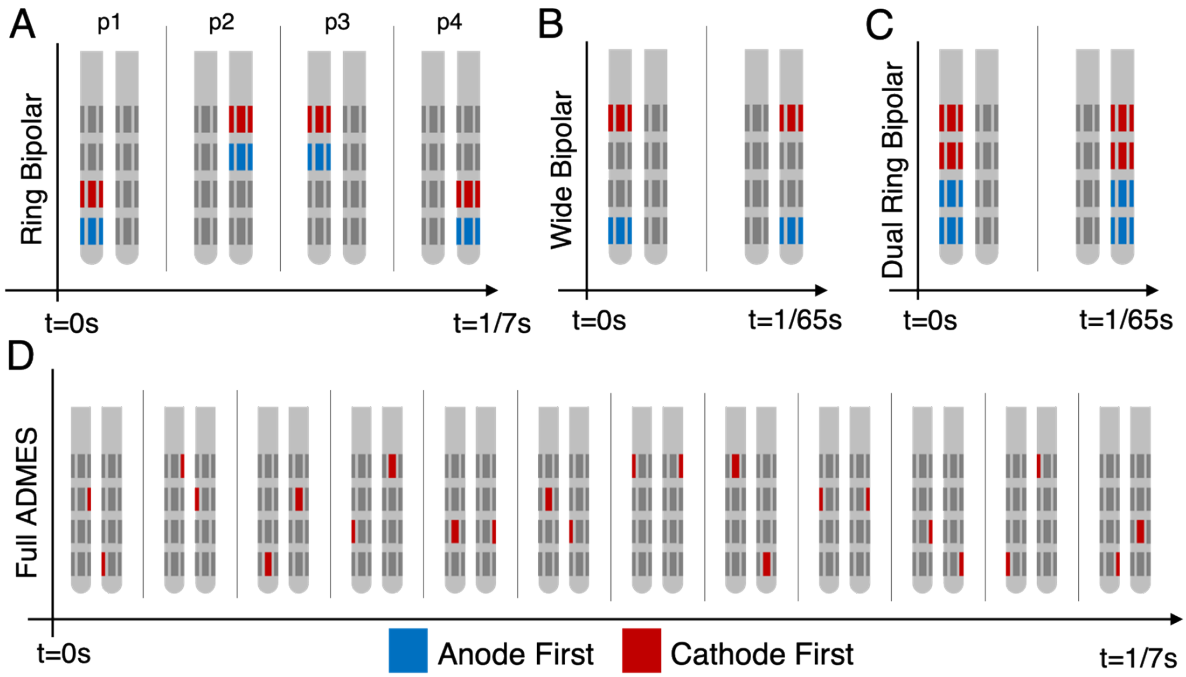

Figure 3. Stimulation patterns. Each stimulation pattern is divided into an ordered set of programs (p1, p2, …). A program defines which contacts are stimulated and with which parameters (amplitude, pulse-width, polarity). This ordered set of programs is repeated at an overall group frequency. (A) Ring Bipolar is divided into four programs with an overall group frequency of 7Hz. In the NHP instantiation, cylindrical ring contacts were segmented into 3 equally spaced equatorial contacts; in each program a set of three segmented contacts were stimulated simultaneously to create a ring of activation closely approximating the human contacts used in intracranial EEG. This ring is paired with an adjacent ring of contacts with the opposite polarity. All four programs are cycled through over 1/7s, with a fixed time interval between programs (~1/7/4s). (B) Wide Bipolar uses the two outer rings of each electrode to create two wide alternating fields of activation. The two programs are specified with a group frequency of 65Hz. This is intended to create a volume of tissue at the seizure focus that is activated at 130Hz. (C) The Dual Ring Bipolar pattern is identical to the Wide Bipolar, except the top and bottom two rings are combined into a “super-contact” (D) This instantiation, where individual segments are stimulated, is not being used in the ring contacts of the human study. The order of contacts stimulated is fixed, but arbitrary.

We will test 3 distinct asynchronous multi-electrode stimulation patterns shown in **Figure 1**:

- Ring Bipolar – 7 Hz stimulation with 4 rings per electrode created by shorting the three segmented contacts
- Wide Bipolar – 65 Hz stimulation using top/bottom 2 shorted ring-contacts
- Dual Ring Bipolar – 65 Hz stimulation using 2 large contact-groups per electrode created by shorting the top and bottom halves of the electrode

Each of these stimulation patterns will consist of symmetric charge-balanced tri-phasic pulse-trains with anodic and cathodic phases of 200μs and an interphase gap of 53μs. The current controlled amplitude will be determined per-pattern according the safety considerations described above. Stimulation will be applied using an intermittent paradigm of 5 seconds of stimulation followed by 5 seconds without stimulation. This approach will be used so that if there were electrographic seizures they could be clearly identified between the stimulation artifacts.

ADMES effects on memory:

We have proposed an electric Wada test to be used to evaluate the safety of stimulation in the human following the parameters of the current UG3 grant.

This procedure will follow the protocol of the well-established Medical College of Georgia (MCG) Wada (behavioral) procedure used with the setting of the intracarotid amobarbital procedure (10). This protocol was designed to introduce novel objects, which could be dually encoded by visual and verbal means during a period of drug anesthesization, with an evaluation of recognition memory to occur after the effects of the drug had cleared. The period of delay tended to average 10 to 15 minutes in this chemical procedure. We will follow the exact same behavioral protocol while replacing the administration of drug with the administration of electrical stimulation. As we are more interested in determining any effect on function, we will apply stimulation during the entire test session (i.e., encoding through recall) rather than just during encoding.

We will establish a baseline with each potential subject by testing them during a period free of electrical stimulation. They will be presented with a set of 8 real objects, presented one at a time in sequential order, while being provided with the name of each object orally in simultaneous fashion (e.g., “remember the fork” when being presented with an actual fork). After all 8 objects are presented, we will have the patient engaged in another task for 15 minutes, and then ask them to freely recall all 8 objects. Next, we will show them the 8 objects intermixed with 16 similar foils, and have them respond “yes” or “no” as to whether or not they have been shown each object. We are primarily interested in the recognition recall score, consistent with the use of the aforementioned chemical Wada.

The same procedure will be used with a different set of objects during the application of stimulation. We will complete two trials at the highest tolerable frequency of stimulation using both asynchronous and continuous stimulation. The encoding phase will likely require approximately one minute. After the novel objects are presented to the subject, they will be asked to engage in additional tasks during the 15-minute delay period. We

will have them perform standard clinical tasks of attention and processing speed during the delay period (e.g., Digit Span, Coding tasks, verbal fluency), as these are tasks that could be affected by stimulation (i.e., tasks of general function that are affected by any general disruption in brain function). Following the delay period, the subject will be asked to recall the 8 target objects, and their verbal responses will be recorded. We will calculate their total score and any false positive errors. Next, we will show the subject the 8 objects intermixed with 16 similar foils, and we will have them respond "yes" or "no" as to whether or not they have been shown each object. This will allow us to calculate scores that reflect the total number of accurate identifications (i.e., total "hits" out of 8) a total number of false positive errors, and a corrected score (i.e., half a point will be deducted from the total hits for each false positive error made).

We will create three sets of objects for this procedure, so that it can be repeated to obtain a baseline as well as to test the asynchronous and continuous stimulation parameters.

Procedures Involved

Clinical assessments

Prior to enrollment in the study, all patients will have had a standard presurgical workup, including video EEG monitoring, brain MRI, PET scan, neuropsychological testing, and functional MRI or Wada (sodium amobarbital) test. As part of their presurgical workup, they will require an intracranial electrode study (stereoelectroencephalography, SEEG) for further seizure focus localization, with multiple hippocampal electrode arrays, either unilaterally or bilaterally.

Patients will be admitted to the hospital for surgical implantation of depth electrodes. After electrode placement and confirmation of electrode location with imaging, patients will be monitored with continuous intracranial EEG recording in the Emory Epilepsy Monitoring Unit. Post-operative imaging (CT or MRI) is used to confirm location of the electrodes. Their seizure medications will be lowered, directed by the epilepsy team, in order to capture and characterize habitual seizures. This "seizure capture" phase typically lasts 7-21 days, with minimum of 3-5 clinical seizures captured during this time.

Prior to the Testing Session

We will schedule a time that is convenient for the patient to undergo testing, typically suggesting that a 1-2 -hour block of time is ideal. On the day of proposed testing, we will again check with the clinical team and patient that the originally proposed time and date is still acceptable.

Patient Instructions

General Instructions. The patient is asked to get comfortable and if they need to use the restroom or need anything to drink. The patient is asked to minimize head movement during the study, as well as to maintain wakefulness. During the majority of the sessions during which patient participation is not needed, they can watch television, read or talk to friends or family members that they permit to remain in the room. During stimulation, patient is continuously monitored clinically, as well as by EEG. If the patient notes anything they are to verbalize this or raise a hand. Stimulation is then immediately stopped

to discuss what was noted. A decision is then made about whether to persist with stimulation of the present electrodes or move to the next. The patient is able to stop the study at any time.

The Testing Session
Not including the time to set-up equipment, the actual testing session is expected to last ~1-2 hour and will not exceed four hours. The patient can take breaks during the session for food, rest, drink or bathroom use. The patient is at liberty to terminate the session at any time. Brain stimulation will be performed as detailed above.

Number of Sessions
Patients will undergo three ~1-2-hour testing sessions occurring on subsequent days.

Debriefing
The patient will be given the opportunity to ask questions of the team at any time, but will be invited to do so at the end of a session. This way, they can learn about what was tested and how this knowledge can lead to better patient outcomes or increased knowledge of brain function. We will not give medical advice during this de-brief and the study is not designed to provide information that is useful in relate to a specific patient's care. Where there is such information, this will be relayed to the clinical team.

Data and Specimen Banking

No physical specimens will be obtained. Data will be kept on encrypted laboratory computers, securely locked in the Woodruff Memorial Research Building and behind the Emory University 'Firewall' maintained by Information Technology Services. Data will consist of video-electrophysiological recordings and records from the testing session. Data will only be accessed and used by study staff. Study staff will de-identify the data by assigning each participant an alphanumeric ID (study number) as a reference. Any publication or presentation that uses the data from patients in this study is reported without identifying information. Data will be kept for at least six years after creation, thus the duration of the IRB and then for as long as required by any funding obtained for the project.

Sharing Results with Participants

This work (with no identifying information) will be shared with the epilepsy community and the work will result in peer-reviewed publication and presentations targeted to the epilepsy community. The epilepsy community consists not only of health care workers, but patients and their families, researchers, educators, administrators, public health and policy workers. Overall, we hope that the data obtained here will improve the care of patients treated with neuro-stimulation devices.

Study Timelines

Duration of individual patient involvement is 3-1-2 hour sessions over the course of 1 week. We estimate enrollment of patients to occur over the course of 1 year. The total study is anticipated to take place over the course of 2 years including data analysis.

Inclusion and Exclusion Criteria

Screening- Patients are known to the PIs in the context of their clinical work.
In order to be eligible to participate in this study, an individual must meet all of the following criteria:

Inclusion criteria

1. Provision of signed and dated informed consent form
2. Male or female, aged 18-65
3. Diagnosed with lesional or non-lesional mesial temporal (hippocampal) seizure onset confirmed on SEEG monitoring
4. Implanted with depth electrodes for localization of seizure onset with multiple hippocampal electrode arrays

Exclusion criteria

1. Any patient who is unwilling or unable to provide consent
2. Women who are pregnant
3. Patients under 18 years
4. Incarcerated persons

Vulnerable Populations

We are not studying a vulnerable group and will not include children, incarcerated persons, persons with cognitive disability or persons who are unable to give consent.

Local Number of Participants

Based on our power analysis, we estimate needing about 18 subjects for our analysis. Other studies in our Unit have typically had consent rates of 80% for eligible patients. We admit about 50 patients per year for intracranial EEG monitoring, with approximately 50% having mesial temporal lobe epilepsy.

Recruitment Methods

All patients entering our Intracranial Monitoring Unit who meet inclusion criteria will be approached for potential participation after recovery from the implantation surgery (>= 72 hours after implantation).

Patients with refractory suspected MTLE undergoing sEEG represent an accessible patient population from which participants may be recruited. As a high volume tertiary care epilepsy center (14 epileptologists, 3 nurse practitioners, and 2 epilepsy neurosurgeons), the Emory Epilepsy Center has unparalleled access to a large and continuously growing population of patients with refractory epilepsy. Emory is the only Level IV epilepsy center (including an active epilepsy surgery program) in the greater Atlanta area (a city of > 6 million people). Atlanta has one of the largest African American populations of any metropolitan area in the United States. 36% of the patients seen in the Emory Epilepsy Clinic are African American, and 56% of the patients seen are women.

Based on our past clinical volume, we estimate that >20 patients with MTLE will undergo SEEG per year. Study subjects will be recruited from this pool of patients. Our recruitment and retention rates for current and recent research protocols in SEEG patients have been ~80%. We are targeting to enroll 18 subjects, which should be feasible given our historic volume and recruitment rates.

Withdrawal of Participants

If the patient requests to withdraw from the study, all research and clinical records maintained outside the clinical record will be erased, with documentation of the original consent and that the subject has withdrawn from the study.

Risks to Participants

Referred Pain from Meningeal and Large Vessel Stimulation. This is unlikely to occur during hippocampal stimulation, but can be minimized and is not uncomfortable when stimulation intensity is increased gradually.

Seizure

The patient is admitted for the capture of spontaneous seizures. Seizure timing is not predictable, so a spontaneous seizure may occur during the testing session. Causing a seizure by stimulation is unlikely, but possible. This has typically only occurred during stimulation sessions, in our clinical experience, when high frequency stimulation is used in the seizure onset zone, or repeated stimulus pulses are delivered sequentially to the hippocampus in a patient in which seizures start in the hippocampus. In a study of 770 research stimulations, stimulation induced seizures were found to occur in 0.39%- 1.82% of sessions, with none were more severe than the patients typical clinic seizures and none adding morbidity or affecting the clinical course [10].

A neurologist or neurosurgeon is present at the bedside, as mentioned as above, during stimulation and can determine if there is a clinical need for an abortive seizure medication. In the unlikely event that a seizure occurs and becomes prolonged, lorazepam (1-2 mg IV) will be administered under the direction of a physician), as per standard epilepsy

monitoring unit protocol. Stimulation will be discontinued if an electroclinical seizure is triggered by stimulation or if requested by the patient.

In the event that the seizure is clearly related to the stimulation itself, or cannot be clearly determined to be spontaneous, this will be reported as an adverse event; when the seizure is judged spontaneous, this is a routine part of clinical care and will not be considered an adverse event. We expect that we will report rare events that we cannot determine definitively as related to stimulation or spontaneous, perhaps in the order of 2-3 such events.

Memory testing
There are minimal risks to memory testing, except for some distress in the case of patient's responding to their difficulty with the task. Again, if the patient is uncomfortable or distressed, the session will be aborted.

General overview of stimulation safety considerations
The stimulation parameters that we will use have been informed by our previous human studies our nonhuman primate data and fall within the range of typically used clinical stimulation parameters. When stimulation of the brain region within which seizures arise is performed, occasional epileptiform discharges can be seen. These are not seizures, but single events lasting < 200 ms that are the sign of increased excitability of the brain. These after-discharges can warn that seizure may occur with more intense stimulation. To avoid seizures, a neurologist will always be performing the stimulation and watching the real time EEG for these discharges. If a particular current intensity causes after-discharges, the current intensity of subsequent stimulations will be reduced.

Electrical stimulation of brain, using our technique and stimulus parameters, has minimal risk. The main risk is that stimulation might trigger a seizure, but this is uncommon given guidance by study staff who are neurologists trained in human electrophysiology and brain stimulation, and, with our stimulation parameters (careful attention to current density and emphasis of low-frequency stimulation; avoidance or appropriate decrease of current density and use of only low frequency stimulation in the hippocampal formation).

In the present study, current density will be kept below the typical safety limit of SEEG stimulation of 30 $\mu C/cm^2$/phase (thirty micro-Coulombs per square centimeter per phase), calculated based on current delivered per second and electrode surface area [8]. Stimulation will be graded, starting at very low current density then gradually increased.

During stimulation, the EEG will be observed by a trained neurologist physician with experience with reading intracranial EEG. If epileptiform discharges appear we will stop the stimulation run. If these continue we will use the ictal disrupt feature. We will then decrease the current and continue, but typically we will already have useful data at a lower current intensity. In the unlikely event that a seizure occurs, and becomes prolonged, lorazepam (1-2 mg IV) will be administered under the direction of a physician (there will always be a physician present), as per standard epilepsy monitoring unit protocol.

There is no evidence or preclinical reason to believe that this stimulation work will affect patient likelihood of having spontaneous seizures - the object of the admission. This study does not add additional physical, psychological, financial, legal, or other risk to each human subject beyond the risks discussed above and the risks already associated with clinical video-intracranial-EEG monitoring. The risk of accidentally disclosing personally identifiable private information will be minimized by (1) the activities of Emory EHIS and (2) the de-identification of patient data in accordance with HIPAA guidelines before release of data, analyses, grants, talks or publications.

Risks associated with the 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 a 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.

Potential Benefits to Participants

People with Epilepsy. This work will guide us in selecting neurostimulation parameters for patients undergoing asynchronous neurostimulation with implantable devices (to be examined in a subsequent early feasibility study). The overall goal is to improve seizure outcomes for patients requiring neurostimulation.

Science. There are several benefits to science, including clinical science with future benefits for medical care. We will (a) improve understanding of the physiologic changes occurring during seizures (b) provide better parameters for electrophysiological stimulation using implantable devices.

Society. We aim to provide a means to better understand and treat epilepsy, hoping to reduce the burden of epilepsy, a common disorder, in the community. This will reduce the psychological, social, financial and productivity burden of epilepsy. We will also communicate our findings to the greater epilepsy community, through publications in peer-reviewed journals when impactful findings are obtained.

Benefit to Subjects. The time with the study group will afford some added time for education about epilepsy, science and neurophysiology. The data obtained from individual subjects will, unfortunately, not provide a direct clinical benefit. Patients often report, however, that participating in research can be intrinsically rewarding, knowing

that the knowledge gained can help future patients with epilepsy. Where some clinical information is gained, this will be relayed to the clinical team.

Data Management and Confidentiality

Plans to protect subjects' privacy during their participation in the study

Our testing will only be performed in the patient's single-patient private hospital room.

Plans to establish and maintain confidentiality of subjects' identifiable data

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 for a patient is de-identified before any non-clinician researcher obtains it for analysis. The confidential data in this study will include medical records, neurological or psychological exams, medical imaging of the brain, EEG, video, and surgical outcomes at typical time points (e.g., 6 weeks, 6 months, 1 year). Study staff will de-identify the data 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 this person, within the limits possible by the contact of medical staff involved in the study who will likely recognize de-identified data, video and audio recordings. Any publication or presentation that uses the data from patients in this study is reported using the study numbers for the patients, with video and audio, where shown, manipulated to obscure the participant's identity.

Provisions to Monitor the Data to Ensure the Safety of Participants

The proposed study is most appropriately considered the equivalent of a basic science interventional clinical trial. The Emory Brain Health Center Data Safety Monitoring Board will provide oversight and annual review of the safety and confidentiality components of the study. All serious adverse events and Unanticipated Problems will be reported to the DSMB within 10 days of learning of their occurrence, and any deaths will be reported within 24 hours. The DSMB includes physicians who are experienced in clinical research, a nurse, and administrative support. Statistical help is available as needed. Study clinicians serving on the DSMB will recuse themselves when the current studied is being reviewed by the DSMB.

Data and Safety Monitoring Plan

a. *Plans for assuring compliance with requirements regarding the reporting of adverse events:*

Definitions:

1. **Unanticipated Problems Involving Risk to Subjects or Others**: any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e., not described in study-related documents such as the IRB-approved protocol or consent form)
- Related/possibly related to participation in the research (i.e.,there is a reasonable possibility that the incident experience or outcome may have been caused by the research)
- Suggests that the research places subjects or others at greater risk of harm
- (including physical, psychological, economic, or social harm)

2. **Adverse Event** (AE): any symptom, sign, illness, or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:
   - Results in study withdrawal
   - Is associated with clinical signs/symptoms or a serious adverse event
   - Leads to additional treatment or to further diagnostic tests
   - Is considered by the investigator to be of clinical significance
3. **Serious Adverse Event**: classified as serious or non-serious. A *serious adverse event* is any AE that is fatal, life-threatening, requires/prolongs hospital stay, or results in persistent or significant disability or incapacity. An *important medical event* is an event that may not be immediately life threatening, but is clearly of major clinical significance.
   Investigators shall submit reports of the following problems to the institutional IRB within 10 working days from the time the investigator becomes aware of the event:
   - Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the
     opinion of the principal investigator, is:
   - Unexpected (its specificity and severity are not accurately reflected in the IRB- approved research protocol and IRB-approved informed consent document)
   - Related to the research procedures (in the opinion of the principal investigator, the event was more likely than not to be caused by the research procedures)

Reporting Deaths: deaths that occur during the course of a research study will be reported more rapidly, as follows:

- Unexpected/unforeseen deaths will be reported within 24 hours
- All other deaths, regardless of whether the death is related to study participation, will be reported within 72 hours

Other Reportable Events:

- Any AE that would cause the PI to modify the protocol or consent form, or would prompt other action by the IRB to assure protection of human subjects
- Breach of confidentiality
- Information that indicates a change to the risks or potential benefits of the research
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team
- Protocol violation (an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects

b. *Monitoring the progress of trials and the safety of participants*

- The medical doctor for the patient holds primary responsibility for monitoring the safety of each subject both during study visits and in between study visits, if complications arise. All complications will be well documented, followed, and submitted to the Emory IRB and reported to the DSMB either annually or ad hoc.

The PIs, Dr. Gross and Bullinger, will be primarily responsible for monitoring patient safety. Additionally, because this work is conducted in the Intracranial Monitoring Unit (ICMU) while each participant is hospitalized, the ICMU staff (including members of this study) and Emory HealthCare IT maintain strict EHC standards for patient confidentiality, safety and data storage. Each time a patient is included in the study, the PI will discuss the patient with both clinical staff and the study personnel involved in research to ensure that there was no additional risk to the patient, no adverse event and that the patient was safe.

If there are any concerns, these will be handled as required by the protocol. Any unanticipated problem or adverse event due to the study will be carefully documented, investigated and submitted to the Emory eIRB under Reportable New Information (RNI). The eRIB submission will also be shared with the DSMB. Copies of each report and documentation of IRB notification and receipt will be kept in the PI's study file.

Monitoring of the following information, and other pertinent permissible data, for study participants will occur continuously for the purposes of the Study Objectives and Safety Monitoring:

- Administrative and scheduling information
- Proper attainment and storage of signed informed consents
- Any adverse events
- Withdrawals from the study
- Experimental data
- Original and processed neurophysiological recordings (including video)
- Medical and surgical records before, during and after the present admission

- Any diagnostic testing pertaining to the patient's epilepsy, neurological status and present admission. neurophysiology or neuroimaging of the brain

Reporting
If any expected or unexpected problem arises as a result of this study, then we will complete and submit a report to the eIRB portal as a Reportable New Information (RNI) in compliance with the Emory University *Clinical Trials Guidebook*. Any RNI will be reported to the DSMB at the time of the site awareness concurrent to IRB submission. Since currently there is no sponsor for the protocol, there is no additional requirement for reporting beyond those of the Emory IRB.

Site Monitoring Plan

Monitoring of study data and study regulatory documents will be conducted periodically to ensure that the study is taking place in accordance with the approved study protocol including study procedures, study intervention, and data collection processes.

The monitoring plan includes:

1. PI-requested monitoring visit(s) by the Clinical Trials Audit and Compliance (CTAC) Department: A CTAC initial audit will be scheduled after 2 subjects have been consented in the study and have completed the study procedures. Additional CTAC audits may be requested during the course of the study and at study close-out.
2. Self-monitoring: the study coordinators will conduct an annual self-monitoring visit using CTAC's self-monitoring tool. Findings will be discussed with the study PIs and reported to CTAC. The scope of the review will include regulatory documents, including IRB fillings, and subject-related data sources to ensure compliance with established study protocol and Emory policies. Every effort will be made to ensure that reviewers involved in self-monitoring activities are not involved in data collection or reporting.

Findings from the internal audits and self-monitoring activities will be shared with the PIs, with CTAC and with IRB, as appropriate. Findings leading to corrective action plans will also be discussed with the PIs, and may be reported to the DSMB, CTAC and Emory's IRB, if and when appropriate.

Provisions to Protect the Privacy Interests of Participants

All data is encrypted and stored on Emory University Computers that are behind the Emory Firewall. All computers used for analysis are also hardware encrypted and password protected in accord with Emory HealthCare IT policies. Each patient is given a unique identifier by the study staff for the purposes of communication: Any publication or presentation that uses the data from patients in this study is reported using the study numbers for the patients. While not presently anticipated, before any researcher not on the protocol uses any data whatsoever, complete deidentification will occur. In this case, the

patients' unique identifiers will be used that can only be linked to identifying information by the study staff (all of which are included on this IRB).

Economic Burden to Patients

There is no cost to the patient. No reimbursement will be given for this study.

Consent Process

We will only obtain informed consent after admission and after the patient has recovered from surgery. While typically lucid the day after surgery, there is often quite marked headache after electrode implantation that takes 48-72 hours to settle. We will plan to approach patients after at least 72 hours of admission and to return at a later time if they do not feel up to hearing about the study. Informed consent will be obtained by a member of the research staff: Drs. Gross, Bullinger, Mahmoudi or Marc Connolly.

We request a HIPAA partial waver to know which patients are admitted to the hospital. The neurologists and neurosurgeons that are participating will know this in the course of clinical practice (we all attend weekly surgical conference), but in order to be certain about these details when we are not directly involved in clinical care of each patient, we request a partial waver. We can then contact the clinical team to coordinate consenting of the patient.

We are not studying a vulnerable group and will not include patients with cognitive disability that preclude autonomous consent and/or participation, as described above.

Informed consent will be validated by: allowing time for questions, having the patient verbally summarize the material, asking subjects to answer a few questions to gauge understanding and then asking the patient if they understand all components of the study to the extent that they can then consent.

Setting

Data collection for this study will take place within the Emory University Epilepsy Monitoring Unit (EUH 3G) where patients are electively hospitalized for the clinical purpose of recording seizures and identifying the brain location of seizure onset. This study will not interfere with or extend the duration of their clinical stay. No physical specimens will be obtained. Data will be kept on encrypted laboratory computers, securely locked in the Woodruff Memorial Research Building and behind the Emory University 'Firewall' maintained by Information Technology Services.

Resources Available

As a high volume tertiary care epilepsy center (14 epileptologists, 3 nurse practitioners, and 2 epilepsy neurosurgeons), the Emory Epilepsy Center has unparalleled access to a large and continuously growing population of patients with refractory epilepsy. Based on our past clinical volume, we estimate that >20 patients with MTLE will undergo SEEG per year. Study subjects will be recruited from this pool of patients. Our recruitment and retention rates for current and recent research protocols in SEEG patients have been ~80%. We are targeting to enroll 18 subjects, which should be feasible given our historic volume and recruitment rates.

Patients included in this study are admitted to Emory University Hospital for clinical purposes by the functional neurosurgery team. Patients are monitored with continuous EEG in our Epilepsy Monitoring Unit (EMU). Emory has an 11 bed EMU. The EMU is staffed by 2 physician epileptologists, 2 or more epilepsy/clinical neurophysiology fellows and 1 nurse practitioner at any given time. 24 hour inpatient support for intracranial monitoring patients is provided by the neurosurgery and general neurology teams in addition to nursing staff. The epilepsy monitoring unit consists of a centralized monitoring pod which is continuously monitored by EEG technicians.

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