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Mihir Ghetiya April 9, 2019

Characterizing the memory deficits from asynchronous distributed hippocampal stimulation

by

Mihir Ghetiya

Robert Gross, MD, PhD
Adviser

Neuroscience and Behavioral Biology

Robert Gross, MD, PhD

Adviser

Melvin Konner, MD, PhD

Committee Member

Joseph Manns, PhD

Committee Member

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Mihir Ghetiya

Robert Gross, MD, PhD

Adviser

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Abstract

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Epilepsy is a group of debilitating neurological disorders characterized by chronic, spontaneous seizures. Temporal lobe epilepsy (TLE) is the most common form of epilepsy in adults. It is characterized by complex partial seizures that arise from the limbic structures of the brain, such as the hippocampus and the adjacent temporal lobe structures. Current treatments for TLE include anti-epileptic drugs (AED) and surgery. However, seizures are controllable with medication in only 70% of patients and surgery is not possible if the epileptic focus is in an eloquent area of brain, such motor, sensory, or memory regions. Neuromodulation through deep brain stimulation (DBS) offers an alternative therapy to suppress seizures in patients with refractory TLE. Various clinical studies have shown the effect of vagus nerve stimulation, anterior nucleus of thalamus stimulation, and direct hippocampal stimulation in suppressing seizures. However, these DBS approaches are limited by low efficacy and significant inter-subject variability in seizure reduction outcome. Recent work suggests that asynchronous distributed multielectrode stimulation (ADMES) delivered at theta frequencies to the hippocampus has the potential for providing a superior therapy for patients with TLE. Preliminary work suggests that 2V and 4V ADMES causes memory impairments in normal rats. However, it is possible that the rats can tolerate certain voltages before showing memory impairments. A simple grid search is straightforward to analyze, but it requires many experiments and does not explain the effect on memory as a continuous function of increasing voltage of stimulation. We utilize a black-box Bayesian optimization (BaO) algorithm to efficiently and quickly explore the voltage parameter space. We performed spatial object recognition memory task to assess spatial memory in rats as they received ADMES stimulation. We obtained subject specific model of memory performance with increasing voltages and observed considerable inter-subject variability in response to increasing voltages. We also did a preliminary grid search looking at the effect of ADMES frequency on memory in normal rats using novel object recognition task. Our BaO approach shows promise for further multi-objective function optimization for fine-tuning stimulation parameters to reduce seizure frequency while preserving memory performance.

Characterizing the memory deficits from asynchronous distributed hippocampal stimulation

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Chapter 1: Background and Introduction

Epilepsy is a group of debilitating neurological disorders characterized by chronic, spontaneous seizures. Seizures are abnormal changes in the electrical activity of the brain that could lead to a brief loss of consciousness, involuntary contractions of muscles, and other symptoms. These seizures can arise from the multitude of brain regions and differ in etiology and pathology. Epilepsy is the fourth most common neurological disorders and affects people of all age. There are estimated 3.4 million people in the US who have epilepsy (Sirven and Shafer, 2014). The spontaneous nature of ictal events and potential loss of consciousness leads to a very restrictive lifestyle for the patients. For example, they are often legally restricted from driving automobiles and are risk of significant injury from tasks as mundane as climbing stairs.

Temporal lobe epilepsy (TLE) is the most common form of epilepsy in adults (Goldberg and Coulter, 2013). It is characterized by complex partial seizures that arise from the limbic structures of the brain, such as the hippocampus and the adjacent temporal lobe structures (French et al., 1993). TLE is associated with distinct clinical, EEG, and pathological features (Goldenberg, 2010). Additionally, patients often report abdominal visceral sensations such as nausea, pressure, rising epigastric sensation, and various other somatosensory auras (French et al., 1993). Memory deficits in TLE have been extensively studied because of the role of hippocampus and adjacent cortical structures in learning and memory. Patients with TLE show significant learning and memory impairments even without the presence of overt damage to the brain, which suggests that the epileptic activity is independently responsible for memory impairments (Giovagnoli and Avanzini, 1999). Hemispheric lateralization plays a role in causing specific memory impairments (Zhao et al., 2014). Patients with left TLE show significant

memory impairments on verbal memory tasks, while patients with right TLE had significant impairments in visual memory tests (Giovagnoli and Avanzini, 1999).

Current treatments for TLE include anti-epileptic drugs (AED) and surgery. AED therapies are aimed at eliminating or reducing the frequency of seizures, helping patents maintain a normal lifestyle, and preventing long-term adverse effects of drugs itself (Goldenberg, 2010). Common AEDs include valproic acid, Topiramate, Lamotrigine, Carbamazepine, Phenytoin, Ethosuximide, Oxcarbazepine, Benzodiazepine (Goldenberg, 2010). AEDs primarily reduce hyperexcitability of an epileptic brain by inhibiting voltage-gated sodium channels, increasing GABA concentrations, and modulating the activity of GABA receptors (Goldenberg, 2010). However, seizures are controllable with medication in only 70% of patients and TLE is the most common form of medication-resistant adult epilepsy (Eadie, 2012; Engel, 1996). When these AEDs fail to adequately control seizures, the next line of treatment is surgery to resect the epileptogenic focus. Apart from the complications related to any surgery, resection of the offending tissue is not possible if the epileptic focus is in an eloquent area of brain, such motor, sensory, or memory regions. Therefore, there is a great need for novel therapies for seizure reduction that have minimal side-effects.

Neuromodulation through deep brain stimulation (DBS) offers an alternative therapy to suppress seizures in patients with refractory TLE. Vagus nerve stimulation (VNS) was the first neuromodulatory option for patients with refractory epilepsy. The first patient underwent VNS for refractory epilepsy and became seizure-free in 1988 (Penry and Dean, 1990). Meta-analysis of 74 VNS clinical studies has shown seizure reduction of usually higher than 50% in

approximately 50% of patients with intractable epilepsy (Englot et al., 2011). VNS is thought to modulate the activity of locus coeruleus and increase the amount of hippocampal noradrenaline, which correlates to suppression of seizures in a pilocarpine rat model of TLE (Raedt et al., 2011). Despite a large number of studies, the mechanism of action of the VNS remains to be fully understood and the patient specific stimulation parameters have not been identified (Dibue et al., 2019).

An alternative approach to DBS for TLE is stimulation of the neural tissue at or upstream to the hippocampus. Significant work has been done to identify new targets for stimulation that can more precisely modulate the activity of the hippocampus. Pathological synchronized electrical activity in the Papez circuit is thought to be the cause of temporal lobe epilepsy (Han et al., 2014). The Papez circuit consists of the anterior thalamic nucleus, hippocampus, mammillary body, and cingulate gyrus. The Papez circuit begins in the hippocampus and reaches the mamillary body through fornix (Shah et al., 2012). From there, the mamillary tracts project into the anterior nucleus of thalamus (Shah et al., 2012). The anterior nucleus of thalamus connects to cingulate gyrus through anterior thalamic radiations (Shah et al., 2012). The cingulate gyrus extensively projects to the hippocampus through entorhinal cortex (Shah et al., 2012). This suggests that electrical stimulation in the Papez circuit structures has the potential to modulate neural activity in the hippocampus and suppress seizures that arise from it.

One of the first targets in the Papez circuit for neurostimulation was the anterior nucleus of thalamus, which was evaluated in the pivotal SANTE clinical trial. The trial used a prospective, randomized, double-blinded, parallel group clinical study design. Bilateral stimulation of the

anterior thalamic nuclei resulted into median seizure reduction of 40.4% after 3 months of stimulation versus a median seizure reduction of 14.5% in no stimulation controls that received only AEDs (Fisher et al., 2010) (Figure 1). The stimulation parameters used by Fisher et al. (2010) were 5 V, 90 μs pulses, 145 Hz, "ON" 1 min and "OFF" 5 mins. During the open label period (4 – 13 months) all participants (N = 110) received active stimulation, up to 7.5 V and 185 Hz (Fisher et al., 2010). After the open label period, the clinicians were allowed to set any parameter provided it did not cause any adverse effects. Long-term follow up showed a median 56% reduction in seizure frequency at the end of 2 years (N = 81). The authors discuss that the exact mechanism that lead to seizure reduction remains unclear. Recent animal studies have show that ANT stimulation may have a neuroprotective effect on the hippocampal neurons (Yang et al., 2015).

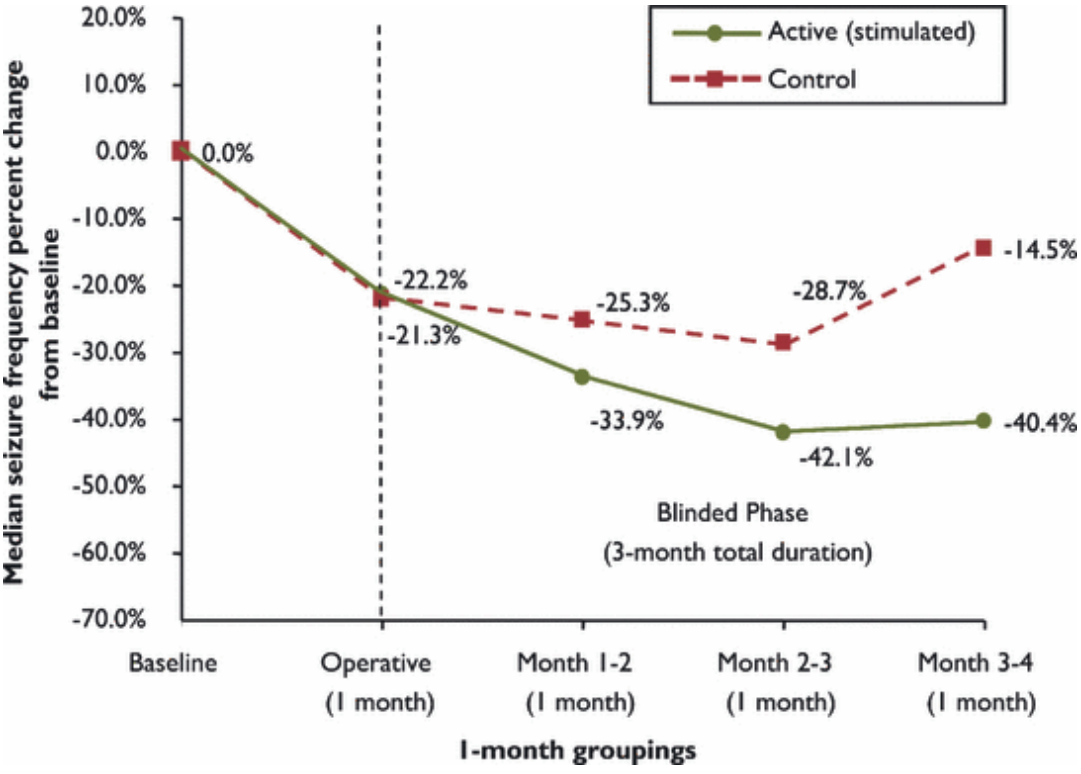

Figure 1: ANT-DBS reduces seizure frequency in patients with TLE. The graph shows unadjusted median seizure frequency percent change during the blinded phase for the no stim implanted controls and active stim patient group. Adopted from Fisher et al. (2010).

Various clinical studies have shown the efficacy of hippocampal stimulation in suppressing seizures in patients with refractory TLE (Boex et al., 2011; Bondallaz et al., 2013; Cukiert et al., 2014; Tyrand et al., 2012). There is also an FDA approved device called responsive neurostimulation (RNS) system developed by NeuroPace Inc. that detects epileptic activity through electrocorticography (ECoG) and provides brief pulse of stimulation in response to the seizure detection. The system uses a cortical strip settled on the surface of the temporal lobe for ECoG recording and a depth electrode that provides stimulation to the hippocampus. A long-term treatment study looking at the efficacy of RNS system showed seizure reduction of 44% at 1 year and 53% at 2 years postimplant, a significantly greater reduction compared to the controls who received only AEDs (Bergey et al., 2015).

Some of the biggest shortcomings of current electrical stimulation approaches for refractory epilepsy include significant inter-subject variability in seizure suppression outcome and low efficacy (Rolston et al., 2012). In SANTE trial, at the end of two years only six of 81 patients were seizure-free for three-month segment and thirteen patients had a reduction of >90%, compared to baseline (Fisher et al., 2010). In the RNS study, 60% of participants had 50% or greater seizure reduction, and only16% of participants were seizure free (Bergey et al., 2015). A significant number of participants in both studies did not have meaningful seizure reduction. This suggests that there is a need for different stimulation approaches with subject specific stimulation parameters and greater efficacy.

The advent of new stimulation electrode designs and controllers that can run complex machine learning algorithms provide researchers with more flexibility in delivering and fine-tuning neuromodulatory therapy. There are a number of parameter settings to the therapeutic electrical stimulation such as voltage, frequency, pulse length, waveform, and the no. of electrodes or leads. This electrical stimulation parameter space, including spatial-temporal stimulation patterns, has remained unexplored (Desai et al., 2015). In Desai et al. (2015), the researchers utilized a microelectrode array (MEA), consisting of 15 microelectrodes, to deliver distributed electrical stimulation to the hippocampus of tetanus toxin rat of epilepsy. Power spectral density analysis on LFP recordings showed that tetanus toxin rats had reduced hippocampal theta oscillations compared to saline-injected controls (theta range for rats is 6-12 Hz and theta peak is between 7-8 Hz) (Desai et al., 2015). Desai et al. (2015) hypothesized that delivering electrical stimulation in the theta range and/or theta peak may have a more beneficial effect in comparison to other frequencies. The efficacy of three different stimulation protocols were tested through the MEA including asynchronous pulse, synchronous pulse, and synchronous sinusoid pulse (Figure 2). Each of the protocols were used to deliver stimulation at theta range and theta peak frequencies in continuous and intermittent manner. They also tested a theta peak and theta range electrical stimulation through a macroelectrode for comparison.

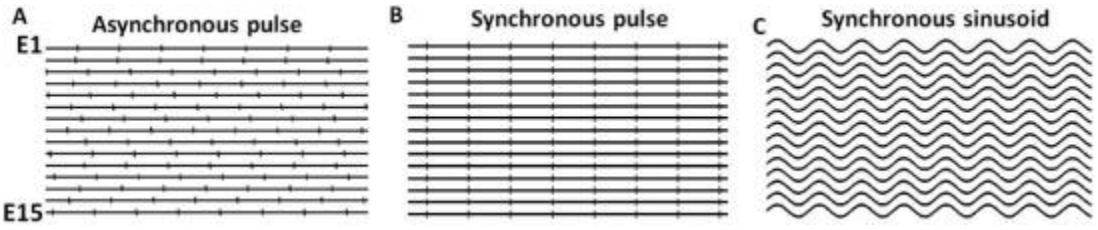

Figure 2: Three different stimulation protocols tested through microelectrode array. Adopted from Desai et al. (2015).

Desai et al. (2015) found that asynchronous theta peak and theta range stimulation through the MEA, in continous and intermittent form, resulted into significant reduction in seiure frequency (Figure 3). All other stimulation protocols did not have significant effect. Desai et al. (2015) hypothesisze that asynchronous distributed multielectrode stimulation (ADMES) could prevent seizures by desynchronizing local neuronal populations. This shows that ADMES delivered at theta frequencies has the potential for being a therapy for patients with TLE.

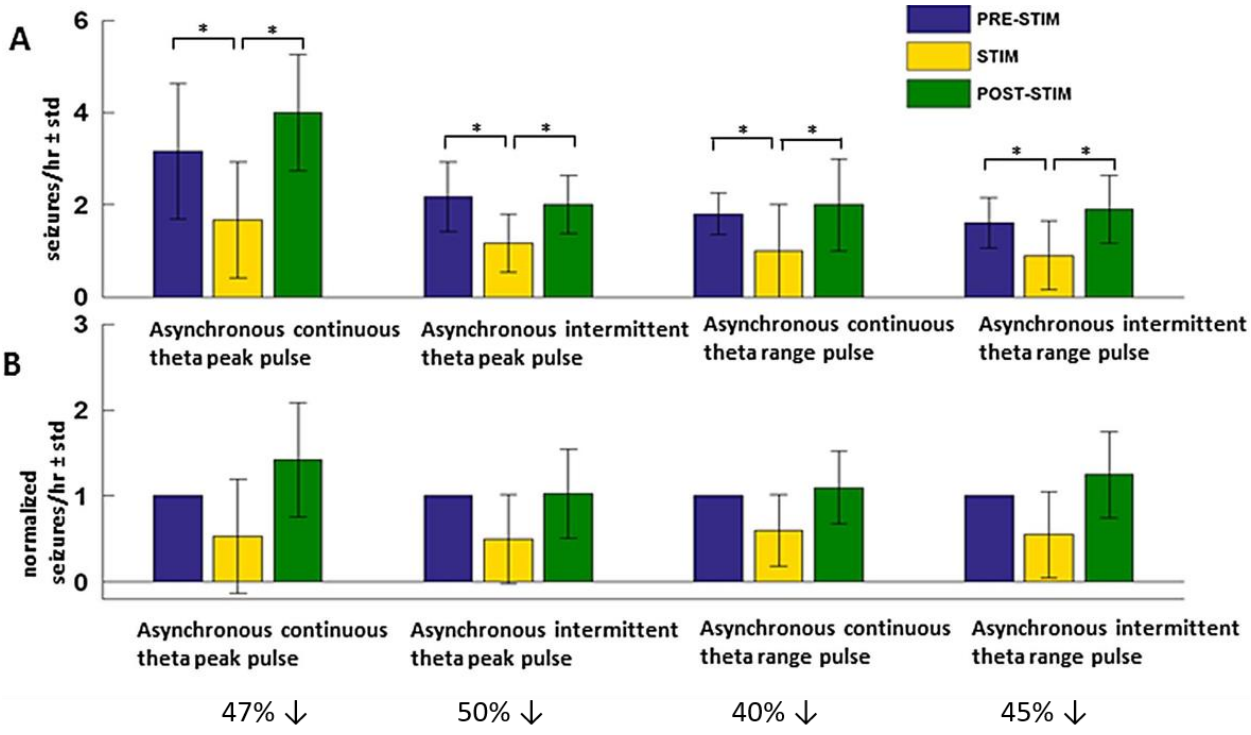

Figure 3: Asynchronous distributed microstimulation in theta frequency reduces seizure frequency. A) Mean ± std of seizure counts during pre-stim, stim, and post-stim phases. B) Mean ± std of normalized seizure counts during pre-stim, stim, and post-stim phases. The percent decrease in seizure frequency for each condition is stated below B. Adopted from Desai et al. (2015).

While effective at reducing seizures, direct hippocampal electrical stimulation has been shown to cause memory impairments in humans (Lacruz et al., 2010; Coleshill et al., 2004). In Lacruz et al. (2010), the researchers found that bilateral stimulation caused 57% decrease in total memory scores, 38% decrease in memory subscores for words, 38% reduction in memory subscores for objects, 81% reduction in memory subscores for geometrical drawings. In Coleshill et al. (2014), the researchers found that unilateral stimulation in the left hippocampus caused memory deficits for words, and unilateral stimulation in the right hippocampus caused memory deficits for faces. This suggests that ADMES could cause memory impairments as it includes direct stimulation to the hippocampus. As the goal is to provide therapeutic benefit while causing minimal side effects, it is crucial to test the effect of ADMES on memory.

Characterizing the effect of ADMES on memory, can be framed as learning the mapping from stimulation parameters to performance on a memory task. One approach is a grid search. Statistically, a grid search is straightforward to analyze, however it requires many experiments and does not explain the effect of memory as a continuous function of the stimulation parameters. Black-box optimization is an alternative approach to efficiently explore a parameter space. Black-box optimization is a problem setup in which the goal is to optimize an objective function, which in our case would be maximization of the memory performance on a specific task. Black-box optimization algorithms makes no assumption about function (e.g. linearity, convexity) and can handle discontinuities in the function. This is especially important because we do not have prior information regarding the nature of the relationship between stimulation parameter and the memory performance, and there is inherent stochasticity associated with the animal's performance on a memory task.

Chapter 2: Bayesian optimization to search for voltage parameters to maximize and minimize memory task performance

Section 2.1: Background and Preliminary Data

Preliminary work suggests that ADMES has negative effect on memory (Figure 4). In a previous experiment, a spatial object recognition task (methods described in the following section) was used to test spatial memory for sham stim, 2V ADMES, and 4V ADMES conditions. Stimulation frequency was set at 7Hz for 2V and 4V ADMES conditions. Each stimulation condition was preceded by a pre-stim control memory experiment and followed by post-stim control memory experiment. The rats showed significant memory impairments in 2V and 4V ADMES conditions compared to sham condition. It is possible that the rats can tolerate stimulation up to certain lower voltages before showing memory impairments. At high voltages, the DS was observed to be less than 0. This indicates preference for the stationary object. We would also like to investigate this favoritism for stationary or familiar object. Therefore, it is important to characterize this reduction in memory performance with increasing voltage. As previously described, a grid search is too time-consuming and inefficient to serve this purpose. Therefore, we utilized black-box Bayesian optimization, to efficiently explore the voltage space.

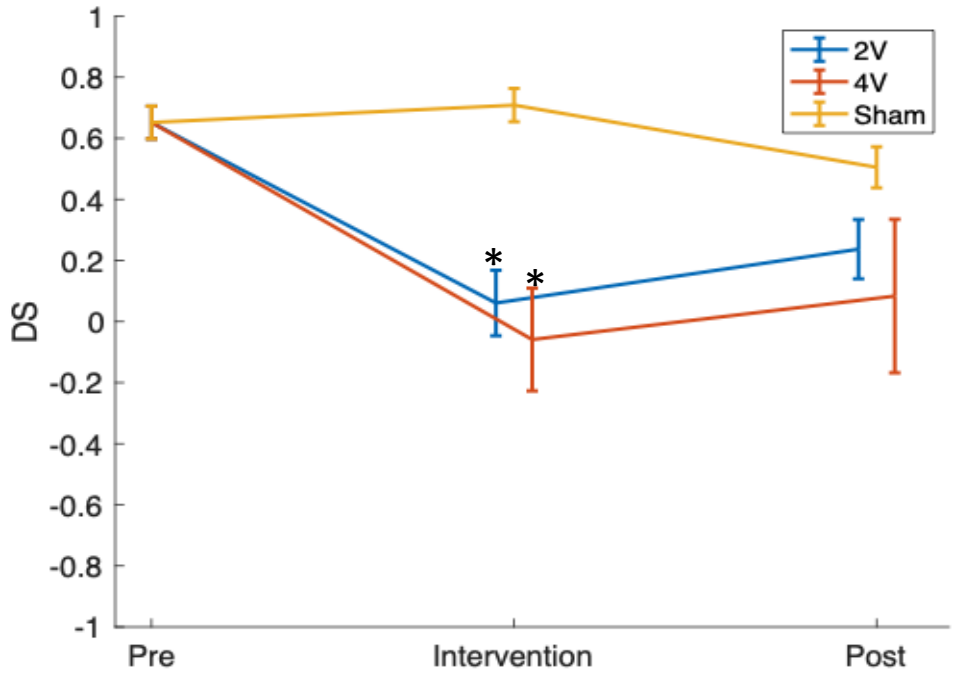

Figure 4: ADMES at 2V and 4V causes significant memory impairments in normal rats. Spatial object recognition task was used to test memory in normal rats during active ADMES stimulation. 2V ADMES causes significant memory impairments versus sham stimulation ($p<0.001$). 4V ADMES causes significant causes significant memory impairments versus sham stimulation ($p<0.01$).

Section 2.2: Methods

Section 2.2.1: Electrode implant

Stimulating and recording electrodes were stereotactically implanted in the CA3 and CA1 regions of the hippocampus in male Sprague Dawley rats under general anesthesia using isoflurane (2-5%). A craniotomy was performed at ML + 2 mm and AP + 3 mm relative to Bregma using a drill. A 16-channel electrode with two rows of 8 leads offset by 1 mm (Figure 5) was lowered to a depth of 2.9 mm using a stereotactic frame. Five screws are implanted in the cranium adjacent to the craniotomy. Ground and reference lead wires are attached to two of the

screws. The electrode is then connected to the TDT electrophysiology system that monitors neural activity in real-time. The electrode is then gradually lowered in the brain. Multi-unit spikes are used to confirm the correct placement of the electrodes in the CA3 and CA1 regions of the hippocampus. Once a desired location is obtained, the electrode is affixed to the skull and support screws using an acrylic-based glue. The glue is allowed to harden before removing anesthesia. The animal is allowed to recover for at least one week before subsequent memory testing.

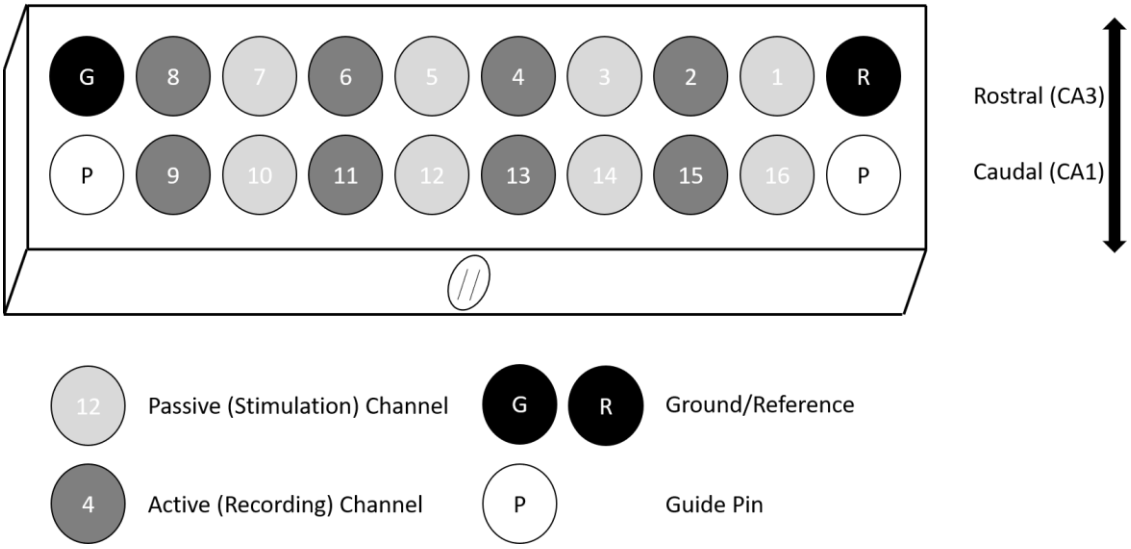

Figure 5: Schematic of the implanted electrode.

Section 2.2.2: Spatial object recognition task

We used a modified version of novel object recognition, called spatial object recognition task (SOR). Spatial object recognition task is a well-established behavioral task to test object recognition memory in various animal species (Antunes and Biala, 2012). The task relies on the innate preference of rats for novel objects and location. Before starting the SOR experiments, the

implanted rats were habituated to the open-field SOR box for 5 minutes per day for three days. Practice objects were placed in the SOR box on the third day to confirm if the rats were willing to explore the objects. Further training was utilized if the rat show hesitation to explore the practice objects. The dimensions of the SOR box are 2’ by 1.5’.

The head-stage is connected to the electrode on the rat, which is in turn connected to a rotating commutator on the other end. This is done to prevent knots to form in the wire of the head-stage and ensure that the head-stage does not disconnect during the experiment or cause discomfort to the rat. The stimulation parameters are set in a propriety MATLAB application that controls the TDT electrophysiology system and the program is executed. Realtime LFP recordings are used to confirm if the electrode is indeed delivering stimulation to the animal.

The task consists of the habituation phase, familiarization phase, and the test phase (Figure 6). In the habituation phase, the rat is moved to the testing room 30 minutes before the NOR task is performed. The exploration area is thoroughly cleaned with 70% isopropyl alcohol to remove any olfactory signals. In the familiarization phase, we placed three objects in different corners of the exploration box and let the rat explore them for 3.5 minutes. The rat is then removed from the exploration area for 3 minutes. During this time, the exploration area and the objects are thoroughly cleaned with 70% isopropyl alcohol to remove any olfactory signals. During the testing phase, the rat is again brought to the exploration area and allowed to explore the objects for 3.5 minutes. The rat is then removed from the exploration area and the head-stage is disconnected from the electrode. This marks the end of the SOR experiment. The orientation of interchanged objects is counterbalanced among the three corners as to prevent any bias. A

camera placed on the top of the box was used to capture the video of the familiarization and the testing phases. SOR experiments were performed in a sound-isolated room and presence of only red light. This was necessary to encourage exploration as rats are nocturnal and light sensitive, but lack receptors for red light. This ensured that minimal stressors affected the animal's performance.

Discrimination score (DS) is used to quantify the animal's memory performance. DS is defined as the difference in exploration time for interchanged objects and exploration time for stationary object divided by the sum of exploration time for interchanged objects and exploration time for stationary object:

$$\frac{(\boldsymbol{Time\ spent\ on\ Object\ B + C}) \ - \boldsymbol{Time\ spent\ on\ object\ A}}{\boldsymbol{Time\ spent\ on\ object\ B + C + A}}$$

The score ranges from -1 to +1. +1 score indicates preference only for the interchanged objects and -1 score indicates preferance only for the stationary object. Score of 0.33 suggests equal preferenence for all three objects and is indicative of no memory. Exploration of an object was defined as the orientation of animals's snout directed towards the object with the whiskers actively twitching, which suggests sniffing. Any other interactions such as running around the object, playing with the object, sitting or climbing on the object was not recorded as exploration.

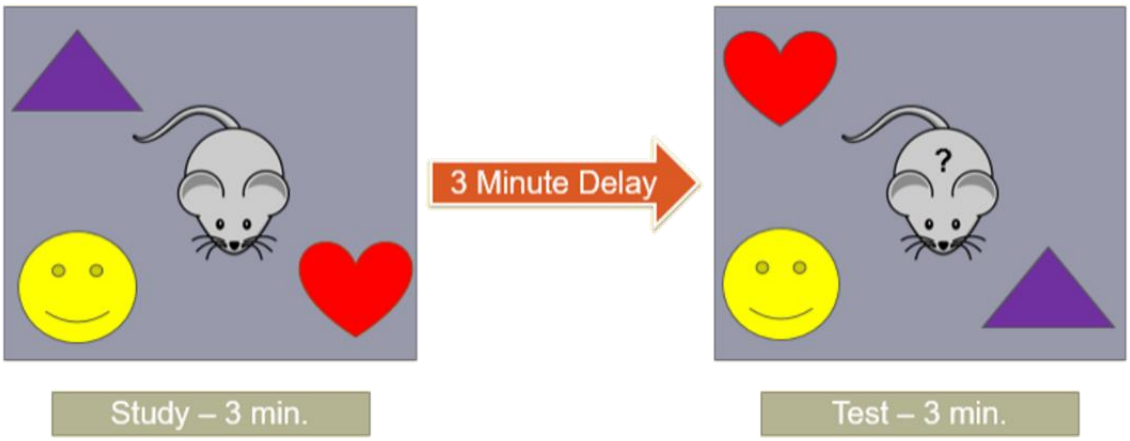

Figure 6: Schematic of the spatial object recognition task. Left panel shows the familiarization phase. In the familiarization phase, the rat is allowed to explore the three objects placed in three different corners of the exploration area for 3.5 minutes. During the 3-minute delay, the location of two objects is interchanged. Right panel shows the testing phase. In the testing phase, the rat is allowed to explore the objects in the exploration area for 3.5 minutes.

Section 2.2.3: Bayesian Optimization

Black-box optimization is an approach to efficiently explore the voltage space and its effect on memory performance. We utilized a technique called Bayesian optimization (BaO) to achieve our objective. An overview of BaO is shown in Figure 7. Bayesian optimization consists of two main components: a statistical model based on a Gaussian process (GP) to model the objective function, and an acquisition function to determine the next point to be sampled (Snoek et al., 2012). Initially, a few points are sampled from a uniform distribution and fed to the algorithm.

The BaO algorithm then fits a GP to this data to build a surrogate model f(x) that approximates the objective function f over the inputs x. A GP is a probability distribution over possible function, which means that there is an uncertainty associated with the f(x) for a given x. This is especially important as there is an inherent randomness associated with the rat's performance on

the memory task. Inferences from the GP are in the form $\{x_n, y_n\}$, where $y_n \sim N(f(x_n), \nu)$ with ν as the measurement noise (randomness) associated with the function observation.

The acquisition function defines the strategy to sample the next point. It is described as $a(x; \{x_n, y_n\}, \theta)$. It considers the mean and the variance in the surrogate model and evaluates the improvement, or potential for improvement for each point, and gives us the next point to sample. This is done via a proxy optimization $x_{next} = \text{argmax}_x a(x)$. In the first experiment the objective function was the maximization of memory. This was to see if some voltage between 0V and 2V could increase memory. In the second experiment the objective function was the minimization of memory. This was to investigate if high voltage stimulation provided an aversive stimulus and resulted into preference for stationary object. Five voltage points were randomly sampled for an initial burn-in for the BaO algorithm. The point suggested by the algorithm was sampled and the model was updated with new data. This process was repeated until sufficient characterization of memory was achieved.

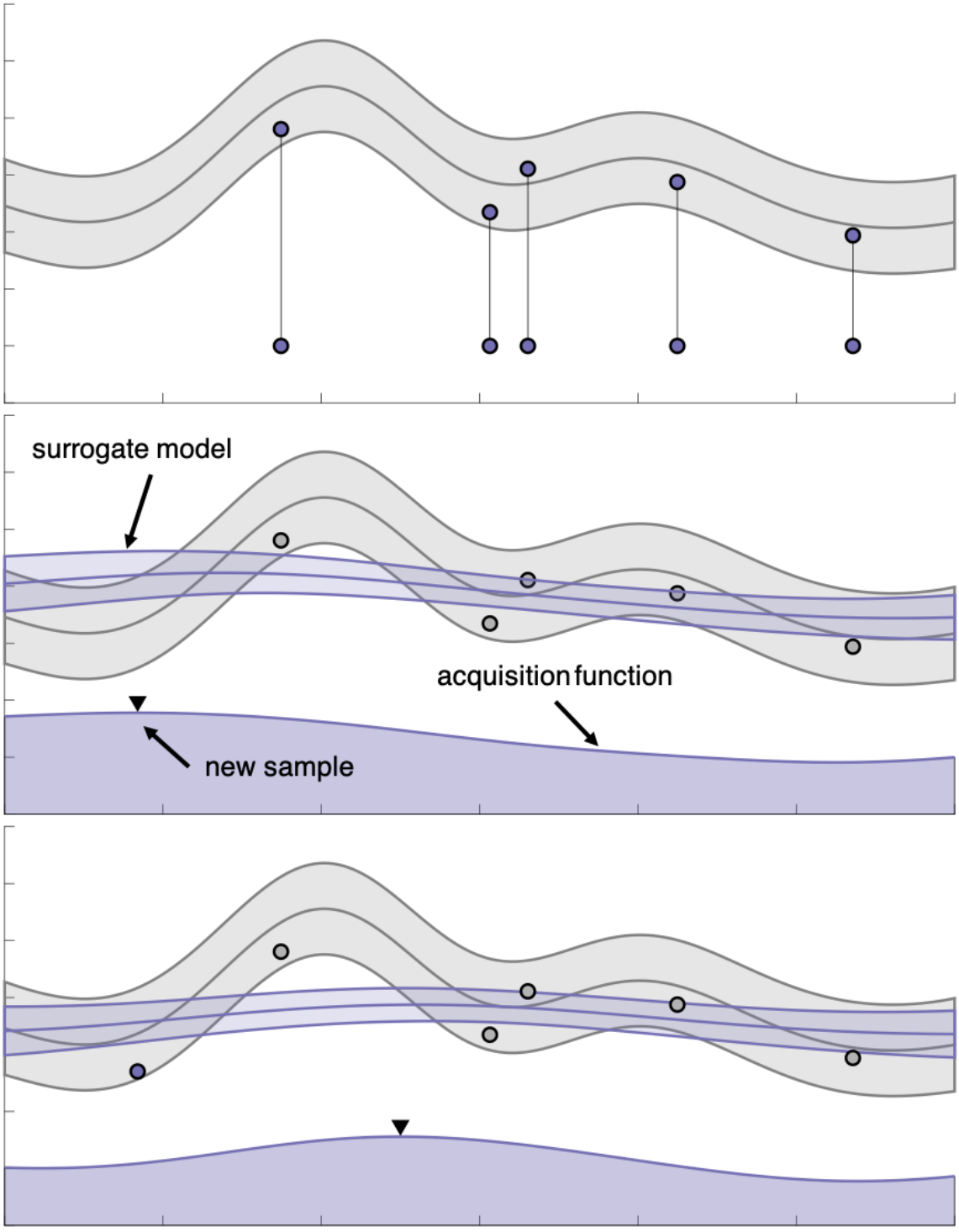

Figure 7: Overview of the Bayesian optimization (BaO) algorithm. A) Few points are sampled from a normal distribution and evaluated. B) A Gaussian process surrogate model is generated to fit the existing data. An acquisition function is then used to propose another point to be sampled based on the expectation and uncertainty estimated by the model. C) After the proposed point is sampled and evaluated, the model is updated with the new data. Steps B – C are repeated.

Section 2.3: Results

Bayesian optimization with objective function of maximization of memory was able to sufficiently characterize the memory performance in 13 iterations for Rat 2 and 11 iterations for Rat 1 and Rat 3 (Figure 8). Bayesian optimization with objective function of minimization of memory was able to sufficiently characterize the memory performance in 14 iterations for Rat 1, 11 iterations for Rat 2, and 12 iterations for Rat 3 (Figure 9). There were no specified convergence criteria, as heretofore insufficient data had been collected. Visualizing the approximation of the objective function learned by the Bayesian optimization algorithm, it can be shown that each rat responded differently to the stimulation. Rat 1 showed memory impairments from any stimulation over 0 V. Rat 2 was able to tolerate voltages up to 1 V before showing memory impairments. Rat 3 was able to tolerate voltages up to 1.5 V before showing considerable memory impairments. Figure 9 makes it clear that increasing voltage stimulation did not result into an aversive stimulus that causes the rats to prefer stationary object.

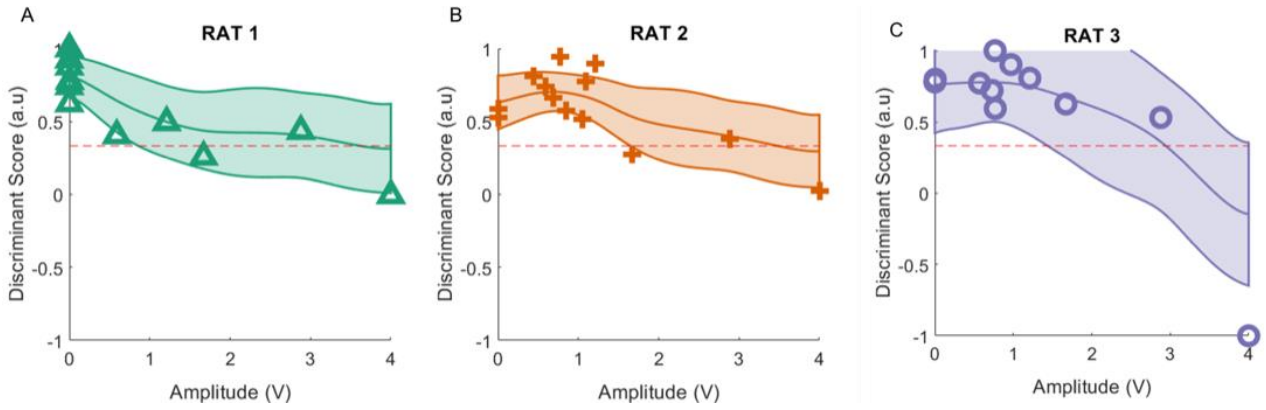

Figure 8: The BaO algorithm with the objective function of maximization of memory. A-C) Memory performance with increasing voltage stimulation for Rats 1-3, respectively. Stimulation frequency was set at 7Hz. Red dotted line indicates a threshold score, performance above which demonstrates memory. Spatial object recognition task was used to test memory in normal rats during active ADMES stimulation. The shaded area indicates the confidence interval of the GP model.

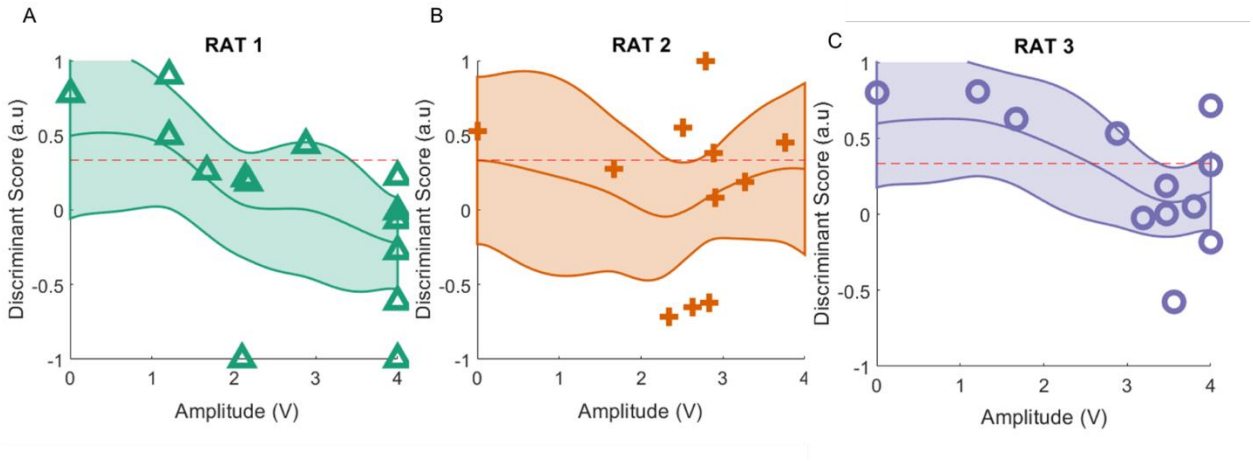

Figure 9: The BaO algorithm with the objective function of minimization of memory. A-C) Memory performance with increasing voltage stimulation for Rats 1-3, respectively. Stimulation frequency was set at 7Hz. Red dotted line indicates a threshold score, performance above which demonstrates memory. Spatial object recognition task was used to test memory in normal rats during active ADMES stimulation. The shaded area indicates the confidence interval of the GP model.

Section 2.4: Discussion

Our preliminary data suggests that 2V and 4V ADMES at 7Hz causes significant memory impairments in normal rats (Figure 4). It is very likely that the rats are able to tolerate stimulation up to certain voltages before showing significant memory impairments. At high voltages, the DS was observed to be less than 0. This indicates preference for the stationary object. This can be explained by our stimulation providing an aversive stimulus that causes them to fear the objects that moved. To determine if this was real, we performed an optimization experiment with the objective function of minimizing memory performance. Based on our metric, this would be defined as a negative DS, or preference for the stationary object. Results from the minimization of memory experiment suggest it was a statistical anomaly as we could not induce a negative DS, or preference for stationary object within our parameter space.

Grid search looking at memory impairments with increasing voltages is relatively straightforward approach to explore the voltage space. However, it requires a large number of relatively difficult memory experiments. One way to solve this problem is to use a black-box optimization algorithm that can efficiently explore this space and provide a good enough characterization of parameter space for our purpose. There are many black-box optimization approaches such as simultaneous perturbation stochastic approximation (SPSA) optimization, cross-entropy method (CEM) optimization, and Bayesian optimization (BaO). SPSA optimization uses several samples to calculate the approximation of the gradient to find either the maximum or a minimum of the function (Spall et al., 1998). SPSA is notorious for its tendency to get stuck in local minima or local maxima and requires significant calibration to get good results (Maryak and Chin, 1999). CEM optimization is a general evolutionary algorithm that initially samples a wide range of points in the parameter space and then selects the points with the greatest performance (De Boer et al., 2005). It continuous this cycle before converging into the global maxima or minima. For our problem, CEM fails to provide a better alternative to a simple grid search as it requires a lot of sample points to work. BaO is a better approach for our problem as it is able to characterize the parameter space with relatively low number of trials. Another strength of the BaO is its ability to handle multi-objective functions (Snoek et al, 2012).

Direct hippocampal stimulation has shown to cause memory impairments in humans (Coleshill et al., 2004; Lacruz et al., 2010). The stimulation of hippocampus in rodents has shown to reduce or enhance memory depending on the specific modulation of underlying neuronal activity (Hampson et al., 2012; Suthana and Fried, 2014). Our results show that there is indeed memory

impairment from ADMES stimulation and there is considerable inter-subject variability in response to increasing voltages.

We were able to characterize the memory performance in the first experiment with the objective function of maximizing memory performance in 11-13 iterations and characterize the memory performance in the second experiment with the objective function of minimizing memory performance in 11-14 iterations. This points to the ability of BaO to efficiently explore stimulation parameter space. BaO approach shows promise for further multi-objective function optimization for fine-tuning stimulation parameters to reduce seizure frequency while preserving memory performance.

Section 2.5: Limitations and Future Directions

One of the limitations of the study relates to the way the BaO algorithm is set up. It was set up to maximize and minimize performance, not to find the tradeoff between voltage and performance. For example, it is possible that the rat can tolerate 1V stimulation, but BaO found that 0V is where the memory is maximized and never decided to sample more around 1V. This limitation can be addressed in future studies with a multi-objective formulation to directly learn the trade-off, or the pareto front.

Another limitation relates to the limited parameter space explored in the experiment. There are a lot of parameters that are selected for a particular stimulation such as the voltage, frequency, pulse width, waveform. High frequency DBS for PD patients often causes side effects such as lower limb dyskinesias, facial contractions, and a subjective sensation of heavy head (Dayal et

al., 2017). Therefore, it is very important to fine tune other parameters to achieve therapeutic effect while minimizing side effects. This is the reason why we address optimizing frequency in the next chapter.

Chapter 3: Exploring voltage and frequency domain of ADMES and its effect on memory

Section 3.1: Introduction

In the previous section we utilized a novel black-box optimization approach to efficiently test memory impairments from increasing voltages of electrical stimulation. Another way to fine-tune electrical stimulation is changing the frequency. All the memory experiments in section 2A utilized electrical stimulation of 7 Hz. As I previously discussed, direct hippocampal stimulation in rodents has been shown to impair or enhance memory. Therefore, it is crucial to test ADMES with different frequencies in normal rats and look at the memory performance. Preliminary SOR tests with the new batch of rats showed very noisy result. Therefore, we decided to switch to a simpler two object NOR task.

In this experiment, we make a departure from the Bayesian optimization approach described in the previous section, and instead use a less efficient grid-search strategy. Unpublished work by Connolly et al. has demonstrated that the performance of an optimization algorithm is sensitive to the configuration of internal algorithm settings or hyperparameters. Correct hyperparameters can be selected through data-based simulation experiments. However, these experiments require pilot data that can be obtained through a grid-search.

Section 3.2: Methods

Section 3.2.1: Electrode implant

Same procedure as the one described in section 2.2.1 is performed here.

Section 3.2.2: Novel object recognition task

The novel object recognition (NOR) task is a well-established behavioral task to test object recognition memory in various animal species (Antunes and Biala, 2012). The task relies on the innate preference of rats for novel objects. Before starting the NOR experiments, the implanted rats were habituated to the open-field NOR box for 5 minutes per day for three days. Practice objects were placed in the NOR box on the third day to confirm if the rats were willing to explore the objects. Further training was utilized if the rat show hesitation to explore the practice objects. The dimensions of the NOR box are 2’ by 1.5’.

The head-stage is connected to the electrode on the rat, which is in turn connected to a rotating commutator on the other end. This is done to prevent knots to form in the wire of the head-stage and ensure that the head-stage does not disconnect during the experiment or cause discomfort to the rat. The stimulation parameters are set in a propriety MATLAB application that controls the TDT electrophysiology system and the program is executed. Realtime LFP recordings are used to confirm if the electrode is indeed delivering stimulation to the animal.

The task consists of the habituation phase, familiarization phase, and the test phase (Figure 10). In the habituation phase, the rat is moved to the testing room 30 minutes before the NOR task is performed. The exploration area is thoroughly cleaned with 70% isopropyl alcohol to remove any olfactory signals. During the familiarization phase, the rat is brought to the exploration area containing two objects each placed in the adjacent corners. The rat is then allowed to explore the area and the objects for 3.5 minutes. The rat is then removed from the exploration area for 3 minutes. During this time, the exploration area and the objects are thoroughly cleaned with 70%

isopropyl alcohol to remove any olfactory signals. One of the familiar objects is then placed in its prior position. A novel object is placed in the position of the other familiar object. The placement of the novel object is counterbalanced between the two corners as to prevent any bias. During the testing phase, the rat is again brought to the exploration area and allowed to explore the objects for 3.5 minutes. The rat is then removed from the exploration area and the head-stage is disconnected from the electrode. This marks the end of the NOR experiment. A camera placed on the top of the box was used to capture the video of the familiarization and the testing phases. NOR experiments were performed in a sound-isolated room and presence of only red light. This was necessary to encourage exploration as rats are nocturnal and light sensitive, but lack receptors for red light. This ensured that minimal stressors affected the animal's performance.

Discrimination score (DS) was used as a metric to determine the animals performacne. DS is defined as the difference in exploration time between novel and familiar object divided by the total time of exploration of the novel and familiar object during the testing phase:

$$\frac{(\boldsymbol{Time\ spent\ on\ novel\ object\ -Time\ spent\ on\ familiar\ object})}{\boldsymbol{Total\ time\ spent\ on\ novel\ and\ familiar\ object}}$$

The score ranges from -1 to +1, where a negative score indicates preferance for the familiar object and a positive score indicating preferance for novel object. Exploration of an object was defined as the orientation of animals's snout directed towards the object with the whiskers actively twitching, which suggests sniffing. Any other interactions such as running around the object, playing with the object, sitting or climbing on the object was not recorded as exploration.

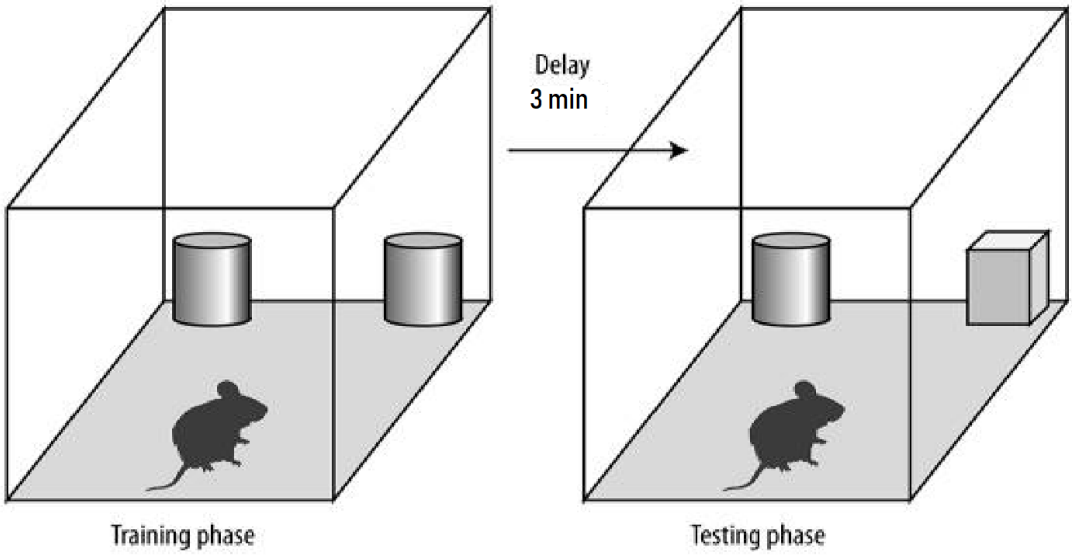

Figure 10: Schematic of the NOR experiment. Adopted from Brodziak et al. (2014). Left panel shows the familiarization phase. In the familiarization phase, the rat is allowed to explore the two objects placed in different corners of the exploration area for 3.5 minutes. During the 3-minute delay, one of the objects is replaced with a novel object. Right panel shows the testing phase. In the testing phase, the rat is allowed to explore the objects in the exploration area for 3.5 minutes.

Section 3.3: Results

The data on object recognition memory effects from different voltage and frequency combinations of electrical stimulation in rats are shown in Figure 11. No significant effects on memory were observed with 1V and 3V stimulation compared to 0V stimulation for both rats. However, a trend of increasing memory performance with 1V, 15 Hz stimulation was found in Rat 5. A trend of decreasing memory performance for 3V, 7 Hz stimulation was found in Rat 4.

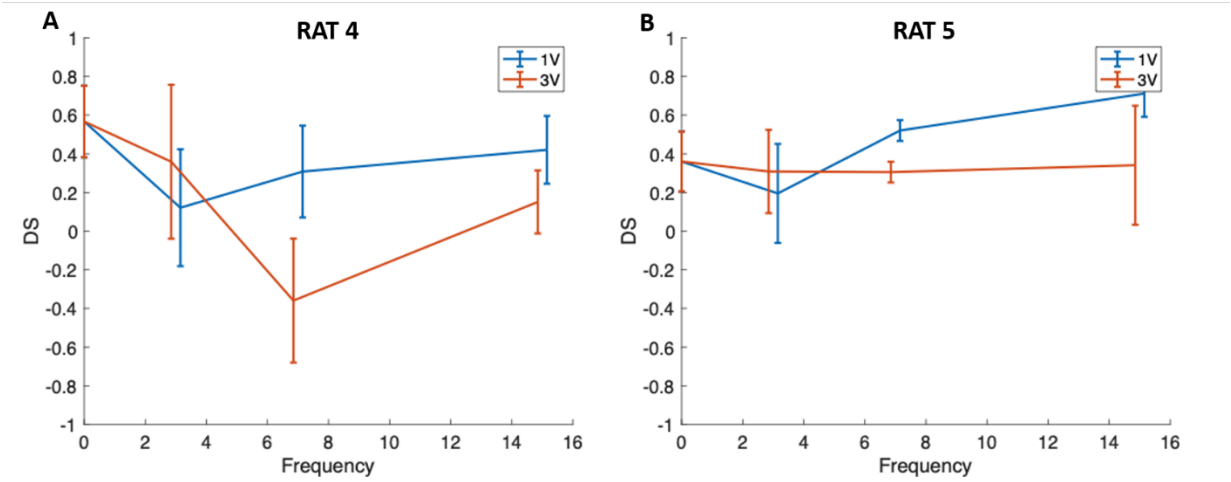

Figure 11: Grid search for voltage and frequency ADMES combinations. A & B) Memory performance for different voltage and frequency combinations of stimulation for Rat 4 and Rat 5 respectively. Novel object recognition task was used to test memory in normal rats during active ADMES stimulation. No significant differences in memory performance within group or between group was observed using two-way ANOVA test.

Section 3.4: Discussion and Limitations

The goal of this experiment was to obtain pilot data on the effect of different voltage and frequency combinations of stimulation on memory performance in normal rats. The NOR task was used to assess memory. No significant effects on memory were observed with 1V and 3V stimulation, with varying frequencies, compared to 0V stimulation for both rats. Two-way ANOVA test was used to test for significance. This is attributed to low number of experiments. However, the data is adequate for conducting the BaO algorithm for future studies.

There is a considerable amount of variation in the sensitivity to stimulation parameters between subjects. In this experiment, Rat 4 could not tolerate even minimal stimulation whereas Rat 5 is generally less sensitive to stimulation, which is why (when rescaled) the effect is minimal.

Insufficiently wide parameter space is another potential limitation of this study, and we will motivate the design of future experiments where the voltage range is either adjusted for each rat or optimized with a safe-opt procedure that would avoid stimulation parameters that risk instabilities.

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