



Effects of Vagal Nerve Stimulation on Seizures and Cognition in a Rodent Model of Temporal Lobe Epilepsy

Lucy Ogbu-Nwobodo, Ali Izadi, Aleksandr Pevzner, Kiarash Shahlaie, Gene Gurkoff

Department of Neurological Surgery, University of California, Davis, CA, USA

UC DAVIS
SCHOOL OF MEDICINE

Introduction

An estimated 5.1 million people in the U.S. are diagnosed with epilepsy resulting in an estimated economic cost of \$15.5 billion yearly¹⁻². Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy. Despite the introduction of newer antiepileptic drugs (AED), ~40% of patients have poor seizure control with medication alone³. Many of these patients also suffer from chronic learning and memory deficits which can be further exacerbated by current AEDs⁴⁻⁵. Therefore, there is a critical need for innovative therapies that both reduce seizures and also address the associated cognitive deficits.

Vagus nerve stimulation (VNS), approved by the FDA in 1997, is used as an adjunctive therapy for medically refractory epilepsy. Concurrent use of VNS and AEDs results in 50% seizure reduction in up to 50% of treated patients⁶. Despite its success in reducing seizures, VNS as it is used clinically has failed to address cognitive impairments in TLE.

One reason why vagus stimulation may not affect cognitive processes is related to the current frequency that is used, which is in the range of 20-30 Hz. Interestingly, studies demonstrate that seizures can be interrupted with stimulation in the 5-12 Hz range⁷. This frequency range, also known as theta, has long been implicated in learning and memory processing⁸. Furthermore, theta oscillations are a dominant rhythm in the hippocampus, a structure that is both involved in the generation of TLE and formation of long-term memories. Consistent with these observations, our lab recently demonstrated that theta stimulation of the septohippocampal system increased seizure threshold and improved cognition in a rodent model of epilepsy⁹.

Consequently, we hypothesize that low frequency stimulation of the vagal nerve (7.7 Hz) will reduce seizures and improve learning and memory in the pilocarpine model of TLE.

Methods

Animals and Surgical Procedures: All animal procedures were carried out in accordance with the UC Davis Institutional Animal Care and Use Committee (IACUC) policy.

Subjects: Adult male Sprague-Dawley rats (300-350g) were housed under standard laboratory conditions.

VNS Construction: VNS electrodes were constructed from stainless steel wires attached to polyvinyl chloride (PVC) tubes as previously described¹⁰.

VNS Implantation: Animals were anesthetized using 4% isoflurane and then intubated. An incision was made on the left side of the ventral neck just lateral to the midline. The sternohyoid and sternomastoid muscles were separated longitudinally until the carotid sheath was visualized. The vagus, contained within the carotid sheath was carefully separated and placed into the lumen of the VNS cuff. Suture was tied to secure the cuff.

Electrode Implantation: Individual tungsten electrodes (0.02 cm diameter; PlasticsOne) were stereotaxically positioned to target the hippocampus (AP -3.3mm, ML ±2.0mm, DV -3.8mm) and the medial septal nucleus (MSN) (AP +0.5mm, ML ±1.3mm, DV -6.8mm; 12.8°) bilaterally. Electrodes were affixed to the skull with C&B-Metabond (Parkell). Electrodes were connected to a 16-channel electrical interface board (Neuralynx) and the interface board implanted in dental acrylic.

Pilocarpine-Induced Epilepsy: Scopolamine methyl nitrate was injected (1mg/kg, IP) 30 min prior to pilocarpine. Seizures were induced by injection of pilocarpine (350 mg/kg, IP) and convulsive seizures were terminated with diazepam (8mg/kg, IP) after 240 min.

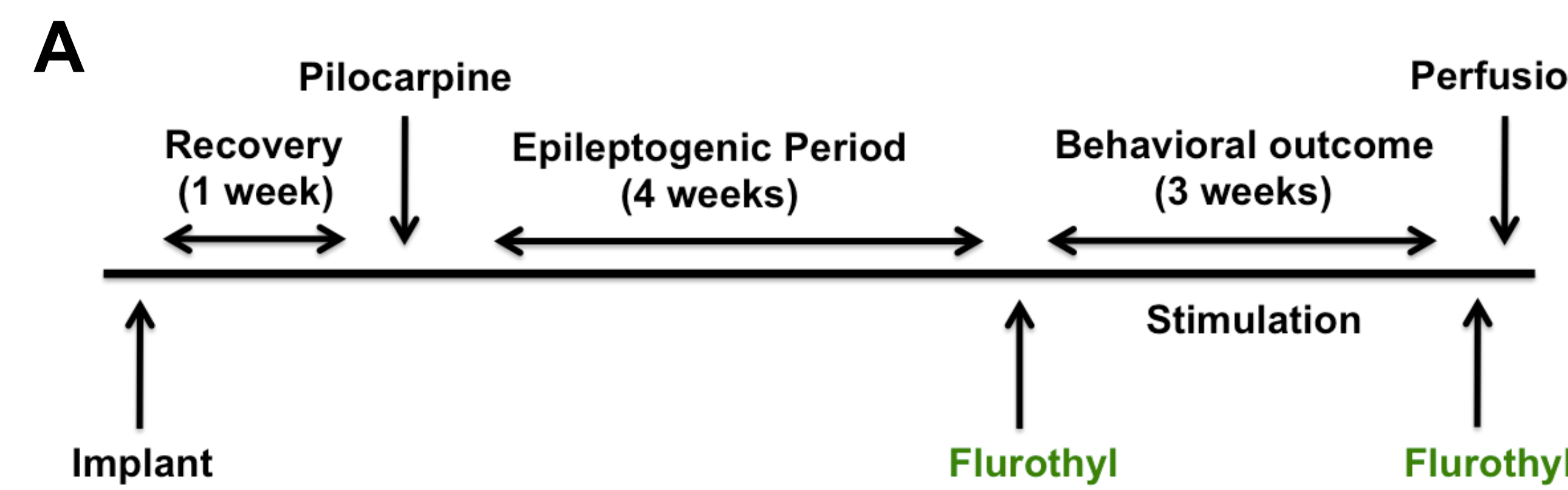
Seizure Threshold Assessments: Seizure threshold was assessed using Flurothyl, a volatile GABA antagonist. A pump (Harvard Apparatus) was used to gradually increase the concentration in the chamber (20µl/min). Time to seize was used as a measure of seizure threshold.

Behavioral Tasks: The Barnes maze is a circular platform (1.5 m diameter) with 22 circular holes (14 cm diameter) equally spaced along the periphery, with four surrounding distal spatial cues. A dark escape box is placed in a fixed location under one hole. Animals were attached to a tether and placed in a start box centered on the maze for 10 sec. The box was lifted and a white noise generator and two ultra-bright LED lights were turned on for the duration of the trial. Latency (sec) to find the hidden escape box was used as a measure of spatial memory.

Data Acquisition: A unity gain amplifier was magnetically clipped directly onto implanted electrode interface board. Amplified EEG was relayed via tether to a 16-channel acquisition system (Cheetah, Neuralynx), band-pass filtered between 0.1-2000 Hz sampled at 32 kHz and referenced to the ground screw in the cerebellum. Local field potentials (LFP) in the theta frequency were analyzed with in-house Matlab scripts (Mathworks) for power, percent time and phase coherence. Based on previous studies, theta was operationally defined as 6-10 Hz.

Experimental Design & Results

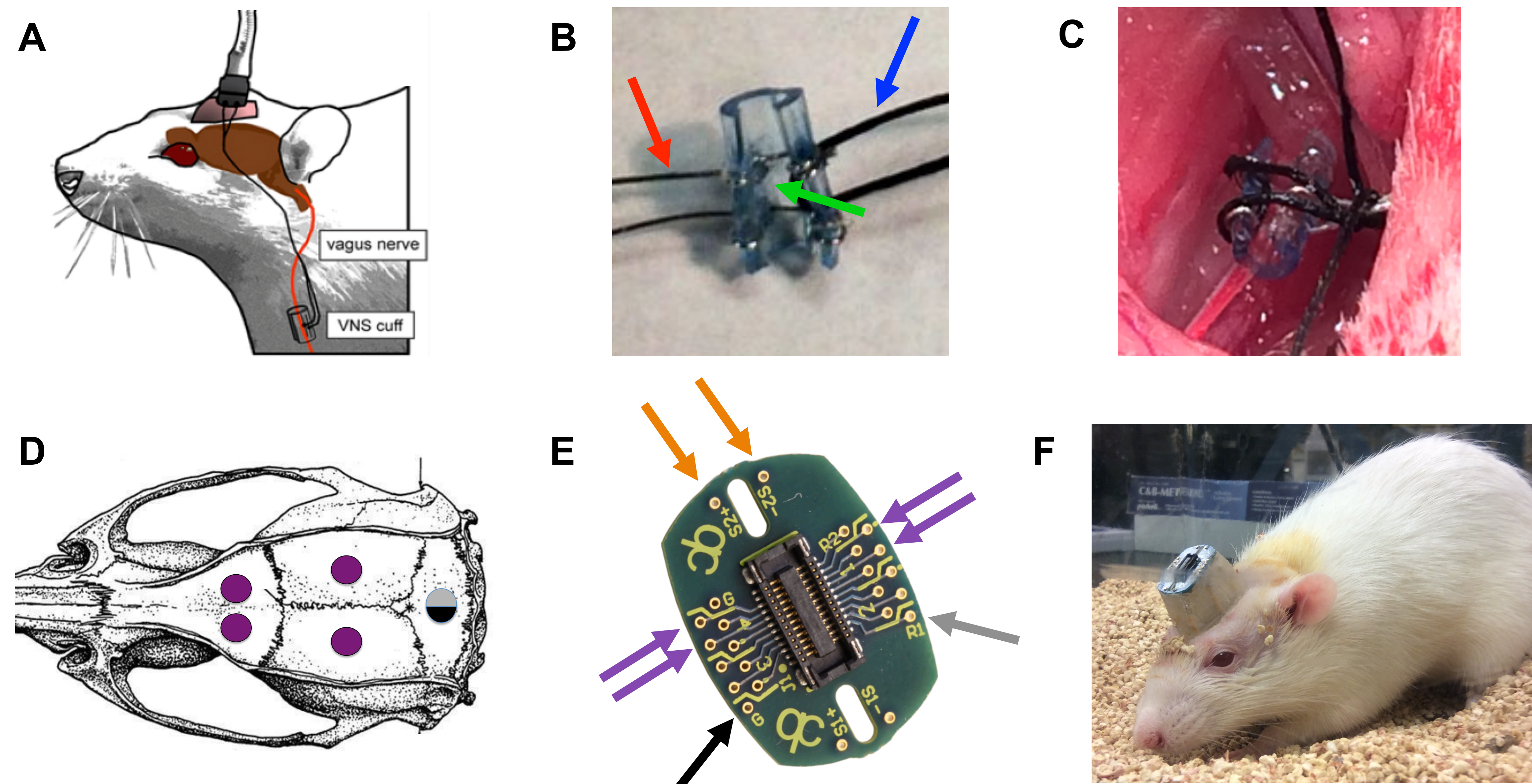
FIGURE 1: EXPERIMENTAL DESIGN



GROUP	EPILEPSY	SEIZURE THRESHOLD	BEHAVIOR
PILO+STIM (7.7 Hz)	X	X	X
PILO+STIM (30 Hz)	X	X	X
PILO+NO STIM	X	X	X
SHAM+NO STIM		X	X

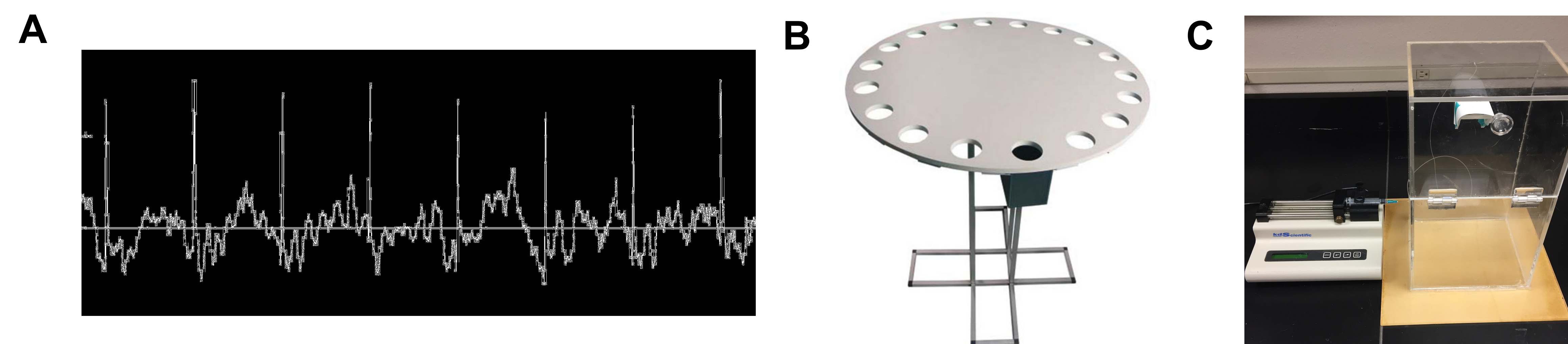
Experimental Design: A) A schematic timeline describing the initiation of seizures with the muscarinic agonist pilocarpine, assessment of cognitive outcomes following an epileptogenic period and evaluation of seizure threshold with the volatile GABA antagonist Flurothyl. B) Description of groups including controls (sham and pilocarpine) and two experimental groups (pilocarpine+7.7 Hz theta stimulation and pilocarpine + 30 Hz stimulation).

FIGURE 2: SURGERY PROCEDURES



A) Illustration of a rat with implanted vagal cuff, head implant and wire tether¹⁰. B) Vagus cuff including exposed contacts for bipolar stimulation (green arrow), silver wire for connection to interface board (red arrow) and sterile suture to manipulate and secure the cuff's position (blue arrow). C) Cuff implanted on left vagus nerve. D) Schematic describing electrode implantation with bilateral septal and hippocampal (purple) recording sites as well as a cerebellar ground/reference screw (black/gray, respectively). E) Electrode interface board (EIB) with specific via for connecting bipolar stimulation electrodes (orange); recording electrodes for bilateral hippocampus and MSN (purple); ground (black) and reference (gray) electrodes connected to a skull screw. F) Rat with a stable implant.

FIGURE 3: OUTCOME MEASUREMENTS



A) Example of hippocampal LFP recorded from a rat while receiving 7.7 Hz stimulation in the medial septum. B) Barnes Maze apparatus as described in methods¹¹. C) Flurothyl chamber for seizure threshold measurement.

Next Steps

1. Evaluate whether 7.7 or 30 Hz VNS stimulation entrains hippocampal theta oscillations (percent time, power, coherence).
2. Compare the effects of 7.7 Hz and 30 Hz stimulation on both Barnes maze spatial learning performance and Flurothyl seizure threshold.
3. Confirm that stimulation, regardless of frequency, does not trigger seizures.

Future Directions

1. Evaluate the effects of VNS on the number of electrographic and convulsive seizures.
2. Additional behavioral tests to assay cognitive function over a range of clinically relevant measures (e.g. recognition memory, temporal ordering).
3. Optimize additional stimulation parameters including stimulation pattern (e.g. cycled vs. continuous, sine vs. square).
4. Determine whether accumulation of stimulation (i.e. multiple weeks of continuous stimulation) results in either neuroprotection or excitotoxicity in specific cell populations relevant to hippocampal oscillations (e.g. hippocampal pyramidal cells and parvalbumin containing interneurons).
5. Clinical safety & feasibility study – UCDMC.

References

1. Centers for Disease Control and Prevention. (2016). Epilepsy. <https://www.cdc.gov/epilepsy/basic/fast-facts.htm>.
2. Kobau R, Luo Y, Zack M, Heimers S, Thurman D. (2012) Epilepsy in adults and access to care-United States, 2012. *MMWR*. 61(45); 909-913.
3. Kwan P, Brodie MJ. (2000) Early identification of refractory epilepsy. *The New England Journal of Medicine*. 342(5):314-219.
4. Bell, B.D., (2012) Route learning impairment in temporal lobe epilepsy. *Epilepsy Behav*. 25(2):256-262.
5. Meador, KJ. Cognitive effects of epilepsy and antiepileptic medications In: Wylie E ed. The treatment of epilepsy: principles and practice, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2001:1215-1225.
6. De Herdt, V., Boon, P., Ceulemans, B., et al. (2007) Vagus nerve stimulation for refractory epilepsy: a Belgian multicenter study. *Eur J Pediatric Neurol*, 11(5), 261-269.
7. Colom L.V., Garcia-Hernandez, A., Castenada, M.T., Perez-Cordova, M.G., Garrido-Sanabria, E.R. (2006) Septo-hippocampal networks in chronically epileptic rats: potential antiepileptic effects of theta rhythm generation. *J Neurophysiol* 95: 3645-3653
8. Buzsaki G. (2002). Theta oscillations in the hippocampus. *Neuron* 33:325-340.
9. Lee, D. J., Izadi, A., Melnik, M., Seidl, S., Echeverri, A., Shahlaie, K., & Gurkoff, G. G. (2017). Stimulation of the medial septum improves performance in spatial learning following pilocarpine-induced status epilepticus. *Epilepsy Res*. 130, 53-63. doi:10.1016/j.eplepsyres.2017.01.005.
10. Childs, J.E., Alvarez-Dieppa, A.C., McIntyre, C.K., Kroener, S. (2015). Vagus nerve stimulation as a tool to induce plasticity in pathways relevant for extinction learning. *J. Vis. Exp.* 102, e53032
11. Barnes maze. Retrieved from <http://www.noldus.com/animal-behavior-research/solutions/research-small-lab-animals/barnes-maze>.

Acknowledgement

This project is supported by the National Center for Advancing Translational Sciences, National Institute of Health, through grant number UL1 TR001860 and linked award TL1 TR001861.