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

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Introduction

An estimated 5.1 million people in the U.S. are diagnosed with epilepsy, resulting in an estimated economic yearly cost of $15.5 billion[1]. 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 alone[2]. Many of these patients also suffer from chronic learning and memory deficits that can be further exacerbated by current AEDs[3]. Therefore, there is a critical need for innovative therapies that both reduce seizures and also address the associated cognitive deficits.

Vagal nerve stimulation (VNS) was approved by the FDA in 1997 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 patients[4]. Despite its success in reducing seizures, VNS, as it is administered clinically, has failed to address cognitive impairments in TLE.

The lack of efficacy of VNS on cognition may be related to the stimulation paradigm; cycled 20-30 Hz. However, seizures can also be interrupted with stimulation in the 5-12 Hz range[5]. This frequency range, also known as theta, has long been implicated in learning and memory processes[6]. Furthermore, theta oscillations are one of the dominant rhythms in the hippocampus, a structure that is both involved in the generation of TLE and spatiotemporal learning. Consistent with these observations, our lab recently demonstrated that theta stimulation of the medial septum increased seizure threshold and improved cognition in a rodent model of TLE[7].

We hypothesize that, similar to DBS, 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. Twelve rats were split into three groups of four: sham (full VNS implant, saline injection), pilocarpine, and pilocarpine with stimulation.

VNS Construction: VNS electrodes were constructed from stainless steel wires attached to polyvinyl chloride (PVC) tubes, as previously described[8].

VNS Implantation: All 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 vagal nerve was carefully isolated 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 lowered, targeting the hippocampus bi-laterally (AP -3.3mm, ML ±2.0mm, DV -3.8mm). Electrodes were affixed to the skull with C&B-Metabond (Parkell). Electrodes were connected to an electrode interface board (Neuralynx) and the interface board implanted in dental acrylic.

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

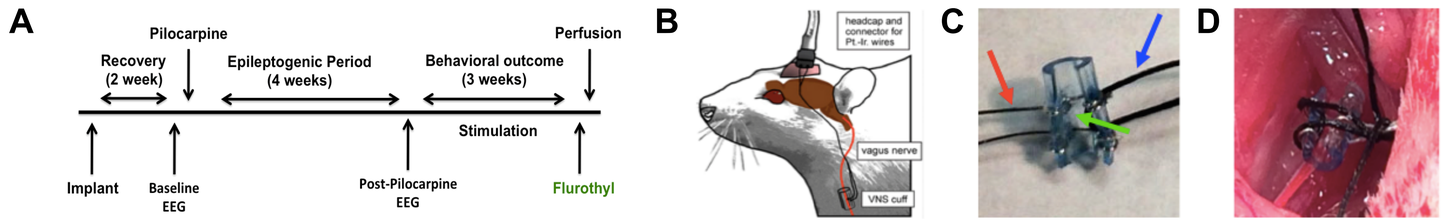
Seizure Threshold Assessments: Seizure threshold was assessed using Flurothyl, a volatile GABA antagonist. A pump (Harvard Apparatus) gradually increased the concentration in the chamber (infused at rate of 20 µl/min). Time to seize was used as a measure of seizure threshold.

Barnes Maze Spatial Learning: 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 connected to a unity gain amplified headstage and tethered to a 16-channel amplifier (Neuralynx) via a motorized commutator and then placed in a start box centered on the maze for 10 seconds. 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 to find the hidden escape box and search strategy were used as a measures of spatial memory. One stimulation animal was removed from the study as it repeatedly fell from the apparatus.

Novel Object Recognition Task: Animals were habituated to a large empty Plexiglas box for 5 minutes. The test was carried out in two five-minute sessions divided by an intersession interval (ISI) of 3 hours. During the first session (familiarization), the animal was free to explore two presented objects. During the second session, (test session), one of the objects was replaced by a novel object. A differential score (novel – familiar) and percent novel object exploration were used to evaluate object preference. Two of the stimulation animals were removed from the novel object analysis, as they did not explore the objects during the familiarization phase.

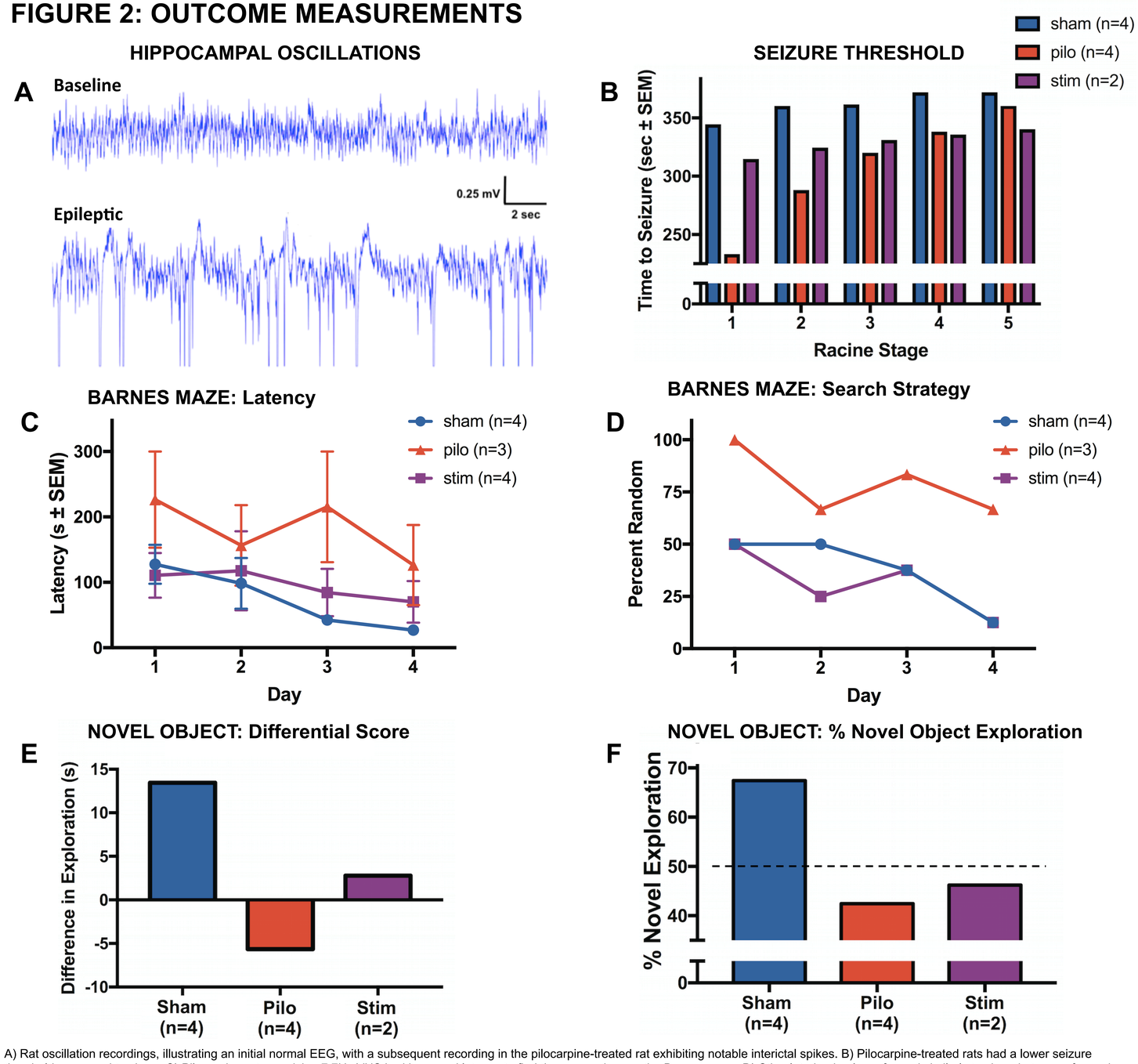
Experimental Design & Results

FIGURE 1: EXPERIMENTAL DESIGN



Experimental Design: A) Schematic describing the timeline of the experiment, including 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) Illustration of a rat with implanted vagal cuff, head implant, and wire tether[8]. C) Vagus cuff, including exposed contacts for bipolar stimulation (green arrow), silver wire for connection to interface board (red arrow), and sterile suture to manipulate position and secure (blue arrow). D) Cuff implanted on left vagus nerve.

FIGURE 2: OUTCOME MEASUREMENTS



A) Rat oscillation recordings, illustrating an initial normal EEG, with a subsequent recording in the pilocarpine-treated rat exhibiting notable interictal spikes. B) Pilocarpine-treated rats had a lower seizure threshold compared to sham. C) Pilocarpine rats receiving 7.7Hz VNS had improved latency to find the escape box on the Barnes maze. D) Stimulated animals performed similarly to sham in terms of search strategy. Non-stimulated rats spent less time exploring a novel object compared to sham rats as determined by both E) differential score and F) percent exploration. One stimulated animal showed preference for the novel object while the other did not, resulting in no overall improvement in novel object performance from stimulation.

Conclusions

1. Preliminary experiments (n=4/group) corroborate previous experiments in which pilocarpine-treated rats developed interictal spikes, a reduction in seizure threshold, and impaired cognitive performance on the Barnes maze
2. Stimulation during the flurothyl test resulted in increased stage 1 and 2 seizure threshold as compared to non–stimulated pilocarpine rats.
3. Stimulation during the Barnes maze (n=3) improved latency toward sham levels and improved search strategy.
4. Stimulation during the NOR (n=2) did not improve novel object recognition.

Future Directions

1. Due to small sample size, we did not run statistics. Power analysis indicates the need for a sample size of 10.
2. Future studies will include extended duration recordings to allow for capture and quantification of electrographic seizures and interictal spikes.
3. Initial stimulation parameters were determined from our previous experience with theta stimulation. Future experiments will also include a cycled 30 Hz stimulation group to compare to current clinical practice.
4. In addition to behavior, we will determine whether stimulation entrains oscillations in the hippocampus and evaluate whether lower frequency stimulation alters hippocampal anatomy, including neuronal number and inflammation.

References

1. Centers for Disease Control and Prevention. (2016). Epilepsy. https://www.cdc.gov/epilepsy/basic/fast-facts.htm
2. Kwan P, Brodie MJ. (2000) Early identification of refractory epilepsy. *The New England Journal of Medicine,* 342(5):314-219.
3. 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
4. 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.
5. 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
6. Buzsaki G. (2002). Theta oscillations in the hippocampus. *Neuron* 33:325-340
7. 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
8. Childs, J.E., Alvarrez-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*

Acknowledgements

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