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Medial septal stimulation increases seizure threshold and improves cognition in epileptic rats

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ABSTRACT

Background: Temporal lobe epilepsy is most prevalent among focal epilepsies, and nearly one-third of patients are refractory to pharmacological intervention. Persistent cognitive and neurobehavioral comorbidities also occur due to the recurrent nature of seizures and medication-related side effects.
Hypothesis: Electrical neuromodulation is an effective strategy to reduce seizures both in animal models and clinically, but its efficacy to modulate cognition remains unclear. We hypothesized that theta frequency stimulation of the medial septum would increase septohippocampal oscillations, increase seizure threshold, and improve spatial learning in a rat model of pilocarpine-induced epilepsy.
Methods: Sham and pilocarpine rats were implanted with electrodes in the medial septum, hippocampus and prefrontal cortex. EEG was assessed days prior to and following stimulation. Sham and pilocarpine-treated rats received either no stimulation, continuous (throughout each behavior), or pre-task (one minute prior to each behavior) 7.7 Hz septal stimulation during the Barnes maze spatial navigation test and also during assessment of flurothyl-induced seizures.
Results: Both continuous and pre-task stimulation prevented epilepsy-associated reductions in theta oscillations over time. Additionally, both stimulation paradigms significantly improved spatial navigation in the Barnes maze, reducing latency and improving search strategy. Moreover, stimulation led to significant increases in seizure threshold in pilocarpine-treated rats. There was no evidence of cognitive enhancement or increased seizure threshold in stimulated sham rats.
Conclusion: These findings have profound implications as theta stimulation of the septum represents a single frequency and target that has the potential to both improve cognition and reduce seizures for patients with refractory epilepsy.

Introduction

Over 3.4 million people have been diagnosed with epilepsy in the United States [1], resulting in an estimated economic impact of 9.5 billion dollars annually [2]. Focal epilepsies represent more than two-thirds of all cases, and temporal lobe epilepsy (TLE) is the most prevalent subtype [3]. Approximately 30–40% of patients with TLE are refractory to anticonvulsant medications, representing 80% of the total economic burden [4–6]. Most patients with intractable TLE experience chronic cognitive deficits due to recurrent seizures as well as drug-related side effects [7]. To date, there are no therapeutic interventions that both reduce seizures and mitigate cognitive performance declines in patients with TLE.

The efficacy of neuromodulation via electrical stimulation to prevent or disrupt seizure activity has been the subject of several clinical studies. For example, deep brain stimulation (DBS) of the anterior nucleus of the thalamus reduced seizures by at least half in 68% of patients over a 5-year period [8]; in a separate study of 29 patients followed for 11 years, a median 70% reduction in total seizures was reported [9]. Responsive neurostimulation similarly reduced seizures by at least half in 70% of patients over a 6-year study period [10]. Interestingly, both approaches resulted in progressive improvements in seizure reduction, with patients having fewer seizures over time [8,10].

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Attempts to use DBS to improve cognition in individuals with chronic learning and memory deficits, however, have yielded mixed results. For example, 50 Hz stimulation of the entorhinal cortex, but not hippocampus, resulted in improved spatial memory in patients with epilepsy [11]. However, in a similar study, stimulation of both entorhinal and hippocampal foci significantly impaired verbal and spatial memory [12]. Additional studies reported significant impairments in memory with direct hippocampal stimulation [13,14]. Indirect modulation, however, may be a means of driving, without disrupting, the hippocampus. For example, electrical stimulation of the hypothalamus and fornix have demonstrated some efficacy in activating the hippocampus and improving cognition [15–17]. Together, these findings emphasize the potential benefit of using a network level approach to identify targets and stimulation parameters to modulate hippocampal function and improve cognition [18].

To date, there is no DBS paradigm that treats both seizures and cognitive dysfunction in TLE. Low frequency theta oscillations play a critical role in septohippocampal networks and are implicated in excitability, seizure generation [19,20], and cognitive processing [21–23]. We hypothesized that theta frequency stimulation of the medial septal nucleus (MSN) would entrain physiologic oscillations, resulting in improved cognitive function and increased seizure thresholds in rats with pilocarpine-induced TLE. A novel therapy that concurrently reduces seizures and improves cognition would constitute a critical advancement in therapy for patients with medically refractory TLE.

Methods

All procedures adhered to NIH guidelines and were approved by the University of California, Davis Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (275–325 g; n = 76) were randomly assigned to control (n = 32) and pilocarpine groups (n = 44). Six rats were sacrificed due to complications following acute status epilepticus and nine others were sacrificed prior to data collection due to failed implants (n = 4 sham, n = 5 pilocarpine). Two animals were sacrificed after completion of the Barnes maze due to failed implants (n = 2 pilocarpine).

In order to induce acute seizures and epilepsy, scopolamine methyl nitrate was injected (1 mg/kg, IP; Sigma Aldrich, St. Louis) 30min prior to pilocarpine (350 mg/kg, IP; Sigma Aldrich, St. Louis) [24]. Convulsive seizures were terminated with diazepam (8 mg/kg, IP; Sigma Aldrich, St. Louis) 240min after injection of pilocarpine. Sham controls were injected with identical doses of scopolamine and diazepam; however, an equal volume of sterile saline was substituted for pilocarpine. Following injections, rats were individually housed for the duration of the study; daily examinations were performed, and animals were given supplemental nutrition for up to three weeks to reduce distress. For all behavioral experiments, investigators were blind to group (pilocarpine vs. sham) but not to stimulation (stimulation needed to be triggered and was visually confirmed on the EEG). Data files were re-coded, and all analyses were performed by a blinded investigator. A timeline of all experiments is illustrated in Fig. 1A.

Flurothyl testing on post-pilocarpine day (PPD) 32 and 56

Seizure threshold was measured using flurothyl (bis(2,2,2-trifluroethyl) ether, (Sigma Aldrich, St. Louis), a volatile antagonist of $GABA_A$ receptors [25] both prior to implantation (PPD32) and again after the conclusion of cognitive behavioral assessment (PPD56). Animals were placed in a small Plexiglas chamber (17.75 × 20.25 × 35.5 cm) with limited room for mobility. Conditions were similar between animals as the chamber was sealed, and placed in a chemical fume hood. Room temperature (~70 F) and humidity (~32%) were also kept constant. Flurothyl was dripped directly onto filter paper at a rate of 1.2 ml/h. All animals progressed from stage 1–5 seizures; animals were rapidly removed from the Plexiglass chamber after reaching stage 5. Exposure time was recorded to compare time to reach each of Racine 1–5 seizures. Flurothyl data collected at PPD32 were used to establish a pre-stimulation baseline for each rat and to counterbalance epileptic and sham animals into stimulation groups with similar average thresholds prior to intervention. PPD32 and 56 values were compared to determine effects of time and stimulation on seizure threshold.

Electrode implantation on PPD33

On PPD33 all animals including sham controls were anesthetized using 2–4% isoflurane in O_2/N_2O(1:2) carrier-gas, intubated, and mechanically ventilated to maintain surgical anesthesia. Following a midline incision, the skin was retracted revealing the skull. Craniotomies (2 mm) were made, and twisted bipolar electrodes (Plastics One MS333/3-B/SP, Roanoke) were stereotactically implanted, in the right medial prefrontal cortex (PFC; AP = +3.2, ML = +1.3, DV = −3.5; 9° angle), right dorsal hippocampus spanning CA3-CA1 (AP = −3.3, ML = +2.0, DV = −3.2) and MSN (AP = +0.48, ML = −1.5, DV = −6.8; 12.8° angle) in both sham and pilocarpine rats. Electrodes were then anchored to stainless steel screws (#0–80) that had been embedded adjacent to each craniotomy using nontoxic superglue. Electrodes and screws were subsequently covered with acrylic to form a rigid implant.

Stimulation paradigm

As previously described, sham and pilocarpine-treated rats were counter-balanced based on PPD32 seizure threshold (Table 1) into one of three stimulation groups: no stimulation (sham-no n = 10; pilocarpine-no n = 10), continuous stimulation (CT; starting exactly 60sec before and lasting throughout behavioral evaluation; sham n = 8; pilocarpine n = 12) and pre-task stimulation (PT; defined as stimulation for exactly 60sec immediately prior to a behavioral evaluation; sham n = 10; pilocarpine n = 11). Stimulation of the MSN occurred exclusively during the Barnes maze (PPD45-48; Fig. 1C) and during PPD56 flurothyl testing (Fig. 1B). A twisted wire cable connected the implanted bi-polar MSN electrode to an isolated pulse stimulator (A-M Systems, model-2100, Sequim). Cathodal stimulation for both CT and PT was 7.7 Hz, 80 μA with 1msec square-wave pulse-width [21,22,26–28].

EEG recordings on PPD42 and PPD55

On PPD42 and 55, animals were individually placed in a small translucent Plexiglas open field (25 × 45 × 50 cm) for 10 min. As little space was available, animal movement was limited to turning around and rearing, with only enough room to take a few steps. EEG was recorded with a preamplifier (Grass model-7P5B, Quincy) and polygraph driver amplifier (Grass model-7DAG) and collected with a computerized acquisition system (Polyview16 v1.1, Astromed, Inc., West Warwick).

A custom Matlab script was used to generate a power spectral density (PSD) graph of the hippocampus comparing all sham and pilocarpine animals during PPD42 EEG recordings. Furthermore, as published previously, data were analyzed using the oscillatory detection algorithm p_episode [26,27,29,30] to quantify the percent time theta oscillations (6–10 Hz) were observed in MSN, hippocampus, and PFC. Using wavelet decomposition and applying strict amplitude and duration thresholds (at least 3 consecutive cycles of

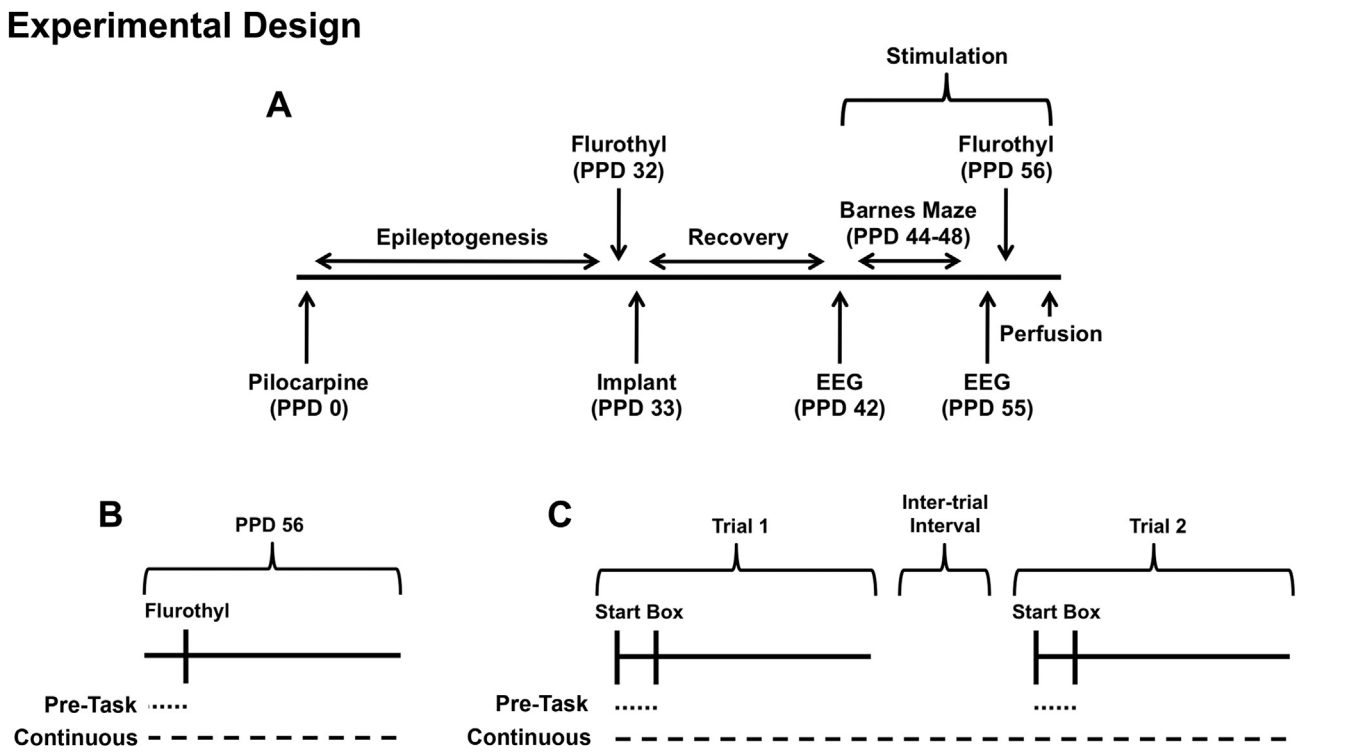

Fig. 1. Timeline of the experimental design (Fig. 1A). Evaluation of seizure threshold (Fig. 1B) occurs on PPD32 and 56. Stimulation only occurs on PPD56; CT stimulation occurs for one minute prior to the start of flurothyl testing and continues through completion; PT stimulation occurs for only one minute prior to the start of flurothyl evaluation. During Barnes maze evaluation (Fig. 1C), CT stimulation occurs continuously through both successive trials each testing day; PT stimulation occurs only for the one minute animals are placed under the start box at the start of each trial for four consecutive days.

theta), p_episode determines whether each peak constitutes an oscillation. The output of p_episode is presented as a percentage of time, accounting for variance in total duration of any given EEG recording due to artifact removal. In this way, p_episode provides a rigorous means of identifying oscillations from artifact.

Barnes maze on PPD44-48

The Barnes maze is a Plexiglas table (122 cm diameter) with 16 peripheral holes (7.5 cm diameter), a goal box under one hole, and four distal cues [26]. On PPD44, rats were placed on the table in ambient light without cues or goal box for 10min to habituate to the environment. Starting on PPD45, animals underwent four consecutive days of training consisting of two 5min trials separated by a 5min inter-trial interval. Each day began by connecting the animal to the stimulator and placing it under a dark start box in the center of the table for 60sec. For the CT group, stimulation was started prior to placement in the start box and was continued without interruption through both trials; for the PT group, the stimulation was started prior to placement in the dark box and terminated before trial initiation (exactly 60sec; Fig. 1C). The box was raised, exposing the rat to the bright light, and rats were allowed 5min to find the goal box; animals failing to locate the target were guided to the hole and into the goal box. All animals were allowed to rest in the goal box for 1min and then placed in a neutral cage for 4min prior to starting the second trial.

Barnes maze data were quantified across each of the days by escape latency (sec), strategy (spatial, peripheral, or random), and efficiency (minimum possible distance/actual distance traveled). For the first day of Barnes maze only trial two was included in analyses as trial 1, regardless of group, was necessarily a random trial. Spatial strategy was defined as the use of a direct path to the

Table 1
Racine stage 3 and stage 5 group seizure threshold averages on PPD32 and PPD56. Groups were counter-balanced using PPD32 seizure threshold measurements. Average cumulative stimulation time over the course of the experiment as measured per group. Corresponding average weights are also reported.

Group	'N'	Day 32			Day 56			Avg. Stim. Time (min)
		Stage 3 (s)	Stage 5 (s)	Wt. (g)	Stage 3 (s)	Stage 5 (s)	Wt. (g)	
Sham-No	10	504.1	584.2	388.7	503.2	565.6	420.6	0
Sham-TC	8	491.1	589.9	403.5	508.6	601.1	432.1	77.9
Sham-PT	10	482.4	589.9	378.1	506.1	555.5	407	13
Pilo-No	10	415.8	461.4	386.3	328.7	350.4	415.1	0
Pilo-TC	12	411.7	464.3	396.4	472.6	516	420.1	73.7
Pilo-PT	11	394.1	458.3	402.8	444.8	514.4	425.6	13

Sham-No: Full Surgery, No pilocarpine Treatment, No Stimulation.
Pilo-No: Full Surgery, Pilocarpine Treatment, No Stimulation.
CT: Continuous Stimulation.
PT: Pre-Task Stimulation.

goal box; peripheral strategy as circling the periphery of the table; and random strategy by exploration of non-consecutive holes.

Histology

Animals were euthanized (Euthasol, Virbac Corp., Fort Worth) and transcardially perfused. Using the NeuN antibody to label neurons, we confirmed electrode position in the hippocampus, MSN, and PFC [27].

Statistical analyses

To evaluate the effect of pilocarpine on seizure threshold prior to assigning animals to stimulation groups (PPD32), all sham and pilocarpine-treated rats were compared using unpaired two-tailed t-tests to compare each of Racine stages 1–5. We used the Bonferroni-Dunn method to correct for multiple comparisons.

To evaluate the effect of stimulation on seizure threshold, we used a repeated-measures ANOVA, which included repeated measures for analyses across two time points (PPD32, 56) for Racine stage 3 and 5. Data across the entire Racine scale was not evaluated, as seizure threshold data were co-linear. To test for differences in the hippocampal PSD between all sham and pilocarpine animals on PPD 42, 6–10 Hz PSD data were first averaged for each animal; the means of each group were then compared using a two-tailed T-test. To test whether stimulation altered percent time oscillating in theta frequency, p_episode outputs were first tested for normality using a chi-square test; subsequently, a repeated-measures ANOVA was used to compare groups between PPD42 and 55; a Bonferroni multiple comparisons test was used to indicate changes in each treatment group across the time points.

For the Barnes maze, data from trials 2–8 were used for all analyses; data from trial 1 was excluded, as performance across all animals, regardless of group, was random. On test days 1–4 of the Barnes maze (day 1 includes only trial 2), average latency per animal was calculated and a repeated-measures ANOVA with a Dunnett's posthoc test was used to compare all treatment groups to non-stimulated pilocarpine animals. For analysis of search strategy, each trial was categorized as spatial, peripheral, or random. Data were collapsed across trials 2–8 and a nonparametric chi-square analysis was used to compare differences in search strategy with the performance of non-stimulated pilocarpine animals used as the test proportion in comparison to all other treatment groups. To determine differences in path efficiency, repeated-measures ANOVA with a Dunnett's posthoc test was used to compare all treatment groups to non-stimulated pilocarpine animals.

We made an *a priori* decision based on our hypothesis to analyze EEG, behavior, and seizure threshold from stimulated sham rats similarly, but separately, from stimulation of pilocarpine rats. We used the same non-stimulated shams for comparison to both the pilocarpine as well as the sham stimulation groups. All data is presented as the mean ± standard error. Statistical significance was assigned to values $p < 0.05$. All analyses were done using GraphPad Prism (GraphPad Software, La Jolla).

Results

Theta stimulation increases seizure threshold

Following pilocarpine injection, all animals experienced acute repetitive motor limbic seizures, including multiple cycles of status epilepticus, and began to exhibit spontaneous seizures in the weeks following injection of pilocarpine. On PPD32, prior to being assigned to stimulation groups, pilocarpine-treated animals ($n = 33$) demonstrated a significantly lower seizure threshold in response to flurothyl across each stage of the Racine scale, as compared with sham animals ($n = 28$; Stages 1–5, $df = 57$, $t = 5.41$, 4.84, 4.49, 5.49, 5.67 respectively, $p < 0.001$ for all comparisons; Fig. 2A).

On PPD56, both CT and PT stimulation of pilocarpine-treated rats improved seizure thresholds. Specifically, there was a significant effect of group and an interaction between group and time when analyzing stage 3 (Group: $F_{(3,37)} = 7.54$, $p = 0.0005$; Interaction: $F_{(3,37)} = 10.94$, $p < 0.0001$) and stage 5 seizures (Group: $F_{(3,37)} = 10.43$, $p < 0.0001$; Interaction: $F_{(3,37)} = 7.96$, $p = 0.0003$) between PPD32 and 56. Post-hoc tests demonstrated no effect of time on seizure threshold in shams (Stage 3: Fig. 2B; Stage 5: Fig. 2C). While non-stimulated pilocarpine rats had a significantly lower seizure threshold on PPD56 compared to PPD32 (Stage 3: $p < 0.001$; Stage 5: $p < 0.005$), stimulated pilocarpine rats did not demonstrate this decrease. In fact, CT stimulated rats had significantly elevated stage 3 seizure thresholds (Stage 3: $p < 0.05$) and PT stimulated rats had a trend towards elevated threshold (Stage 3: $p = 0.056$) after correcting for multiple comparisons.

Theta stimulation preserves oscillatory activity

Fig. 3A depicts an example EEG trace from the hippocampus of a sham animal. A power analysis on PPD42 found no significant difference in hippocampal theta power between sham and pilocarpine rats prior to stimulation (Fig. 3B) or after behavioral analysis (data not shown). There were also no differences in theta power in the MSN or PFC (data not shown). P_episode was compared between baseline (PPD42) and PPD55 to assess potential changes in theta oscillations across time following behavioral analysis and stimulation. While there was no main effect of stimulation on percent time in theta for group or for time in either the hippocampus or PFC, we did find a significant group-by-time interaction in both the hippocampus ($F_{(3,74)} = 2.81$, $p < 0.05$) and PFC ($F_{(3,74)} = 2.82$, $p < 0.05$), indicating that the effects of stimulation differed over time. Post-hoc tests demonstrated that non-stimulated pilocarpine-treated rats had a significant reduction in the percentage of time oscillating in theta on PPD55 as compared to PPD42 (Fig 3C; $p < 0.05$). Yet, similar to sham, CT and PT stimulation groups showed no change across time, accounting for the interaction. In the MSN, there was a significant main effect of group ($F_{(3,74)} = 3.55$, $p < 0.05$) and time ($F_{(1,74)} = 6.50$, $p < 0.05$), but there was no statistical interaction. As in the hippocampus and PFC, non-stimulated pilocarpine rats were the only group that experienced a reduction in septal theta over time. These findings indicate that theta oscillations decreased over time in pilocarpine-treated rats and that stimulation prevented this pilocarpine-induced decrease across the hippocampus, PFC and MSN. This effect persisted days after the stimulation was applied and thus cannot be considered an artifact of the stimulation itself.

Theta stimulation improves spatial learning

There was a significant main effect of group on latency to find the goal box in the Barnes maze ($F_{(3,39)} = 4.12$, $p < 0.05$; Fig. 4A). Dunnett's posthoc tests indicated that pilocarpine-treated animals had significantly longer latencies as compared to sham controls. Importantly, latency in non-stimulated pilocarpine rats was also significantly longer than both CT ($p < 0.05$) and PT ($p < 0.05$) stimulation groups. In addition to latency, there were also significant differences in search strategy. As animals are naïve to the maze on trial one, search strategy is necessarily random. While non-stimulated epileptic animals continued to utilize predominantly random search strategies on trials 2–8, sham and stimulated animals used more spatial or peripheral strategies. By the final trial, none of the sham or stimulated pilocarpine rats used a random approach to find

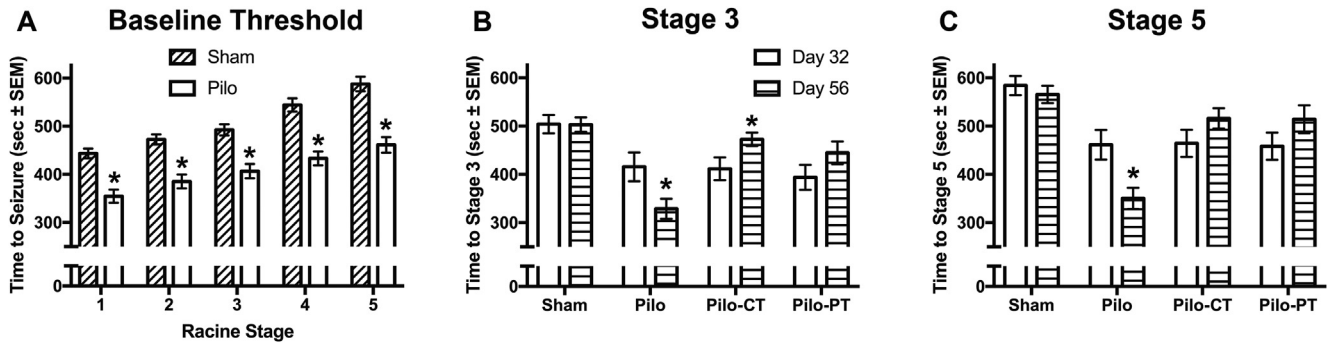

Fig. 2. On PPD32, pilocarpine-treated animals had significantly lower seizure thresholds across all stages of the Racine scale as compared with non-stimulated sham animals (*$p < 0.001$; Fig. 2A). There was a significant reduction of stage 3 (*$p < 0.001$; Fig. 2B) and stage 5 (*$p < 0.005$; Fig. 2C) seizure thresholds in pilocarpine-treated animals when comparing days 32 and 56. Seizure thresholds did not decrease across time points in stimulated pilocarpine animals and non-stimulated sham controls. In fact, CT rats had significantly elevated stage 3 seizure thresholds ($p < 0.05$) and PT stimulated rats had a trend towards elevated threshold ($p = 0.056$) when comparing days 32 and 56.

the target. Non-parametric chi-square analyses across trials 2–8 indicated that non-stimulated epileptic rats used significantly different search strategies compared to shams ($\chi^2(2), = 96.3$, $p < 0.0001$; Fig. 4B), CT ($\chi^2(2), = 98.4$, $p < 0.0001$) and PT stimulated animals ($\chi^2(2), = 100.7$, $p < 0.0001$). Improved search strategy resulted in improved path efficiency (Fig. 4C), as indicated by a significant main effect of group ($F_{(3,39)} = 4.40$, $p < 0.01$). Although posthoc tests indicated that pilocarpine rats were not statistically different than sham controls ($p = 0.063$), both CT and PT stimulated pilocarpine animals demonstrated significantly improved efficiency compared with non-stimulated pilocarpine animals ($p < 0.01$).

Theta stimulation does not enhance behavioral outcome in control animals

Stimulation did not increase seizure threshold or improve cognitive performance in sham animals. Specifically, when comparing seizure thresholds in sham treatment groups (no stimulation, CT and PT), there were no significant effects of group ($F_{(2,25)} = 0.09$, $p = 0.92$) or time ($F_{(1,25)} = 0.53$, $p = 0.47$) and also no group-by-day interaction ($F_{(2,25)} = 0.17$, $p = 0.84$).

There was a significant main effect of stimulation across sham groups when evaluating escape latency on the Barnes maze ($F_{(2,25)} = 3.433$, $p < 0.048$; Fig. 5A); in fact, a Dunnett's posthoc test indicated that PT stimulated sham animals took significantly longer to find the escape box than non-stimulated shams. CT stimulated animals demonstrated a trend towards increased latency ($p = 0.10$). Both CT and PT stimulated sham animals also used random search strategies significantly more than non-stimulated controls (CT: $\chi^2(2), = 24.7$, $p < 0.0001$; PT: $\chi^2(2), = 29.4$, $p < 0.0001$; Fig. 5B). There was no effect of stimulation on path efficiency when comparing sham groups ($F_{(2,25)} = 1.77$, $p = 0.19$; Fig. 5C). As previously noted, the same non-stimulated sham controls were used separately as a comparison for both the pilocarpine with stimulation and sham with stimulation analyses.

Discussion

Current epilepsy treatments are designed specifically to limit seizures, and if they play any role in cognition, they tend to further impair learning and memory [7]. While separate studies have indicated that electrical neuromodulation can reduce the number of epileptic seizures [8,10] and, using different frequencies in distinct neuroanatomical targets, has the potential to improve learning and memory in patients with neurological disorders [11,16], there is no single target or set of stimulation parameters that can treat both phenotypes concurrently. Here, we demonstrate that 7.7 Hz stimulation of the MSN preserves low-frequency oscillations across the duration of the study, improves performance on the Barnes maze, and increases seizure threshold in pilocarpine-treated rats. Improved cognition and elevated seizure threshold were not observed in stimulated sham controls, suggesting that these effects represent a recovery of function rather than non-specific behavioral enhancement.

Network pathology: altered theta oscillations and impaired cognition in TLE

The hippocampus is critical for the formation and recall of memories. Moreover, cognitive processes require the integration of

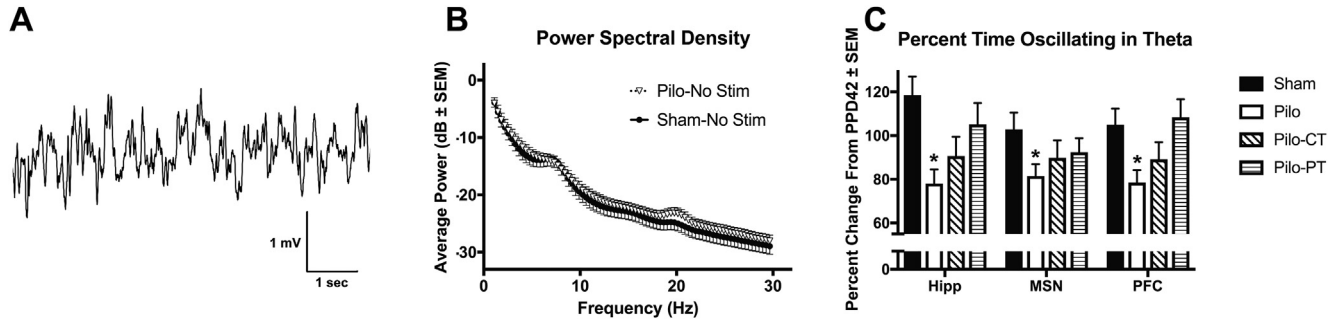

Fig. 3. An example EEG trace from the hippocampus (Fig. 3A). There was no difference in the hippocampal PSD when comparing all sham and pilocarpine animals prior to stimulation (PPD42; Fig. 3B). Theta oscillations (P-episode) measured on PPD55 were compared to PPD42. Pilocarpine-treated animals had a significant reduction in percent time in theta as compared to PPD42 (*$p < 0.05$) across all regions. There is no change in theta oscillations in non-stimulated shams or pilocarpine-treated animals receiving stimulation.

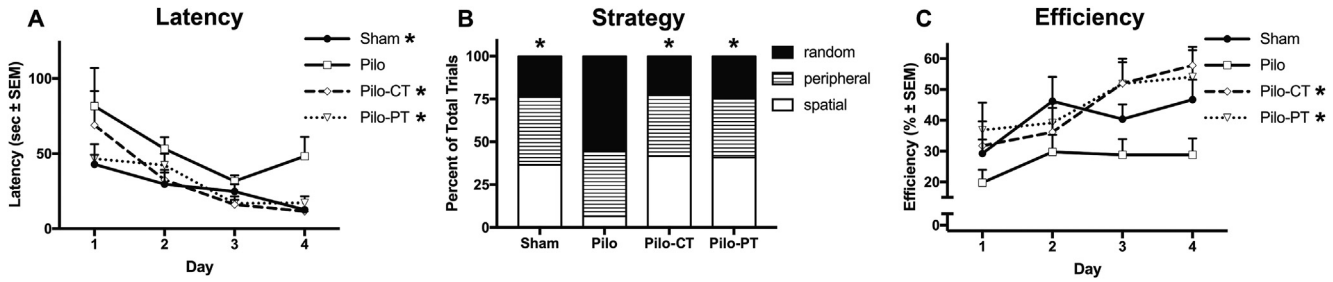

Fig. 4. On days 44–48 of Barnes maze testing, non-stimulated pilocarpine-treated animals demonstrated significantly increased latency to find the escape box as compared with non-stimulated sham animals. Pilocarpine animals with CT and PT stimulation performed significantly better than non-stimulated pilocarpine rats (*$p < 0.05$; Fig. 4A). When looking at all trials, non-stimulated pilocarpine-treated animals utilized spatial search strategies on significantly fewer trials compared with non-stimulated sham and stimulated pilocarpine animals ($p < 0.0001$; Fig. 4B). Non-stimulated pilocarpine-treated animals trend towards being less efficient on the Barnes maze as compared with sham animals; stimulated pilocarpine-treated animals demonstrated significantly improved efficiency as compared with non-stimulated animals (*$p < 0.01$; Fig. 4C).

activity across multiple brain regions. It is hypothesized, in both human [29] and in the rat, that slow wave theta oscillations not only facilitate the timing and integration of information within the hippocampus [31–33], but also across key nodes of the cognitive circuit [34,35]. In fact, when the MSN is lesioned either pharmacologically or chemically, resulting in a decrease of theta oscillations, impaired cognitive performance is observed [22,36,37]. Moreover, disruption of septohippocampal theta also decouples neuronal firing patterns between the PFC and hippocampus [38,39]. If lesions do not disrupt theta oscillations, spatial learning remains intact [23]. Similarly, pilocarpine-induced epilepsy results in significant cell loss, including degeneration of GABAergic septal neurons, GABAergic hippocampal interneurons, as well as other septohippocampal neuronal populations critical for the generation and modulation of theta oscillations such as hippocampal CA1 pyramidal neurons [19,40–42]. As a result, pilocarpine-treated rats typically experience a significant reduction in hippocampal theta power and amplitude [19], and there is evidence of reduced hippocampal theta power and altered theta peak frequency during exploration both acutely post-status and in chronically epileptic animals [28,43].

In the current study, the percent time oscillating in the theta frequency decreased over time within the core of the septohippocampal circuit (MSN, hippocampus), as well as in the PFC. Along with decreased oscillations, pilocarpine-treated rats experienced significant spatial memory deficits on the Barnes maze, demonstrating significantly longer escape latencies as compared to sham controls. Longer latencies resulted from a greater utilization of random search strategies and inefficient paths to the escape box. In fact, pilocarpine rats had a similar path length across four days of testing even as their latencies decreased, indicating that they learned to run faster as opposed to more efficiently. These data support and extend our understanding of the relationship between oscillations and cognition and, importantly, how lesions that disrupt theta result in deficits in learning and memory.

A novel target: 7.7 Hz stimulation of the MSN

During navigation and learning, hippocampal theta is observed primarily in the 6–10 Hz range [21–23,44–46]. In particular, there are increases in theta power in the 7.5–8.5 Hz when rats explore a novel environment [21]. Previous studies have demonstrated that 7.7 Hz is the optimal septal stimulation frequency to most efficiently restore physiologically relevant hippocampal theta oscillations [21,45]. In fact, 7.7 Hz stimulation of the fornix has been shown to significantly increase hippocampal theta oscillations and improve cognitive outcome following pharmacological inactivation of the MSN [22]. Furthermore, our group has also previously demonstrated that 7.7 Hz stimulation improves spatial memory performance in rats following traumatic brain injury [26,27] as well as acute status epilepticus [28].

While we could not record oscillations during stimulation due to artifact, pilocarpine-treated rats receiving stimulation did not experience a reduction in theta oscillations across the duration of our study as determined by comparing EEG in an open-field between both before (PPD42) and after completion of stimulation (PPD55). Even very limited stimulation (PT stimulation, 13 total minutes) was effective at entraining oscillations across the MSN, hippocampus, and PFC while there was a reduction over this same time in non-stimulated pilocarpine rats. In addition to preserving oscillatory activity, both CT and PT stimulation of pilocarpine animals significantly improved performance on the Barnes maze with both paradigms resulting in significantly shorter latencies to find the goal box, decreased utilization of random search strategies, and increased search efficiency.

Although we were unable to record EEG during stimulation and during Barnes maze exploration, it is possible that fixed frequency

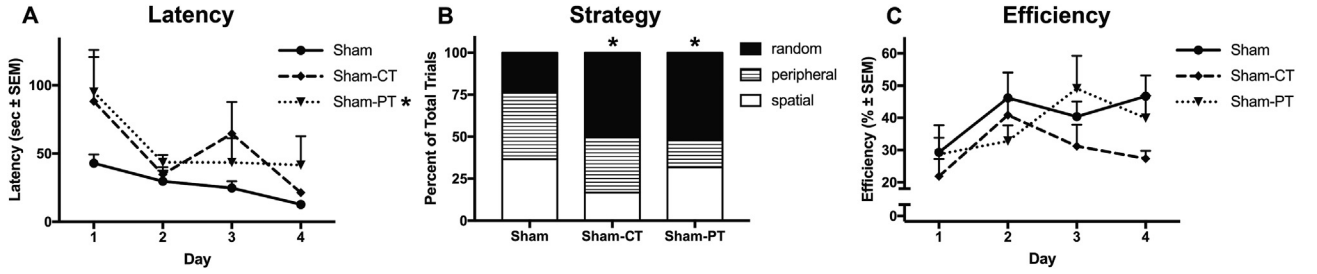

Fig. 5. On days 44–48 of Barnes maze testing, PT stimulated sham animals demonstrated significantly longer latency to the escape box as compared to non-stimulated sham controls (*$p < 0.05$; Fig. 5A). There was no significant difference between CT and non-stimulated sham controls. Sham stimulated animals utilized spatial search strategies on significantly fewer trials as compared with non-stimulated sham controls ($p < 0.0001$; Fig. 5B). There were no differences in path efficiency between sham groups (Fig. 5C).

stimulation entrains oscillations, resulting in more physiological theta as measured by frequency and power, and in turn, improved cognition. MSN theta generation is also necessary for precise temporal firing patterns of individual neurons that phase-lock to theta oscillations both within the septohippocampal circuit and in cortical regions necessary for spatial memory. During epileptogenesis, septal bursting neurons that typically phase-lock to hippocampal theta demonstrate decoupling [47]. Therefore, theta stimulation may reset normal phase-locked firing patterns in the surviving population of rhythmically bursting septal neurons [34,35]. Finally, septal stimulation could be modulating levels of excitation/inhibition, allowing for more efficient activation of ensembles and ultimately promoting plasticity. In order to elucidate the precise mechanism(s), future experiments will need to incorporate single or multi-unit activity and also evaluate additional characteristics of theta oscillations including potential alterations to frequency, spectral coherence, and cross-frequency coupling in epileptic and control rats.

The potential mechanisms responsible for improved memory performance resulting from theta frequency stimulation are not well understood. However, while beneficial for epileptic animals, stimulation of sham rats did not improve Barnes maze performance. In fact, PT stimulated sham animals demonstrated significantly longer latency to the goal, and both stimulation groups used significantly more random strategies as compared to non-stimulated shams. Therefore, 7.7 Hz stimulation does not enhance cognitive function in otherwise normal rats. Instead, these data support the hypothesis that 7.7 Hz septal stimulation is effective as a means of recovery of function in epileptic rats. Moreover, these data indicate that while a non-physiologic fixed frequency stimulation pattern can improve outcome in the damaged hippocampal circuit, altering physiologic oscillations with tonic stimulation has the potential to be disruptive in control animals.

Increased seizure threshold resulting from theta stimulation

Previous studies have indicated that theta frequency stimulation reduces evoked seizures. For example, electrical stimulation of the MSN to evoke theta inhibits pentylenetetrazol-induced seizures [20] while microinjections of carbachol also induces theta oscillations and inhibit behavioral and electrographic seizures in acutely exposed pilocarpine rats [19,20]. Furthermore, both spontaneous occurrences of theta and induced theta via tail pinch reduce markers of epileptiform activity [19]. Although the associated mechanisms are unclear, there is significant evidence demonstrating the potential of theta oscillations to reduce hyperexcitability and induced-seizures. Accordingly, we hypothesized that in addition to ameliorating cognitive impairments, stimulation of the MSN to drive theta oscillations would also concurrently increase seizure threshold in response to flurothyl exposure in pilocarpine-treated rats.

In the current study, pilocarpine rats had a significantly lower seizure threshold compared to shams at PPD32. By PPD56, three weeks later, a further significant decrease was observed in non-stimulated pilocarpine animals, while no change was observed in shams. Importantly however, seizure threshold did not decrease in either CT or PT stimulated pilocarpine groups. In fact, there was no significant difference in seizure threshold at PPD56 when comparing pilocarpine-treated animals receiving stimulation and sham animals. Elevated seizure threshold in epileptic animals may be a result of influencing the level of inhibition in a hyperexcitable network. Critically, the beneficial effects of stimulation were again limited to epileptic animals, as stimulation of sham controls did not alter seizure threshold.

Limitations

In the current study we were unable to continuously monitor EEG and therefore quantify seizures. Furthermore, signal-to-noise of the EEG during exploration of the Barnes maze was inadequate to rigorously analyze EEG during navigation. Moreover, significant artifact from stimulation further complicated evaluation of EEG during ongoing stimulation. It is critical, therefore, that future studies include continuous EEG and behavioral monitoring to not only quantify seizures in animals injected with pilocarpine, but also to determine whether a limited period of stimulation, as described in this manuscript, has lasting effect on reducing seizures, in addition to altering seizure threshold, and preserving EEG in pilocarpine-treated rats.

Conclusion

Electrical neuromodulation is an FDA-approved strategy to reduce seizures in patients with refractory TLE, and there are ongoing efforts to develop neuromodulation strategies to improve cognitive abilities in patients across a range of neurological disorders. Our goal was to identify a single target and stimulation paradigm that would both reduce seizures and improve cognitive performance in a rat model of TLE. There is clear evidence that altered theta oscillations play a critical role in both epilepsy and cognitive processes and that septohippocampal theta oscillations coordinate local and distal neural networks, modulating the excitability of neurons and the timing of their activity. This coordination is important for preventing the uncontrolled synchronized activation of neurons observed during seizures, as well as facilitating neuronal processes critical to plasticity and cognition. As epileptogenesis alters theta oscillations and the underlying network, we hypothesized that entraining septohippocampal theta oscillations could concurrently reduce excitability and improve learning. Our data suggest that theta stimulation of the MSN preserves oscillatory activity over the duration of the study, improves cognitive performance, and increases seizure threshold during flurothyl exposure. While clinical translation of septal stimulation to treat epilepsy will require further testing and confirmation, a recent review by Fisher and colleagues also demonstrated the potential benefits of MSN DBS to treat TLE [48]. Therefore work done by our lab as well as others together emphasize that theta frequency stimulation of the MSN represents a promising potential therapeutic option to significantly improve the quality of life for millions of patients with refractory temporal lobe epilepsy.

Declarations of interest

None.

Potential conflicts of interest

There are no potential conflicts of interest by any author involved in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2019.01.005.

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