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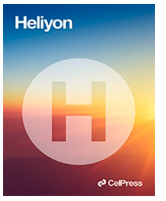

Research article

Effects of acute hippocampal stimulation in the nonhuman primate penicillin model of temporal lobe seizures

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ABSTRACT

Asynchronous distributed multielectrode stimulation (ADMES) is a novel approach to deep brain stimulation for medication resistant temporal lobe epilepsy that has shown promise in rodent and *in vitro* seizure models. To further evaluate its effects on a pre-clinical model, we characterized the effect of unilateral ADMES in an NHP model of temporal lobe seizures induced by intra-hippocampal injection of penicillin (PCN). Four non-human primates were used for this study in two contemporaneous cohorts. One cohort (n = 3 hemispheres) was implanted with the Medtronic RC + S stimulation (GIN cohort) and recording system connected to two 4-contact ring electrodes to evaluate three unilateral stimulation patterns: 7 Hz Ring ADMES, 20 Hz Dual Ring, and 125 Hz Dual Ring (analog of clinical stimulation). In an additional cohort (EPC cohort, n = 2), two 12-contact segmented electrodes were implanted in the right hippocampus and connected to an externalized recording and stimulation system to allow more flexibility in the stimulation pattern. In this second cohort, 4 variations of stimulation were evaluated (7 Hz Full ADMES, 7 Hz Ring ADMES, 31 Hz Wide Ring, and 31 Hz Dual Ring). In the GIN cohort, we found an increase in seizure frequency and time spent in seizure during the 7 Hz Ring ADMES stimulation compared to the respective post-stimulation. A similar post-stimulation effect was found in the EPC cohort. We also found an increase in seizure frequency during the 7Hz full ADMES compared to the respective post-stimulation. However, we did not find a difference between pre-stimulation and stimulation conditions suggesting a possible post stimulation effect of the 7Hz hippocampal stimulation. In conclusion, in the NHP PCN model of temporal lobe seizures, acute asynchronous hippocampal stimulation was not therapeutic, however, our findings related to the post-

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stimulation effect can support future studies using hippocampal stimulation for the treatment of temporal lobe epilepsy.

1. Introduction

Epilepsy affects up to 1 % of the population [1] with seizures originating from temporal lobe most likely to be drug resistant [2]. Focal temporal lobe seizures can be associated with auras, oral automatisms, loss of consciousness, memory deficits [3–5], and can generalize to other parts of the brain [6]. While surgery is an option for some patients, many are not eligible as it may adversely impact memory and cognitive function [7,8]. For these patients, brain stimulation therapy can be an attractive alternative.

There are currently two clinically approved brain stimulation therapies for temporal lobe epilepsy – responsive neurostimulation (RNS) [9], and deep brain stimulation (DBS) of the anterior nucleus of the thalamus (ANT) [10]. In addition, early clinical studies on continual hippocampal stimulation have shown promising outcomes for patients with temporal lobe epilepsy [11–14]. While these therapies are effective, achieving >50 % seizure reduction in most cases, very few patients become seizure free. This treatment gap highlights the need for new approaches to brain stimulation for temporal lobe epilepsy that are more effective at achieving the gold standard of seizure freedom.

Asynchronous distributed multielectrode stimulation (ADMES) is a recently developed approach to deliver brain stimulation for epilepsy. ADMES is based on the hypothesis that asynchronous stimulation distributed across multiple electrodes can desynchronize the neural circuit and prevent seizures. Prior work demonstrated that asynchronous stimulation across a microelectrode array can decrease spontaneous bursting in cortical neuron cultures [15]. More recently, we found that ADMES at theta frequencies (4–7 Hz) also decreased seizures in the rat tetanus toxin model of temporal lobe seizures [16]. Based on this success, ADMES was well positioned for further validation in a nonhuman primate (NHP) model of temporal lobe seizures, which has also been shown to respond to ANT DBS [17]. Here we report the results of the first study of ADMES and various pattern of hippocampal stimulation in an NHP model of temporal lobe seizures. We tested the effectiveness of hippocampal stimulation across two cohorts, utilizing either an implanted clinical research neurostimulator capable of stimulation and recording, or an externalized stimulation platform that allowed for greater flexibility in delivering different stimulation pattern.

2. Methods

This study was conducted at two centers: The Grenoble Institute of Neuroscience (GIN) and the Emory National Primate Research Center (EPC). All procedures at GIN were carried out in accordance with European Communities Council Directive of 2010 (2010/63/UE) as well as the recommendations of the French National Committee (2013/113). Authorization for conducting these experiments was approved by the Committee on the Ethics of Animal Experiments (#00132.01, #04). All procedures at EPC were performed in accordance with the U.S. Public Health Service Policy on the humane care and use of laboratory animals, including the provisions of the "Guide for the Care and Use of Laboratory Animals" [18]. All studies were approved by the Biosafety and Institutional Animal Care and Use Committees of Emory University.

A total of 4 adult rhesus macaques were used in this study, 2 at GIN (2 males) and 2 at EPC (1 male and 1 female). Subjects weighed between 5 and 12 kgs with ages between 3 and 7 years. All subjects were acclimated to being handled and trained to sit in a primate chair.

2.1. Surgery

The 2 NHPs at GIN (GIN-1 and GIN-2) underwent surgery under general anesthesia using intramuscular injection of ketamine (Imalgene, Merial laboratory, France) with 0.4 mg at induction and 0.2 mg per hour for maintenance, along with xylazine 2 %, 0.2 ml at induction followed by 0.1 ml per hour (Rompun, Bayer Healthcare AG, Germany). In addition, local anesthesia was provided by subcutaneous injection of lidocaine hydrochloride to the scalp. Prophylactic antibiotics, analgesics, and anti-inflammatories were provided preoperatively. Two leads with 4 annular contacts (lead length, 40 mm; outer diameter, 0.8 mm; with contacts of 0.5 mm spaced 0.5 mm apart; DIXI, France) were implanted in the right hippocampus (GIN1-R and GIN2-R) under ventriculographic control (2 mL water soluble iodine contrast medium, Braco Imaging, France). A guide cannula was implanted between the electrodes to enable repeated injections of PCN for seizure induction. All hardware was embedded in an acrylic cap. The back of the NHP was prepared for a skin incision and a subcutaneous space was made to place the implantable pulse generator (IPG; Model RC + S, Medtronic, USA). Lead extensions were tunneled through the subcutaneous space of the neck and back and connected to the IPG and implanted electrodes. During the testing phase, Subject GIN-1 experienced an infection at the lead extension site. After explantation and recovery, 2 new electrodes were implanted in the left hemisphere (GIN1-L).

The 2 NHPs at EPC underwent an aseptic survival surgery under general anesthesia with 1–3% isoflurane. Using a stereotaxic frame and based on preoperative MRI imaging, an MRI compatible recording chamber (Crist Instrument, Hagerstown, MD; USA) aiming at the right hippocampus ~10 mm anterior to the ear bar zone (EBZ) was placed along with a headpost for head fixation, and 7 epidural screws (diameter 0.25 mm, length 0.4 mm). The epidural screws were placed over the motor and frontal cortices of each hemisphere for recording cortical activity. Two additional screws were placed over one eye for bipolar electrooculographic recording, and one reference electrode was placed over the occipital lobe. The epidural screws were wired to a connector (A22017–001, Omnetics,

Minneapolis, MN, USA), secured in a custom designed 3D printed enclosure [19]. All the hardware was embedded in an acrylic cap. Animals were given analgesics and prophylactic antibiotics for one week following the surgery. After recovery, a post-operative MRI was used to map the placement of the chamber. Single unit microelectrode recording was used to confirm the depth and boundaries of the hippocampus. After determination of the HPC coordinates, a microdrive was used to lower two macro-stimulation electrodes to the target depth. The electrodes were oriented along the AP axis and separated by 3.3 mm. Each electrode consisted of 12 contacts arranged in four rings separated into three segments (outer diameter, 0.8 mm, contacts of 0.5 mm in length spaced 0.5 mm apart; Heraeaus, Hanau, Germany). In both cohorts NHPs were allowed to recover for at least 10 days before beginning experiments.

2.2. Electrophysiological recording

In the GIN cohort, neural data was recorded using the Medtronic RC + S sensing capabilities. Local field potentials (LFP) were recorded from a single contact on one of the electrodes at a sampling rate of 500 Hz.

In the EPC cohort, neural data was recorded using an externalized data acquisition system (Cerebus, Blackrock Neurotech). Both electrodes were connected to a Y-adaptor for continuous recording from each of the contacts during stimulation. LFPs were recorded from each contact at a sampling rate of 2000 Hz.

2.3. Stimulation paradigms and safety thresholds

2.3.1. Stimulation paradigms

Stimulation experiments were initiated after subjects recovered from surgery and each animal received unilateral hippocampal stimulation. In the GIN cohort stimulation was applied using the RC + S. We evaluated three stimulation settings: 7Hz Ring ADMES, 20Hz Dual Ring, and 125Hz Dual Ring similar to 'clinical' stimulation (Fig. 1). All stimulation waveforms were current controlled, biphasic square waves with a pulse width of 200 μs. The 7Hz Ring ADMES stimulation was composed of 4 ordered programs that cycled at fixed intervals over 1/7 s (Fig. 1A). Each program defined a bipolar stimulation setting between two adjacent contacts. The 20Hz and 125Hz Dual Ring stimulation settings applied bipolar stimulation across two groups of contacts (Fig. 1B–C). Minor fluctuation in the amplitude of the stimulation during the 7Hz Ring ADMES stimulation were noted, however it is unlikely that this might have affected the effect of the stimulation (Sup Fig. 1).

In the EPC cohort, we evaluated 7 Hz Full ADMES, 7Hz Ring ADMES, 31 Hz Wide Ring, and 31 Hz Dual (Fig. 2). The stimulation waveforms were current controlled, symmetric, biphasic square waves with a pulse width of 200 μs. The 7Hz Full ADMES stimulation was composed of 12 ordered programs that cycled at fixed intervals over 1/7 s (Fig. 2A). Each program defined a monopolar stimulation setting using the patient ground connected to the primate chair to reproduce the stimulation pattern used in our original study in rodents [16]. The 7Hz Ring ADMES stimulation was composed of 4 ordered programs that cycled at fixed intervals over 1/7 s (Fig. 2B). Each program defined a bipolar stimulation setting between two adjacent pseudo ring contacts. Both the Wide and Dual Ring stimulation were composed of 2 ordered programs that cycled at fixed intervals over 1/31 s (Fig. 2C–D). Each program defined a bipolar stimulation setting between the most dorsal and ventral pseudo ring contact for Wide Ring and for the Dual Ring, the two dorsal and ventral pseudo rings were grouped into two super contacts. In both cohorts, the stimulation amplitude was determined independently for each stimulation setting (Table S1).

2.3.2. Determining subject- and pattern-specific safety thresholds for EPC and GIN cohort

The subject-specific amplitude threshold was evaluated for each type of stimulation. Stimulation was applied for 2 min in a 5 s-on/5 s-off pattern followed by 2 min of washout. To titrate the amplitude, each 2-min block of stimulation was applied with increasing current, starting with 100 μA with increments of 100–200 μA. During each stimulation, the LFP was monitored for after discharges or other abnormal electrophysiological signals and the subject was closely monitored for any behavioral changes time-locked with the

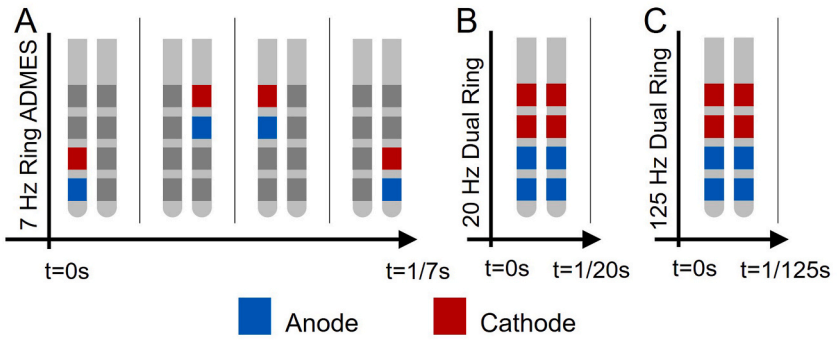

Fig. 1. Stimulation patterns evaluated with the implanted RC + S (GIN, cohort 1). (A) The 7Hz Ring ADMES stimulation pattern consisted of 4 different stimulation programs delivered within 1/7s, resulting in one stimulation every 1/28s. The order of stimulations programs was fixed. For the 20Hz (B) and 125Hz Dual Ring stimulation (C), pulses were delivered simultaneously across both electrodes.

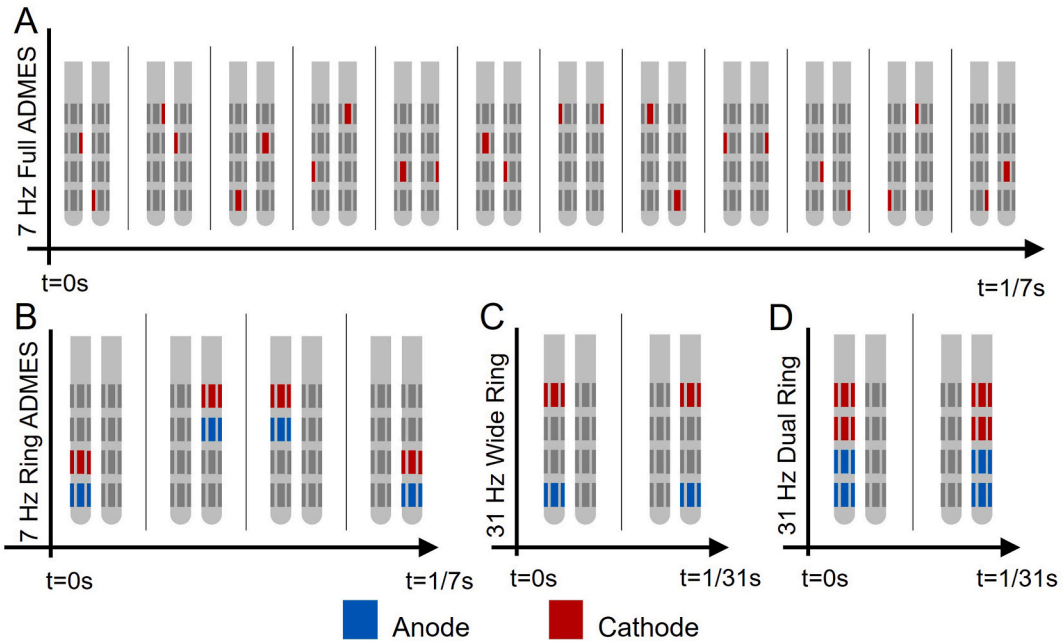

Fig. 2. Stimulation patterns evaluated with externalized system (EPC, cohort 2). (A) Full ADMES leverages the segmented contacts of the electrode to distribute the asynchronous stimulation across 12 independent contacts per electrode. Due to hardware limitations, two contacts, one on each electrode, were stimulated simultaneously. The order of contacts stimulated was fixed, but arbitrary. The Full ADMES pattern was composed of 12 ordered programs that cycled at fixed intervals over 1/7 s. Each program defined a monopolar stimulation setting using the patient ground connected to the primate chair to reproduce the stimulation pattern used in our original study in rodents [1]. (B) Ring ADMES consisted of stimulation at 4 different pairs of pseudoring contacts (three segmented contacts stimulated simultaneously) used to apply bipolar stimulation with an overall group frequency of 7Hz. (C) Wide Ring 31Hz consisted of stimulation at the dorsal and ventral pseudorings of each electrode. Stimulation was delivered from each electrode at a group frequency of 31 Hz. (D) The Dual Ring 31Hz pattern was identical to the Wide Ring, except the top and bottom two rings are combined into a "super-pseudoring".

stimulation (indicated by agitation, sudden aggressiveness or changes in vocalization). The stimulation amplitude that elicited a behavioral or electrophysiological response was designated as suprathreshold. The stimulation amplitude was then iteratively decremented by 100 μA until the absence of abnormal behavioral and electrophysiological responses was confirmed. The stimulation amplitude was set to 80 % of the side effect threshold. This process was repeated for all stimulation patterns. The stimulation amplitude used for each animal and stimulation pattern were set before the induction of seizure and kept the same for each experimental day. These parameters can be found in Supplementary Table S1.

2.4. Seizure experiments

At both GIN and EPC, seizures for an experimental session were induced via injection into the hippocampus of PCN-G (GIN: SIGMA, P3032; EPC: Panpharma) diluted in sterile water for injection (Millipore Sigma, Merck, Germany) with a final concentration of 1000 units/μl. At EPC, the two electrodes were first lowered to the target depth using a microdrive. Then using an implanted guide cannula (GIN) or an injection cannula guided by the microdrive (EPC), PCN was injected at a rate of 0.1 μL/min controlled with a motorized pump connected to a microsyringe (CMA, Harvard Biosciences). Prior to the start of stimulation experiments, the baseline volume of PCN was titrated individually for each NHP. As previously described, PCN injection typically induced seizures within 20–60 min, after which spontaneous self-terminating seizures presented every 5–20 min for 4–6 h before subsiding completely [20]. Once the seizure rate stabilized, we recorded a 20-min baseline before initiating the stimulation paradigm.

At GIN, each trial consisted of 20 min of pre-stimulation sham, followed by 20 min of stimulation, followed by another 20-min post-stimulation sham period (Fig. 3A). One of the three stimulation settings was randomly selected and applied using an intermittent pattern of 5-s stimulation/5 s off to ensure that seizures could be visualized despite the presence of the stimulation artifact. At EPC, each trial consisted of 10 min of pre-stimulation sham, followed by 20 min of stimulation, followed by another 10-min post-stimulation sham period (Fig. 4A). One of the four ADMES stimulation settings was randomly selected and applied using the same 5 s intermittent stimulation pattern. During each PCN session 2–5 stimulation settings were tested.

2.5. Seizure metrics

Data were imported using Spike 2 (CED, Cambridge, UK) to annotate the seizures offline. The seizure onset and offset times were

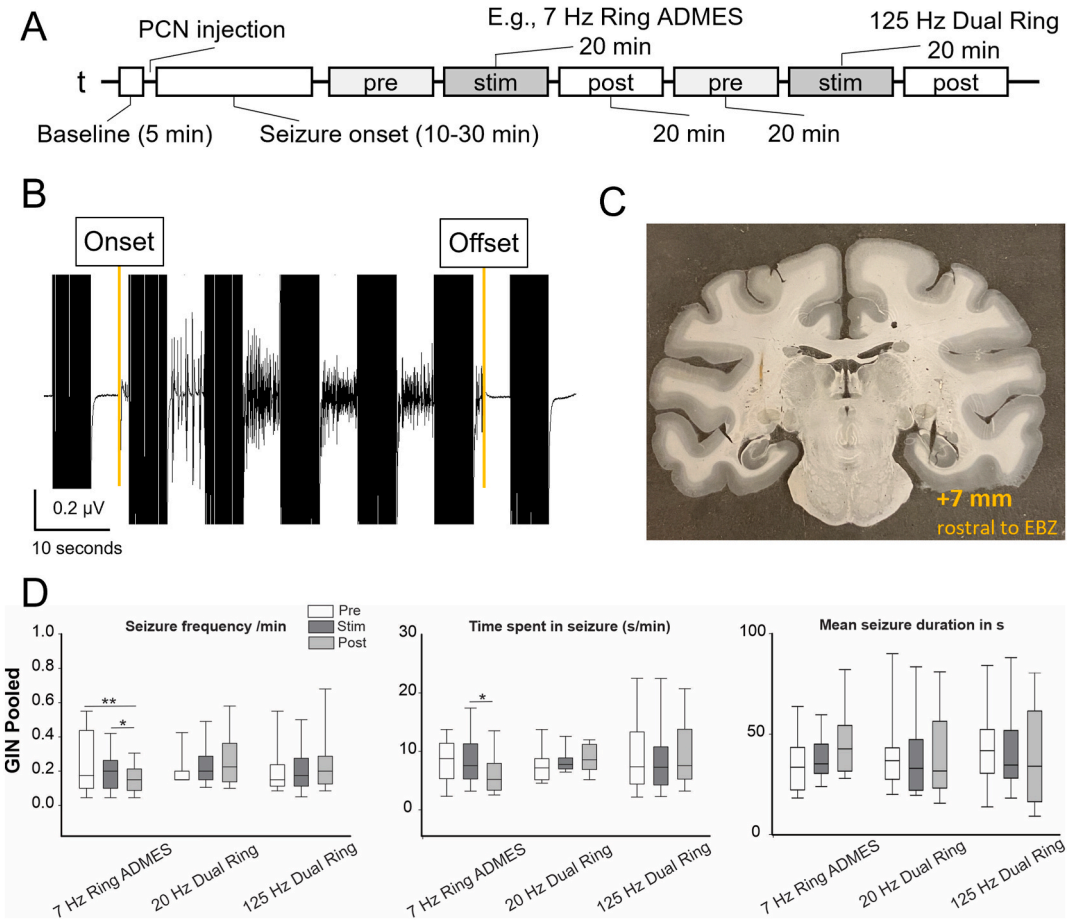

Fig. 3. Seizure outcomes for the GIN cohort. (A) Timeline of a PCN injection experiment. (B) Recording of a single seizure visible between the intermittent 5-s blocks of stimulation. (C) Wet-mounted coronal section of GIN 1 showing the left and right electrode trajectories targeting the hippocampus. (D) Effect of stimulation on seizure metrics. Line represents the median and boxes span from the 25th to 75th percentile. Effect of stimulation on seizure metrics were tested with GEE model and pairwise Sidak error for posthoc analyses, *p < 0.05 ** < 0.01.

manually annotated by a blinded experimenter (Fig. 3B and 4B). If the onset or end of the seizure was obscured by the stimulation artifact, the beginning of the stimulation was labeled as the seizure onset/offset. For each pre, stim and post periods, the following metrics were calculated: frequency of seizure per min, total time spent in seizure in s per min, and the mean seizure duration in s.

2.6. Statistics

To test for differences between the three repeated sequential measurements of seizures during the period immediately before stimulation (pre-phase), during stimulation (stim phase) and immediately after (post-phase), Generalized estimating equations (GEE) was used which adjusts for the non-independence of the multiple trials within each animal. Pairwise posthoc analyses were also performed using Sidak error rate adjustment to compare pre-to-stim, pre-to-post and stim-to-post {Hedeker, 2006 #46}. An additional analysis (between groups analysis of variance, ANOVA) was performed to compare the seizure metrics during the stim and post/sham periods independently across stimulation settings. While distributional assumptions were checked, given the smaller sample sizes, non-parametric tests (Friedman's ANOVA for the repeated measures and Kruskal-Wallis ANOVA for the between subject's tests) were also run to check the robustness of the findings and p-values <0.05 are noted. All statistical analyses were performed using SPSS v.27.

2.7. Sacrifice and Postmortem examination

The animals received an overdose of Nembutal (25 mg/kg) and were perfused with Ringer's solution followed by 4 % para-formaldehyde in phosphate buffer (0.2 M, pH 7.4). After removing the brain from the skull, brains were post fixed in 4 % para-formaldehyde for 2 days and cryoprotected in ascending sucrose solution (10, 20, and 30 %). Coronal sections (50 µm) were obtained using a freezing microtome and collected in series in cold phosphate-buffered saline (PBS, 0.01 M, pH 7.4), and subsequently stored at −20 °C in an anti-freeze solution (30 % ethylene glycol/30 % glycerol in PB) until further processing. Sections were mounted on glass

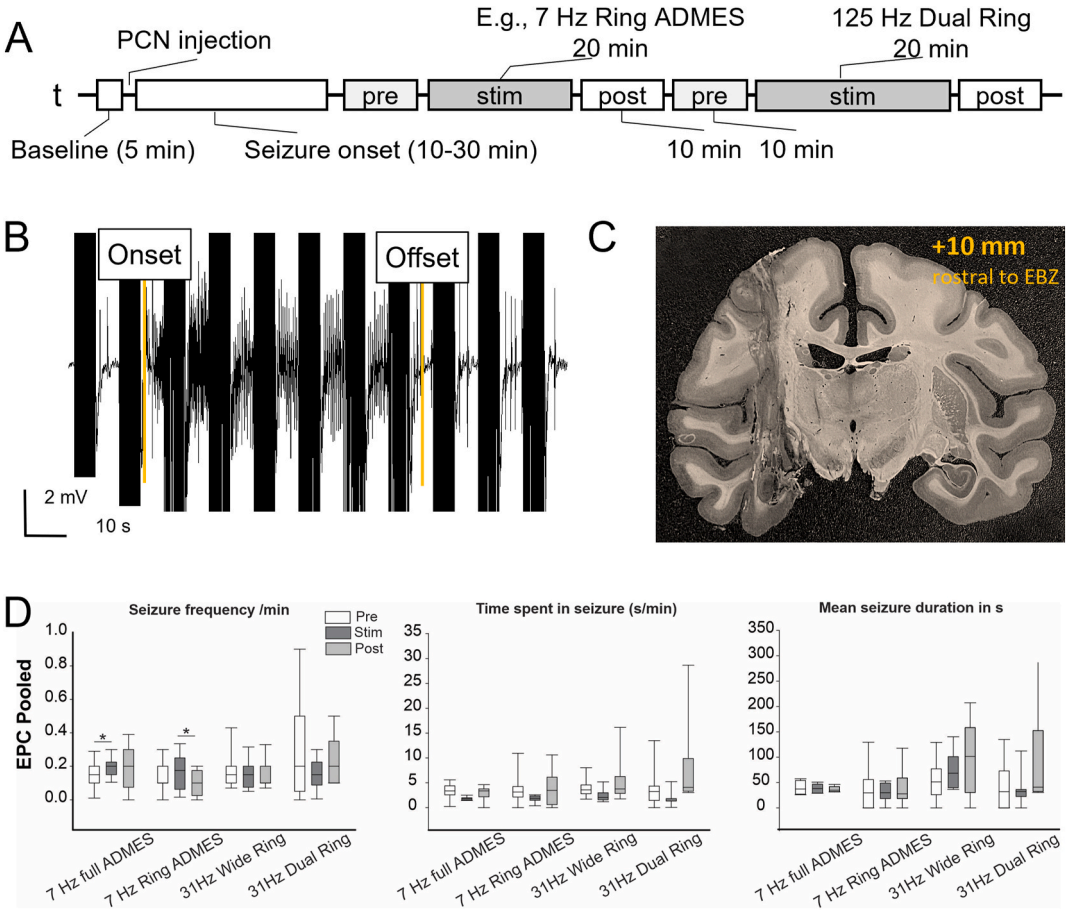

Fig. 4. Seizure outcomes for the EPC cohort. (A) Timeline of a PCN injection experiment. (B) Recording of a single seizure visible between the intermittent 5-s blocks of stimulation. (C) Coronal section of EPC 1 showing the electrode trajectories. (D) Line represents the median and boxes span from the 25th to 75th percentile. Effect of stimulation on seizure metrics were tested with GEE model and pairwise Sidak error for posthoc analyses, *p $<$ 0.05.

slides and photographed while wet to confirm electrode placement (Fig. 3C and 4C).

3. Results

3.1. 7 Hz Ring ADMES, 20 and 125 Hz Dual Ring stimulation did not show a significant beneficial effect on seizures in chronically implanted NHPs

In the GIN cohort, we performed 12 PCN seizure induction sessions across 3 hemispheres in 2 NHPs for a total of 43 blocks of pre/stim/post. The number of blocks varied between sessions. The mean volume of PCN per injection was 3.5 μL. All seizures induced in the GIN cohort were associated with rotation of the neck towards the ipsilateral shoulder. All three stimulation settings were evaluated at

Table 1
Changes in seizure metrics in pre, stim, and post period for each stimulation condition in the GIN cohort.

		Pre	Stim	Post
Seizure frequency	7 Hz Ring ADMES	0.25 ± 0.19	0.22 ± 0.14	0.14 ± 0.10
	20 Hz Dual Ring	0.19 ± 0.09	0.24 ± 0.12	0.25 ± 0.16
	125 Hz Dual Ring	0.22 ± 0.20	0.22 ± 0.19	0.26 ± 0.19
Time spent in seizure	7 Hz Ring ADMES	8.08 ± 3.76	8.06 ± 5.03	6.65 ± 4.55
	20 Hz Dual Ring	7.59 ± 2.93	8.22 ± 1.90	8.77 ± 2.37
	125 Hz Dual Ring	9.08 ± 6.32	8.54 ± 6.14	9.08 ± 5.76
Mean seizure duration	7 Hz Ring ADMES	38.96 ± 14.01	33.96 ± 10.76	50.85 ± 21.12
	20 Hz Dual Ring	43.21 ± 19.57	41.13 ± 18.89	43.31 ± 22.02
	125 Hz Dual Ring	45.98 ± 20.05	44.90 ± 22.12	42.66 ± 24.28

least 3 times in each hemisphere, except for the 20 Hz Dual Ring stimulation, which was only evaluated in one hemisphere of each subject. The histological analysis of the GIN cohort confirmed the placement of the electrode and injection cannula within the hippocampus area (Fig. 3C).

Combining the data from all three hemispheres, we found no significant difference between pre and stim periods for any of the simulation patterns tested (Fig. 3D). However, we found that the total time spent in seizure per minute was significantly higher during 7 Hz Ring ADMES than during post-stimulation (mean ± std: stim 8.06 ± 5.03 vs. post 6.65 ± 4.55 s spent in seizure/min, p-value = 0.042; Fig. 3D and Table 1; GEE model results and data for each animal are available in Table S2 and Table S3, respectively). Similarly, the frequency of seizures was significantly higher during 7 Hz Ring ADMES compared to post stimulation (mean ± std: stim 0.22 ± 0.14 vs. post 0.14 ± 0.10 seizure/minute, p-value = 0.030; Fig. 3D). However, the seizure frequency during the post 7 Hz Ring ADMES period was also lower than the pre-stimulation period (p-values = 0.005).

We did not find any significant difference when comparing seizure metrics between stimulation settings. This analysis confirmed the absence of difference between the pre and post stimulation periods across the stimulation setting tested (Table S3). No stimulation setting demonstrated a significant therapeutic effect on either seizure metric, including clinically established settings.

3.2. *Neither synchronous nor asynchronous hippocampal stimulation induces a therapeutic effect on seizure metric*

In the EPC cohort, 16 PCN seizure induction sessions were performed in 2 NHPs, for a total of 48 blocks of pre/stim/post. Each stimulation setting was evaluated at least 3 times per subject. The mean volume of PCN per injection was 12.0 μL. Seizures were electrographic and localized to the temporal lobe with no overt behavioral effects, in contrast to the GIN subjects. Histological analysis confirmed that for both EPC subjects, the electrodes tracts terminated in the hippocampus between 14 and 8 mm rostral to EBZ. However, in both subjects there was substantial mechanical damage to the column of tissue dorsal to the hippocampus due to the multiple penetration (Fig. 4D).

With the combined data, we found an increase in the seizure frequency in the 7 Hz Full ADMES stimulation period compared to the pre-stimulation period (number of seizures/min change from 0.17 ± 0.07 in the pre to 0.20 ± 0.06 in the stim, p-value = 0.036, Fig. 4 and Table 2, GEE model results data for each animal are available in Table S2 and Table S5, respectively). Similar to the GIN cohort, we found a decrease of the seizure frequency after the 7 Hz Ring ADMES (from 0.17 ± 0.11 seizure/min during stimulation to 0.10 ± 0.07 during post-stimulation periods, p = 0.049).

Overall, these findings suggest that 7 Hz Ring ADMES might induce a reduction in seizure frequency in the post-stimulation period (similar to the one noted in the GIN cohort). Similarly to the GIN cohort, we did not find any significant difference when comparing seizure metrics between stimulation settings (Table S4).

4. Discussion

In this study, we evaluated the effect of hippocampal stimulation in the NHP PCN model of temporal lobe seizures. We sought to build on our previous rodent experiment showing moderate success using asynchronous stimulation at theta frequencies [21]. In this NHP PCN model, we did not observe a therapeutic effect on seizure metric with any of the stimulation tested. However, we found an increase in seizure frequency during the 7Hz full ADMES stim in the EPC cohort and a decrease of seizure frequency in the post-stimulation period after 7 Hz Ring stimulation in both cohorts. These results contradict our previous finding obtained in a rodent model but are aligned with results obtained from patients. Thus, theta-burst stimulation has been found to induce generalized and partial seizures in patients [22–24]. While we did not find any therapeutical effect, our findings related to the post-stimulation effect can support future studies using hippocampal stimulation to improve the treatment of temporal lobe epilepsy.

One consideration to advance the effectiveness of hippocampal stimulation observed in this study is the electrode orientation. Drawing contrast with clinical examples of hippocampal stimulation where the electrode is typically implanted along the longitudinal axis of the hippocampus [25], we used a transdorsal approach to avoid the large occipital muscle in macaques. Rather than an electrode along the axis of the hippocampus with the electrodes directed radially outward, our electrodes traversed the hippocampus at

Table 2
Changes in seizure metrics in pre, stim, and post period for each stimulation condition in the EPC cohort.

		Pre	Stim	Post
Seizure frequency	7 Hz Full ADMES	0.17 ± 0.07	0.20 ± 0.06	0.18 ± 0.13
	7 Hz Ring ADMES	0.13 ± 0.10	0.17 ± 0.11	0.10 ± 0.07
	31 Hz Wide Ring	0.16 ± 0.10	0.16 ± 0.09	0.15 ± 0.10
	31 Hz Dual Ring	0.20 ± 0.17	0.19 ± 0.10	0.21 ± 0.14
Time spent in seizure	7 Hz Full ADMES	6.07 ± 3.17	7.33 ± 2.98	6.30 ± 4.30
	7 Hz Ring ADMES	6.33 ± 5.47	6.70 ± 5.09	5.52 ± 5.42
	31 Hz Wide Ring	6.89 ± 6.63	7.69 ± 5.79	8.74 ± 7.70
	31 Hz Dual Ring	8.17 ± 6.29	7.07 ± 4.07	11.54 ± 8.57
Mean seizure duration	7 Hz Full ADMES	36.86 ± 10.29	36.06 ± 7.03	28.58 ± 15.77
	7 Hz Ring ADMES	41.24 ± 33.72	35.78 ± 14.47	39.88 ± 35.26
	31 Hz Wide Ring	39.57 ± 27.89	50.17 ± 29.86	59.33 ± 54.75
	31 Hz Dual Ring	39.33 ± 39.77	36.69 ± 14.60	73.20 ± 83.44

two rostral-caudal points along its axis. While this difference in electrode orientation may exert some influence over the effects of hippocampal stimulation, the transdorsal approach has successfully been used clinically [26], and recent work in NHPs found that hippocampal stimulation using a transdorsal approach could alleviate seizures in the kainic acid model of temporal lobe seizures [27].

Another consideration is the acute, rather than chronic, stimulation used in our study. Our experiment was designed to detect acute effects of stimulation, and so stimulation was applied intermittently over sessions of 3–5 h. However, it is now well-established that clinical DBS for epilepsy can take 3–6 months before the therapeutic effects are fully realized – even for RNS, which was designed to rapidly detect ictal events and apply brief bursts of stimulation to interrupt potential seizures [28–30]. Chronic stimulation of either the hippocampus or ANT has also been shown to be effective in the NHP kainic acid model of temporal lobe seizures [31]. In two studies, chronic hippocampal or ANT DBS at 130 Hz resulted in a >50 % decrease in the number of seizures and no generalized tonic-clonic seizures over an observation period of at least three months. Moreover, it was found that while administration of kainic acid caused apoptosis and cell death in the hippocampus, chronic stimulation attenuated the changes in proteins related to apoptosis and resulted in decreased neuronal loss compared to unstimulated controls [27,32]. Follow-up studies have also found evidence that chronic stimulation of the hippocampus may exert neuroprotective effects by reversing abnormal gene expression [33], while chronic ANT DBS may induce autophagy [34]. Using microdialysis, it was found that ANT DBS applied chronically over 90 days was associated with a change in neurotransmitters relative to unstimulated controls that became significant after 30 days post injection of the kainic acid [35]. There is one example of hippocampal stimulation for idiopathic epilepsy in an NHP with temporal lobe seizures. In this case, hippocampal stimulation delivered chronically for up to 15 days did not significantly reduce the number of seizures [36]. In this context, it is possible that the effects that we observed with acute stimulation might improve or worsen over time with chronic stimulation.

The PCN model could also be a suboptimal model for testing the effects of ADMES and may offer an alternative explanation for pro-ictal effects of the 7 Hz Ring ADMES observed in this study. We selected the PCN model because it exhibits numerous non-convulsive seizures on demand allowing us to test the effects of complex stimulation patterns using an externalized system [20]. Unlike the kainic acid model of temporal lobe epilepsy [31], the PCN model may be more akin to non-convulsive status epilepticus than the spontaneous onset seizures observed in the in patients with temporal lobe epilepsy. However, in our prior work using an experimental design like the one described here, we found that 40 Hz ANT DBS significantly reduced the number of seizures and total time in seizure [17]. This suggests that these PCN induced seizures can be acutely modulated by electrical stimulation of targets in the same neural circuit as the seizure focus, but at potentially different stimulation frequencies.

One other factor to take into consideration is that rodent models do, in fact, respond to acute stimulation, potentially using different mechanisms than in clinical DBS for temporal lobe seizures. Rodents have been used to evaluate different forms of hippocampal DBS for temporal lobe seizures across a variety of models including kainic acid [37], kindling [38], and 4-AP [39]. While these models can aid in our understanding of how seizures propagate, there are several factors that limit their utility for DBS in human temporal lobe epilepsy. While the hippocampus is relatively conserved between rodent and primates with the exception of its spatial orientation, temporal lobe seizures propagate to other parts of the brain, such as the thalamus [40] and basal ganglia [41,42], that do have substantive functional and anatomical differences between rodents and primates [43]. Combining our findings with previous clinical and preclinical research on DBS for temporal lobe seizures highlights several discernible patterns. First, the most clinically consistent effects of hippocampal (or ANT) stimulation for temporal lobe seizures in NHP have been observed in studies using chronic stimulation and the kainic acid model [27,31–35]. While potentially an indictment of the PCN model, it will be informative to evaluate how the PCN model responds to chronic stimulation. Given that both are models of focal seizures, similar approaches to block seizure activity from propagating and manifesting symptomatically should be similarly effective. Finally, while orientation specific effects of stimulation have been observed and should be accounted for when evaluating the effects of stimulation [44], this is less likely to be a critical component of effective hippocampal stimulation.

4.1. Limitations

There are potential effects of hippocampal stimulation that were not evaluated in this study. First, this study was not designed to detect changes in the threshold of PCN necessary to induce seizures. Second, the primary outcome measures of this study were limited to electrographic seizures measured at the seizure focus. It is possible that stimulation limited the propagation of the seizure to regions of the brain that were not recorded. Similarly, it is possible that stimulation allowed for continued awareness or suppressed other subtle symptoms that were not measured in our experimental design. Additionally, while in this model the frequency of seizures was shown to be stable for a couple of hours [17], we were not able to distinguish between an increased rate of spontaneous seizure related to the dynamic of the PCN model and an increased rate triggered by the stimulation. Another potential limitation is that we only tested unilateral stimulation. Stronger effects have been found with bilateral compared to unilateral hippocampal stimulation in rodents as well as in patients [45]. Another consideration is that the volume of tissue stimulated may have varied between subjects and stimulation settings. In future work, we will consider using computational modeling to better relate the current distribution to the effects of stimulation. In addition, we observed some tissue damage observed in the EPC subjects within and dorsal to the hippocampus. While this potentially had some influence on the overall results, we did not observe any change in seizure pattern or any spontaneous seizures between PCN sessions. Finally, the low sample size limits the power of the analysis and the ability to test for sex-based differences.

4.2. Conclusion

In this model, we observed no therapeutic impact from hippocampal stimulation. Nonetheless, our discoveries regarding the post-

stimulation effects could provide valuable insights to enhance future studies employing hippocampal stimulation for treating temporal lobe epilepsy.

CRediT authorship contribution statement

Mark J. Connolly: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **Brigitte Piallat:** Data curation, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **Mohammad Sendi:** Investigation, Software, Writing – review & editing. **Babak Mahmoudi:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Melinda K. Higgins:** Formal analysis, Methodology, Writing – review & editing. **Claire-Anne Gutekunst:** Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing. **Annaelle Devergnas:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision. **Robert E. Gross:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

Mohammad Sendi provides consulting services for Niji Corp. Robert E. Gross serves as a consultant to Medtronic, which manufactures products related to the research described in this manuscript and receives compensation for these services. He also receives support for unrelated research. The terms of this arrangement have been reviewed and approved by Emory University in accordance with its conflict of interest policies.

Robert E. Gross leads a clinical trial NCT04710004 "Electrophysiological Biomarkers in MTLE Patients." This clinical trial aims to apply asynchronous distributed multi-electrode stimulation in the hippocampus of subject with medial temporal lobe epilepsy to identify biomarker related to the pre-ictal state.

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The data that support the findings of this study are openly available in DANDI:001054/0.240701.1903 at https://dandiarchive.org/dandiset/001054?pos=1.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34257.

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